# GLUE AND GELATIN

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BY

JEROME ALEXANDER

AUTHOR OF "COLLOID CHEMISTRY, AN INTRODUCTION"

American Chemical Society
Monograph Series

*BOOK DEPARTMENT*

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# GENERAL INTRODUCTION

## American Chemical Society Series of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic Monographs on chemical subjects. At the same time it was agreed that the National Research Council, in coöperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, Secretary of the American Chemical Society, Washington, D. C.; John E. Teeple, Treasurer of the American Chemical Society, New York City; and Professor Gellert Alleman of Swarthmore College. The Trustees have arranged for the publication of the American Chemical Society series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company of New York City.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed the editors, named at the close of this introduction, to have charge of securing authors, and of considering critically the manuscripts prepared. The editors of each series will endeavor to select topics which are of current interest and authors who are recognized as authorities in their respective fields. The list of monographs thus far secured appears in the publisher's own announcement elsewhere in this volume.

The development of knowledge in all branches of science, and especially in chemistry, has been so rapid during the last fifty years and the fields covered by this development have been so varied that it is difficult for any individual to keep in touch with the progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and such compendia as Beilstein's Handbuch der Organischen Chemie, Richter's Lexikon, Ostwald's Lehrbuch der Allgemeinen Chemie, Abegg's and Gmelin-Kraut's Handbuch der Anorganischen Chemie and the English and French Dictionaries of Chemistry, it often takes a great deal of time to coördinate the knowledge available upon a single topic. Consequently when men who have spent years in the study of important subjects are willing to coördinate their knowledge and present it in concise, readable form, they perform a service of the highest value to their fellow chemists.

It was with a clear recognition of the usefulness of reviews of this character that a Committee of the American Chemical Society recommended the publication of the two series of monographs under the auspices of the Society.

Two rather distinct purposes are to be served by these monographs. The first purpose, whose fulfilment will probably render to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a readable form, intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs will enable such men to form closer contact with the work of chemists in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well digested survey of the progress already made in that field and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, it is intended to include extended references to the literature, which will enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection will be made of those papers which are most important.

The publication of these books marks a distinct departure in the policy of the American Chemical Society inasmuch as it is a serious attempt to found an American chemical literature without primary regard to commercial considerations. The success of the venture will depend in large part upon the measure of coöperation which can be secured in the preparation of books dealing adequately with topics of general interest; it is earnestly hoped, therefore, that every member of the various organizations in the chemical and allied industries will recognize the importance of the enterprise and take sufficient interest to justify it.

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# PREFACE

The manufacture of glue and gelatin is important not only because of the magnitude of the industry itself, but also because these products are essential to the production of many others, thus making it a key-industry. Furthermore glue and gelatin are typical jelly-forming colloids and they have been used in numberless experiments on and investigations into the nature and behavior of colloids. As a consequence there is an embarrassing wealth of publications from which to draw material for a monograph. For the same reason, a discussion of the behavior of glue and gelatin involves the consideration of many moot points in colloid chemistry, and gives the subject an interest rather broader than the title would indicate.

The theoretical aspect has been treated more at length than has been usual with books on glue, for a more complete understanding of the nature of a product must in the end be useful to its makers and users. Where opinions vary, the different views are given, often in the very words of their principal advocates. Nor have I withheld my own views in such cases.

In the technical sections elaborate descriptions of well-known apparatus have been avoided, because new forms and modifications are continually appearing, and any one can have the latest particulars from their manufacturers. Such descriptions use space to no good purpose, and may give a reader at some later date the erroneous idea that the machines described are then the best of their kind. However the principles involved in manufacture, testing, and use, have been particularly stressed.

On the other hand many excellent papers have been omitted or referred to but briefly, some because the points involved were treated adequately by others, some because they are beyond the scope of this book, and some perhaps because of inadvertence or inaccessibility. No attempt has been made to pass on questions of priority, and the fact that a certain author is quoted as expressing certain views, does not *necessarily* imply that he was the first to express such ideas. For the benefit of those

who wish to look further into such papers, reference may be made to the bibliographies and indexes mentioned below.

I am indebted to many authors for books and reprints, and to a still larger number for scattered items of information. In all cases I have striven to make due acknowledgment in the text for material used, and to refer wherever possible to the original sources of information.

JEROME ALEXANDER.

## Bibliography.

An extensive bibliography on Glue, comprising about 150 titles, was published by Rudolf Ditmar in the *Kolloid Zeitschrift,* 1906, Vol. 1, p. 80. Another bibliography of about the same extent was published by Robert H. Bogue in *Chemical and Metallurgical Engineering,* 1920, Vol. 23, No. 5. The First Report of the British Adhesives Research Committee contains a descriptive bibliography of gelatin (75 pp.) by T. Slater Price, which gives a resumé of much important work.

The indexes of the principal chemical, physical, biological, and technical journals may be consulted, and it must be remembered that many experiments with gelatin are apt to be found in papers or texts indexed under such headings as "Proteins," "Colloids," "Jellies," "Diffusion," etc. The collective indexes of *Chemical Abstracts,* of the *Journal of the Society of Chemical Industry,* and the indexes of the *Kolloid Zeitschrift* will be found particularly useful.

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# GLUE AND GELATIN

## Chapter 1.

## Introduction.

**Definitions.**

*Glue* is an organic colloidal substance of varying appearance, chemical constitution and physical properties, obtained upon drying the solutions resulting from boiling with water properly prepared animal matter such as skin and bone. The jellies which form on chilling soups, stews, boiled chicken and the like, represent very impure glue solutions.

Glue appears in commerce in a wide variety of forms and colors, some of which are commonly, but erroneously, believed to be criteria of quality. The colors range through all shades of white, brown and yellow, and it may be transparent, translucent, or opaque. Gelatin colored red with aniline or vegetable coloring matter is used as a top dressing for cold meats, and specially colored glue compositions are used for making paper pads or blocks, etc.

In European countries glue is usually marketed in the form of oblong cakes about 3 x 6 inches (Cologne shape) or in sheets about 10 inches square (French glue). "Scotch" glue comes in very thick cakes, about 6 x 10 inches, with a string through one end. In America the bulk of the glue is used in ground form, though considerable is sold in thin, broken flakes, and some is made in the form of "noodles" or "ribbons," which special forms have no advantage other than a higher cost. Powdered glue is also used principally in mixing with whiting to make calsomine. For Eastern countries there is made a form known as "bazaar glue," which consists of a poor quality of glue in square sticks about 8 inches long and 1 inch in section.

*Gelatin* is made from bones and skin or hide fragments,

selected, cleaned and treated with especial care so that the resu ing product is cleaner, purer and generally clearer and light in color than glue. Glue is in fact impure gelatin, and any gl possessing suitable strength and appearance may be terme gelatin, although of course all gelatins are not suitable for foo purposes.

It is surprising that such authoritative reference books as th Standard Dictionary and Murray's Oxford Dictionary perpetuate the popular error that hoofs and horns yield glue. Hoofs and horns consist of keratin and are always removed by the glue maker, although of course the feet of animals and the interior bony support of the horn (horn pith) yield glue or gelatin (e.g. calves' foot jelly).

**Philology.**

The word glue has been traced back to the unused Latin verb *gluere,* meaning to draw together, and the form *glus,* glue, was used by Ausonius. It is allied to the Latin *gluten,* glue, and *glutus,* tenacious (*cf.* the English gluten, glutinous), to the Greek *gloios,* mud, gum, and to the old French *glu,* birdlime. Birdlime is a sticky substance which exudes from the holly tree, and is used for snaring birds. In this word *lime* has the significance of the German *leim,* glue, being in no way related to *lime,* calcium oxide. The word *gelatin* comes from the Latin verb *gelare,* to congeal (*cf.* the Latin *gelu,* frost, and the English chill, gel, gelid, jelly). Thus Virgil in describing the awe inspired in the Trojans by the Cumæan Sybil, says:

> *. . . Gelidus Teucris per dura concurrit*
> *ossa tremor . . .*

The word gelatin came into the English through the French *gélatine,* from the Italian *gelatina.* Gelatin is often termed *glutin* by chemists, a practise that should be abandoned, owing to the similarity of this word to *gluten,* the composite protein of rye, wheat, etc. Gelatin is often erroneously termed "isinglass," the confusion being due to the fact that gelatin under the name "patent isinglass" came into use as a substitute for the true singlass (see p. 219) and resembles it in appearance and working roperties. Popular error goes so far as to apply the term

isinglass to the mineral mica, which also appears in thin, transparent, flexible sheets.

Since the essential meaning of glue is that which draws or sticks together, while gelatin means essentially that which gelatinizes, it is but natural that, in popular parlance, the use of these words have, by similitude, been extended to many substances which are not glue or gelatin at all. Thus solutions of gums, dextrins, converted starches, etc., are often called glues, the modifying adjective "vegetable" being generally used. Silicate of soda is sometimes termed "mineral glue," solutions of rubber, pitch and the like are called "marine glue," and those of casein are called "casein glue." Several varieties of sea weed, including Gelideum corneum[1] or agar agar, form, when cleaned and dried, a cord-like product having enormous gelatinizing power, which appears in commerce under various names, such as vegetable or Japanese gelatin, vegetable or Japanese isinglass, Chinese moss, gelose, etc. Our well-known fruit jellies, when pure, contain no gelatin, their gelatinization being due to a jelly-forming carbohydrate known as pectin.

The correct uses of the terms above discussed are not always academic. They are often of importance in determining the operation of tariffs and other legislation. Thus the regulations of the Official Southern and Western Freight Classification (Rule 14, section 2): "Fiberboard and Pulpboard used in making Fiberboard or Pulpboard boxes, without frames, must be three-ply or more, all plies firmly glued together . . ." The ruling of the chemist in charge was that the word "glue" meant glued with animal glue. This narrow construction of the rule still stands, although boxes meeting the tensile and other requirements are passed, even if they are not glued with animal glue.

**Historical.**

Many of our most important discoveries have come as the result of some keen mind noting an incidental or accidental result. Though we may doubt the correctness of Charles Lamb's story as to the origin of roast pig, discovery of British gum and dextrin is said to have followed the observation that some starch, which had been roasted or torrified during a fire in a

[1] Gelidium gracilaria yields a similar product.

Manchester warehouse, yielded a sticky, gummy solution when wet with water.

In all probability the discovery of glue grew out of the fact that stews, especially those containing bones or skins, yield a sticky solution and gelatinize when cold. It is to Egypt that we must look for the oldest record of the use of glue, the discovery of which evidently antedates the Exodus, as may be seen from the following quotation taken from Wilkinson.[2]

"Among the many occupations of the carpenter, that of veneering is noticed in the sculptures of Thebes, as early as the time of the third Thothmes, whom I suppose to be the Pharaoh of the Exodus; and the application of a piece of rare wood of a red colour, to a yellow plank of sycamore or other ordinary kind, is clearly pointed out. And in order to show that the yellow wood is of inferior quality, the workman is represented to have fixed his adze carelessly in a block of the same colour, while engaged in applying them together. Near him are some of his tools, with a box or small chest, made of inlaid and veneered wood, of various hues; and in the same part of the shop are two other men, one of whom is employed in grinding something with a stone on a slab, and the other in spreading glue with a brush.

"It might, perhaps, be conjectured that varnish was intended to be here represented; but the appearance of the pot on the fire, the piece of glue with its concave fracture, and the workman before mentioned applying the two pieces of wood together, satisfactorily decide the question, and attest the invention of glue[3] 3,300 years ago. This is not, however, the only proof of its use at an early period, and several wooden boxes have been found in which glue was employed to fasten the joints."

The manufacture of violins and similar musical instruments during the Middle Ages and Renaissance, especially in Italy, indicates that glue was known and used at that period, and there are indications that early painters used glue size in preparing their canvases.

[2] Sir John Gardner Wilkinson, "Manners and Customs of the Ancient Egyptians," John Murray, London, 1879, Vol. 2, pp. 198-199.

[3] Rosellini seems to think that the application of color is here represented; but the presence of the pot, containing the brush, upon the fire, will scarcely admit of this, though the figure grinding on the slab might appear to strengthen his conjecture. He has placed this subject with the painters of Beni-Hassan, but it is at Thebes. Pliny ascribes the invention of glue to Dædalus, as well as of the saw, the axe, the plumb-line, and the auger. (*Plin.*, vii, 56.)

Murray (New Oxford Dictionary) gives a number of references to the early use of the word *glue* by English writers. Thus in the "Squire's Tale" (line 174), Chaucer (about 1386), in describing the wonderful brass horse on which a royal messenger appeared, says:

> "The horse of brass that may not be remewed,
> It stant as it were to the ground yglewed."

Further in Lanfranc's "Chirurgeon" (about 1400) it is stated (p. 135): "As it were two bordis weren ioyned togidere with cole or with glu."

Glue and gelatin, like most other manufactures of early days, were produced by individual artizans for their own use, and even to-day some paper and textile mills boil their own glue size from rawhide cuttings. From these somewhat primitive methods, the real glue and gelatin industry emerged about the beginning of the nineteenth century. In France the industry started in the vicinity of Lyons, and for many years these factories were the most important of their kind in Europe. During the Napoleonic era extravagant claims were made as to the food value of gelatin, and probably this was one reason why the industry was fostered.

Germany apparently appreciated the importance of the manufacture of glue and gelatin as a key industry, for a German company organized in 1895 with three plants, expanded until in 1912 it controlled the output of seventeen plants, and had also factories in Austria, Russia, Belgium, Switzerland and France.

**The Glue Industry in the United States.**

According to Rufus W. Powell [4] it is very difficult to obtain exact information about the glue manufacturing industry of the United States prior to 1860; but those long in the business reported that outside of regular glue manufacturers, a great many tanners boiled up their own stock in open kettles. The principal factories seem to have been in the vicinity of Boston, New York, Philadelphia and later on Cincinnati. One of the pioneer factories was at Marblehead, Mass., and probably secured its stock from the tanneries at Salem and Lynn. Peter Cooper's

[4] "Glue Statistics," Brooklyn, 1893.

factory was on Newtown Creek, Long Island, now in the Borough of Queens, New York City.

Powell gives the following table based upon reports given the Glue Manufacturer's Association, 1887-1888, which accounts for the commencement of the 92 factories then reporting.

| *Location of Factories* | | *Before 1830* | *1830 to 1840* | *1840 to 1850* | *1850 to 1860* | *1860 to 1870* | | *1870 to 1880* | | *1880 to 1887* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *Hide* | *Bone* | *Hide* | *Bone* | *Hide* | *Bone* |
| New England | 26 | 2 | 1 | 1 | 3 | 4 | 6 | 3 | 2 | 3 | 1 |
| Middle States | 35 | 2 | 1 | 1 | 1 | 8 | — | 7 | 3 | 10 | 2 |
| Western States | 24 | — | — | — | 2 | 5 | 1 | 6 | 2 | 6 | 2 |
| Pacific Coast | 7 | — | — | — | — | 1 | — | 3 | — | 3 | — |
| Total | 92 | 4 | 2 | 2 | 6 | 18 | 7 | 19 | 7 | 22 | 5 |

The Census of 1880 showed that there were then 82 plants producing glue as a principal or by-product. They employed 1,801 hands and a capital of $3,916,750.

Powell estimates that the total production of glue in the United States for the fiscal year 1886-1887 was 38,032,000 lbs., of which 27,743,000 lbs. was from hide, fur and neat's-foot stock, and 10,289,000 lbs. was from bone, bone liquor, and pigs' feet.

Some idea of the range of glue prices during this early period may be gleaned from the following table abridged from Powell (prices given in cents per pound).

| | *1844 1848* | *1858 1860* | *1863* | *1867* | *1876* | *1887* | *1892* |
|---|---|---|---|---|---|---|---|
| A Extra | 40 | 35 | 37 | 60 | 38 | 25 | 23 |
| 1 Extra | 34 | 30 | 32 | 53 | 35 | 22 | 19 |
| No. 1 | 30 | 26 | 27 | 47 | 30 | 19 | 17 |
| 1x | 25 | 24 | 24 | 41 | 25 | 17 | 15 |
| 1¼ | 21 | 22 | 21 | 36 | 21 | 16 | 14½ |
| 1⅜ | 19 | 20 | 19 | 32 | 19 | 15 | 14 |
| 1½ | 18 | 19 | 18 | 29 | 17 | 14 | 13 |
| 1⅝ | 17 | 18 | 17 | 27 | 15 | 12 | 11 |
| 1¾ | 16 | 17 | 16 | 25 | 14 | 11 | 10 |
| 1⅞ | — | — | — | — | — | — | 9 |
| 2 | 14 | 16 | 15 | 23 | 13 | 10 | 8 |

The total value of glue and gelatin produced in the United States for 1914 was $19,725,703, of which 40,844,650 lbs. valued at $3,088,764 was produced by the packing houses. The 1919 figures given below do not include 36,603,000 lbs. of glue valued at $4,489,774, produced by the meat-packing industry, but may include some gelatin. No separate figures are obtainable for gelatin, and that produced by the packers is included under a

heading "All other products." Various other industries incidentally produced glue to the value of $1,039,794.

The United States Census gives the following tabulated information regarding *glue, not elsewhere specified:*

| *Year and State* | *Number of establishments* | *Wage earners (average number)* | *Primary horse-power* | *Capital (expressed in thousands)* | *Wages (expressed in thousands)* | *Cost of Materials (expressed in thousands)* | *Value of products (expressed in thousands)* | *Value added by manufacture (expressed in thousands)* |
|---|---|---|---|---|---|---|---|---|
| *United States* | | | | | | | | |
| 1919 | 62 | 4,264 | 16,979 | 27,237 | 4,777 | 19,280 | 32,134 | 12,854 |
| 1914 | 57 | 3,129 | 13,304 | 17,162 | 1,854 | 9,368 | 13,733 | 4,365 |
| 1909 | 65 | 3,265 | 15,596 | 14,289 | 1,571 | 7,525 | 13,718 | 6,193 |
| 1904 | 58 | 2,864 | 14,280 | 10,673 | 1,529 | 6,186 | 10,035 | 3,849 |
| 1899 | 61 | 1,618 | 6,806 | 6,144 | 685 | 3,767 | 5,387 | 1,622 |
| 1889 | 62 | 1,697 | 4,912 | 4,859 | 676 | 2,511 | 4,270 | 1,759 |
| 1879 | 82 | 1,801 | —— | 3,917 | 600 | 2,786 | 4,324 | 1,538 |
| 1869 | 70 | 860 | 1,051 | 1,955 | 310 | 883 | 1,710 | 827 |
| 1859 | 62 | 875 | —— | 1,053 | 306 | 537 | 1,186 | 649 |
| 1849 | 47 | 391 | —— | 520 | 99 | 372 | 652 | 280 |
| *States, 1914* | | | | | | | | |
| Illinois | 9 | 968 | 3,316 | 5,552 | 614 | 2,385 | 3,751 | 1,346 |
| Indiana | 3 | 71 | 355 | 356 | 30 | 157 | 280 | 123 |
| Massachusetts | 11 | 563 | 1,481 | 2,956 | 294 | 1,789 | 2,589 | 800 |
| New York | 9 | 381 | 2,082 | 2,459 | 249 | 1,942 | 2,483 | 541 |
| Pennsylvania | 8 | 519 | 1,628 | 2,820 | 290 | 1,418 | 2,029 | 611 |
| All other States. | 17 | 627 | 4,442 | 3,019 | 337 | 1,677 | 2,621 | 944 |

The United States Department of Commerce has kindly supplied the following figures regarding the imports and exports of glue, gelatin, and glue stock, which indicate that large amounts of foreign glues and gelatins are consumed here.

| | *1921* | | *1922* | |
|---|---|---|---|---|
| *Imports* | *Pounds* | *Dollars* | *Pounds* | *Dollars* |
| Gelatin (unmanufactured) | 2,396,645 | 1,231,035 | 2,527,198 | 997,896 |
| Glue and Glue Size | 3,561,831 | 762,557 | 4,174,785 | 574,311 |
| Hide cuttings, raw, and other Glue Stock | 36,104,659 | 2,272,847 | 25,322,414 | 1,149,883 |
| *Exports* (domestic) | | | | |
| Glue | 5,991,872 | 1,148,666 | 2,101,328 | 348,643 |

These figures indicate the well-known fact that different varieties of glue are made in different factories, and move according to their fitness for certain uses.

**Great Britain.**

There are 57 glue manufacturers and 21 gelatin manufacturers in the United Kingdom, but figures regarding production are not available. For 5 months ending May 31, 1921, the following figures were kindly furnished by Mr. L. E. Bernays, British Consul at New York.

| | *Cwts.* | *Value, £* |
|---|---|---|
| *Imports*—Glue, Gelatin, and Size..... | 33,666 | 226,912 |
| *Exports*—Gelatin ..... | 1,676 | 33,673 |
| Glue and Size..... | 14,808 | 61,678 |

**France.**

In France there are about 60 factories, the main centers being Paris, Lyons, Marseilles, Dijon, LaPallice and Nantes. Approximately 3,000 workers are employed. Before the war France produced about 11,000 metric tons of pressured bone glue and gelatin, 4,000 tons from acid treated bone, and 3,000 tons from by-products and waste. About 100,000 tons of bones were employed annually.

The following figures were obtained through the United States Department of Commerce.

*1913*

| | *Imports* | | *Exports* | |
|---|---|---|---|---|
| | *Metric tons* | *Value (francs)* | *Metric tons* | *Value (francs)* |
| Fish glue ..... | 77.7 | 2,020,200 | 118.1 | 3,070,600 |
| Glue from bones and other animal waste ..... | 1,883 | 2,259,600 | 7,367 | 8,840,400 |
| Gelatin in powder sheets, etc. | 237.6 | 653,400 | 461 | 1,267,750 |

In 1913, 20 tons of fish glue were imported from the United States, and 9 tons were exported to that country. Exports of bone glue to the United States amounted to 414 metric tons, while exports of gelatin to the United States were approximately 54 metric tons.

*1920*

| | *Imports* | | *Exports* | |
|---|---|---|---|---|
| | *Metric tons* | *Value (francs)* | *Metric tons* | *Value (francs)* |
| Fish glue ..... | 90.1 | 858,000 | 422.9 | 26,960,000 |
| Glue from bone and other animal waste ..... | 1,266.8 | 5,882,000 | 4,394.6 | 21,755,000 |
| Gelatin in powder sheets, etc. | 83.6 | 1,434,000 | 765.8 | 4,545,000 |

**Germany.**

As before remarked, the glue and gelatin industry of Germany is a most important one, and has recently interested American capital. Besides supplying the large home market, an extensive export business is done. Figures are not at present obtainable.

Belgium, Switzerland, Holland, and other countries also produce glue and gelatin, and notable quantities are produced in Japan, Argentina, Canada and Australia.

# Chapter 2.

## The Position of Gelatin among the Proteins, and the Nature of the Forces Binding Together Its Constituents.

Glue and gelatin belong to that large and important group of nitrogen-containing colloidal organic substances known as the *proteins,* which are found in nature as essential components of plants and animals and as products of their metabolism. More specifically they belong to the sub-group of proteins known as *albuminoids* by American and Continental chemists, and as *scleroproteins* by the Chemical and Physiological Societies of England, because the group includes the substances which are the chief organic constituents of the animal skeleton and of the skin and its appendages, i.e. elastin (from tendon), collagen (from bone and hide), and keratin (from horn and hoof).

This new use of the term albuminoid (literally albumin-like) must be distinguished from its now obsolete meaning, for in the past it was used as synonymous with "proteid," and therefore at that time included albumin and its congeners as well as gelatin and allied substances. The term albuminoid thus replaces *"proteoids,"* which was at one time applied to "proteids" (now proteins) of the gelatin group.

It is to be regretted that all scientists have not yet accepted the new meaning for the term albuminoid. Thus according to W. O. Atwater,[1] the American Association of Agricultural Colleges and Experiment Stations subdivide protein compounds into albuminoids, gelatinoids, and extractives. The first group (the albuminoids) includes white of egg, lean meat, casein, and wheat gluten; whereas the second group (the gelatinoids) includes collagen and ossein from which gelatin is made. This confusion in terms is to be deprecated, and perhaps the best way to do would be to drop the term albuminoid entirely, sub-

[1] Farmer's Bulletin 142, U. S. Dept. of Agriculture, reprint January, 1921, p. 4.

stituting in its place the more descriptive term scleroprotein (proteins of the skin and skeleton).

The position of the albuminoids or scleroproteins among the proteins may be seen from the following tabular classifications, which include also the products of hydrolysis.

**Classification of Proteins.**

The American Classification, adopted by the American Physiological Society and the American Society of Biological Chemists, is:

I. SIMPLE PROTEINS:

*Albumins*—i.e. egg albumen; serum-albumin. Soluble in distilled water and in salt solutions; their acid and basic functions are almost equal, and they are salted out by saturation of their solutions with ammonium sulphate.

*Globulins*—i.e. egg-globulin separated from egg white by dilution with distilled water; edestin from the seed of hemp (*Cannabis Sativa*). Insoluble in distilled water, but soluble in dilute solutions of strong acids or bases, or of inorganic salts. They are salted out by half saturation of their solutions with ammonium sulphate, or by complete saturation with magnesium sulphate. They are rather more acid than basic.

*Glutelins*—i.e. glutenin from wheat; oryzenin from rice. Insoluble in distilled water or in dilute salt solutions, but soluble in dilute solutions of strong acids or bases.

*Prolamins*—i.e. *gliadin* from wheat and rye; *hordein* from barley; *zein* from corn. Soluble in 70 to 90 per cent. alcohol, and in dilute solutions of strong acids or bases, but practically insoluble in distilled water. On hydrolysis they yield a large percentage of proline.

*Protamines*—i.e. *salmine* from salmon spermatazoa. The simplest natural proteins, usually found in combination. Predominantly basic substances soluble in water, and forming with acids compounds precipitated by alcohol. On hydrolysis they yield considerable diamino acids.

*Histones*—i.e. the histone of *hemoglobin* which is there combined with the colored acid radicle *hematin*. Soluble in

dilute solutions of acids or of strong bases, but precipitated from acid solutions by ammonia. Less markedly basic than the protamines.

*Albuminoids* (Scleroproteins)—This large heterogeneous group is tentatively sub-divided as follows:

(A)—COLLAGENS OR JELLY-FORMING ALBUMINOIDS:

| | |
|---|---|
| (1) *Collagen* and *gelatin;* from skins, bones, white fibrous connective tissue. | Dissolve more or less readily in boiling water, yielding solutions which gelatinize on cooling. Contain little or no sulphur. |
| (2) *Chondrigen* and *chondrin;* from permanent cartilages. | Chondrigen and chondrin are really glycoproteins, but are mentioned here because they occur in glue and gelatin and in the materials from which they are made. |
| (3) *Isinglass;* from the swimming bladder of fishes.<br>(4) *Sericin* (silk-gum); from silk. | |

(B)—FIBROIDS:

| | |
|---|---|
| (1) *Elastin;* from elastic ligaments.<br>(2) *Fibroin;* from silk and spiders' webs. | Undissolved by dilute acids, boiling water, or boiling very dilute alkali. Dissolved by stronger alkali. Contain no sulphur. Have high tensile strength. |

(C)—CHITINOIDS:

| | |
|---|---|
| (1) *Chitin;* from external shells of beetles, crabs or lobsters.<br>(2) *Chonchiolin;* from shells of mussels and snails.<br>(3) *Spongin;* from sponges. | Insoluble in boiling water or in alkalis (spongin dissolves in concentrated alkali). Contain no sulphur. |

(D)—KERATINS:

| | |
|---|---|
| (1) *Keratin;* from hoofs, horns, feathers, hair, wool, etc.<br>(2) *Neurokeratin;* from brains. | Insoluble in water, salt solutions, or dilute acids or alkalis. Difficultly soluble in strong alkali. Contain sulphur. |

II. CONJUGATED PROTEINS:—Protein combined with a non-protein radicle termed the *prosthetic group.*

*Nucleoproteins*—from nuclei of cells. Compounds of a protein (acting as a base) with one of the nucleic acids (substituted phosphoric acids containing carbohydrate and nitrogenous radicles). Insoluble in distilled water; soluble in dilute alkalis, such solutions being precipitated by weak acids such as acetic acid and carbon dioxide.

*Glycoproteins*—here the prosthetic group is (1) an amino-carbohydrate; (2) a polysaccharide derivative of glucosamin or its acetylated derivatives; (3) chondroitin-sulphuric acid.

There are three subdivisions:

(1) *Mucins*—from mucous, snail-slime, etc., yield extremely viscous solutions from which the mucin is precipitated by acetic acid.

(2) *Mucoids*—i.e. ovomucoid from egg white, are not as viscous in solution as mucins, and are not precipitated by acetic acid.

(3) *Chondroproteins*—from cartilage, amyloid tissue, etc., are insoluble in water. Their solutions in dilute alkali are precipitated by an excess of acetic acid or on neutralization with strong acids. The *chondroitin-sulphuric* acid they yield on hydrolysis is composed of one molecule of sulphuric acid with one molecule of *chondroitin,* itself a compound of glucosamin and glucuronic acid, which physically resembles gum arabic.

*Phosphoproteins*—i.e. casein. Predominantly acid proteins, yielding phosphoric acid on hydrolysis.

*Hemoglobins*—i.e. hemoglobin, in which hematin, an iron-containing complex organic acid, is the prosthetic group, united with a histone-like predominantly basic protein *globin.*

*Lecithoproteins*—here the prosthetic group is a phospholipin. It is questionable whether the phospholipin is chemically combined or is simply an adsorbed impurity.

III. DERIVED PROTEINS:

(A) PRIMARY PROTEIN DERIVATIVES:

(1) *Proteans.*—Insoluble products formed by the incipient

action of water, very dilute acids or enzymes, e.g. myosan from myosin, edestan from edestin (Hawk).

(2) *Metaproteins.*—These result from further action of acid or alkali, are soluble in very weak acid or alkali but insoluble in neutral fluids, e.g. acid metaprotein (acid albuminate); alkali metaprotein (alkali albuminate).

(3) *Coagulated Proteins.*—Insoluble products resulting from the action of heat or alcohol on protein solutions.

(B) Secondary Protein Derivatives:

(1) *Proteoses.*—Soluble in water; not coagulated by heat; precipitated by saturation of their solutions with ammonium or zinc sulphate; e.g. protoproteose, deuteroproteose, *gelatoses.*

(2) *Peptones.*—These differ from proteoses in that they are not precipitated by saturating their solutions with ammonium sulphate, i.e. antipeptone, amphopeptone, *gelatones.*

(3) *Peptides.*—These are really peptones whose structure is known; that is, the polypeptides of Fischer; e.g. glycylglycine, etc.

The classification of proteins adopted by the British Medical Association is as follows:

I. Simple Proteins:

*Protamines*—e.g. salmine, clupeine.
*Histones*—e.g. globin.
*Albumins*—e.g. serum albumin.
*Globulins*—e.g. ovoglobulin.
*Glutelins*—e.g. glutelin.
*Alcohol soluble Proteins*—e.g. zein.
*Scleroproteins*—e.g. elastin.
*Phosphoproteins*—e.g. casein.

II. Conjugated Proteins:

*Glucoproteins*—e.g. mucin.
*Nucleoproteins*—e.g. nucleohistone.
*Chromoproteins*—e.g. hemoglobin.

III. PRODUCTS OF PROTEIN HYDROLYSIS:

*Infraproteins*—i.e. acid or alkali albuminates formed by gently heating albumins in acid or alkali. Insoluble in distilled water; e.g. acid albuminate.

*Proteoses*—e.g. protoproteose.

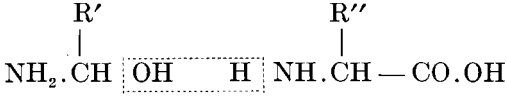
*Peptones*—e.g. antipeptone.

*Polypeptides*—e.g. dipeptides.

T. Brailsford Robertson [2] criticizes both the American and the British systems of classification as being based upon variations in physical behavior which do not necessarily correspond to differences in chemical structure, whereas on the other hand there are many proteins or protein-like substances whose intermediate characteristics make their inclusion in any group a more or less arbitrary matter. His criticism is well-founded.

**Molecular Structure.**

This naturally raises the question as to the nature of the combination which holds together the various amino-acids in the molecules of different proteins. From the complexity of the amino-acids yielded on the drastic hydrolysis of gelatin and allied proteins (see table on p. 30), it is obvious that the combination is by no means as simple as ordinary two dimensional formulæ on paper would indicate. The classical work of Emil Fischer on the polypeptides shows that with these relatively simple (as compared with proteins) compounds, the linkage takes place according to the scheme first suggested by Hofmeister:




That is, the COOH and $NH_2$ groups of different molecules (the molecules may themselves be alike or different) combine with the elimination of water.

But this relatively simple conception can not be carried over literally to proteins. In a trenchant criticism of Wolfgang Pauli's "Kolloidchemie der Eiweisskörper," Wolfgang Ostwald [3]

[2] "Principles of Biochemistry," p. 125.

[3] *Kolloid Z.* 27, 143 (1920).

quotes Emil Fischer as saying: "It can not with certainty be predicted whether a 20-polypeptid or a substance of like complexity of constitution would behave physico-chemically like albumin or not. It may happen sometimes, but not always. When things become so complicated the way they are constituted is not so easily explained. They become so indefinite. . . . In the course of time I have built up ever larger molecules. The colloid chemists would do well, like Perrin, to reverse this and from relatively large particles come down to molecules."

We must approach the subject without bias or fixed preconceived theories, and with minds flexible enough to fit all the facts of Nature, even though some be recently discovered facts. The complexity, frangibility, and even the variability of the so-called chemical elements, are established facts. The radioactive elements are spontaneously decomposing. Rutherford has shattered nitrogen by the impact of $\alpha$ particles shot out from radium at a speed of about 10,000 miles a second. By positive ray analysis Aston and others have demonstrated the existence of isotopes of many elements.[4] We have awaked to a realization of the fact that, just as there is no sharp line of demarcation between colloidal solution and true molecular solution or dispersion, so too no sharp line can be drawn between physical and chemical attraction.

Slight variations may mean much, e.g., the decimal in the atomic weight of hydrogen 1.008 represents electrons. The astronomer Herschel remarked that "the perfect observer will have his eyes, as it were, opened, that they may be struck at once with any occurrence which, according to received theories, *ought not* to happen, for these are the facts which serve as clues to new discoveries."

If the attractive forces existing between atoms (or atomic groups) were entirely satisfied or balanced by their chemical combination consequent upon the principal electronic attractive forces, or forces of primary valence as they are called, then every chemical compound would behave like a perfect gas so far as concerns the factor $a$ in the equation of van der Waals. But in all chemical compounds there exist residual attractions or stray

[4] Thus there are three kinds of chlorine (atomic weights 35, 37 and 39 respectively) and six kinds of mercury; consequently there are 18 different mercuric chlorides and 63 possible mercurous chlorides (Harkins).

fields of force, which exert a controlling influence upon what we ordinarily call the physical properties of the compound—its state (gaseous, liquid or solid), its cohesion, solubility, melting point, freezing point, dielectric constant, conductivity for heat and electricity, etc. These residual attractions are responsible for adhesion, adsorption, and the mechanical strength of materials, and their effective range of action (of the order of $10^{-8}$ cm.) is usually much less than the diameter of a molecule.

Practically all molecules (and even all atoms) are *polar* and exhibit dissymmetry. They therefore *tend* to orient themselves so that their attractive forces may reach an equilibrium. This is particularly evident in cases of adsorption at surfaces where, as Langmuir [5] observes, the molecules usually orient themselves in definite ways in the surface layer, since they are held to the surface by forces acting between it, and particular atoms or atomic groups in the adsorbed molecule.[6]

Where the residual attractive forces reach an equilibrium, the molecules (or atoms) become more or less regularly distributed in the space lattice, and the compound (or element) is crystalline, and usually shows its regularity of orientation when examined by the X ray spectrometer of Bragg and Bragg.[7] In many cases, however, this tendency towards definite orientation is never realized. This is especially so where there are large and cumbersome molecules involved, as with the proteins, and even with metals and alloys where quick chilling tends to preserve the random or haphazard distribution of the atomic groups.[8] The experiments of P. Scherrer [9] with the X-ray spectrometer show that colloidal gold particles too small to be seen even in the ultramicroscope, nevertheless show the same space lattice as macroscopic gold crystals. Old specimens of silicic acid and stannic acid gels exhibit well marked crystal interference figures in addition to the characteristics of amorphous substances, probably representing substances on the point of crystallizing. But typical organic colloids such as gelatin, albumin, casein, cellu-

[5] *J. Am. Chem. Soc., 40,* 1363 (1918).

[6] See also Harkins, Clark and Roberts, *J. Am. Chem. Soc., 42,* 706 (1920).

[7] Bragg and Bragg, "X Rays and Crystal Structure," London, 1915.

[8] See Jerome Alexander, "The Colloidal State in Metals and Alloys," *Trans. Am. Inst. Mining and Met. Eng.,* Vol. 69 (1921); presented at Columbus, O., meeting, October, 1920; *Chem. Met. Eng.,* January, 1922.

[9] *Nachr. Ges. Wiss. Göttingen,* 96-100 (1918).

lose, and starch appear to be amorphous. The colloid particl therefore, probably consist of large individual molecules or groups of irregularly oriented molecules.

Considering the information at present available, it woul appear that the forces binding the relatively simpler molecula units into a "molecule" of gelatin are largely what have here tofore been considered "physical" forces. Perhaps the difficul ties of nomenclature may be to some extent avoided if we adopt the suggestion of P. E. Wells.[10]

(1) *Electronic forces*—maintain positive nucleus and negative or valence electrons in equilibrium as a single system.

(2) *Atomic forces*—maintain two or more atoms in equilibrium as a single system.

(3) *Molecular forces*—maintain two or more molecules in equilibrium as a single system.

(4) *Molar forces*—maintain two or more masses in equilibrium as a single system.

"Each group of forces may be regarded as the residual fields of force remaining unsaturated in the smaller systems constituting the components of the system under consideration. . . . Molecular systems have lost so much of their discreteness that combinations of molecules do not follow the laws of definite and multiple proportions. In such phenomena as molecular association and surface structure, the discreteness of atomic constitution begins to give way to statistical continuity. Moreover, even in these phenomena, the forces are relatively so weak that molecules are not usually regarded as permanently grouped together." (Wells, *loc. cit.*)

What are generally called "chemical forces" are the atomic forces in the above classification, whereas what are generally called "physical forces" are the molecular forces therein mentioned. A careful consideration of the experimental facts of physical chemistry, i.e. ionization, hydrolysis, adsorption, differential diffusion, association, and dissociation, clearly show that it is just as impossible to draw a sharp line of demarcation between physical and chemical compounds as it is to separate by fixed lines the several primary colors of the spectrum.

It must not be supposed that this difficulty of definition is a new matter. Thus in 1884 in discussing a paper on adsorp-

[10] *J. Wash. Acad. Sci.* 9, 361 (1919).

tion by John Uri Lloyd,[11] Prof. Prescott said: "It has been asked whether we should refer Prof. Lloyd's results to chemical action within molecules or to those forces which may be classed under physical action. Now we do not know a great deal about these modes of force, or their essential nature; but I think we know this much, that there is no sharp line of demarcation between *chemical* action *within* the molecules, and the *physical* action *between* the molecules. They grade off into each other. We must look for the interference of *adhesion* in a great many operations called chemical."

The same view has been voiced by many others, and recently by P. P. von Weimarn,[12] although but a short while before, he had regarded the condensation gas → liquid → solid as a *chemical* phenomenon.

In conclusion it may be said that, while the formation of amino-acids and even of some of the more complicated polypeptides is controlled by the action of atomic forces, with increasing complexity of constitution, molecular forces appear and eventually predominate. The view held by many chemists that *all* attraction must follow the same simple laws that govern the formation of simpler compounds, must be abandoned; for, as Poincaré remarked, Nature is not as simple as all that.

Once we realize the fact that gelatin has a coarser physical, as well as a finer chemical structure, we may be able to understand its hydrolysis and degradation without having recourse to ingenious but weird purely chemical explanations or formulas which have no counterpart in the actual facts, although in some cases they may not be disproved by the present experimental evidence.

[11] See *J. Am. Pharmaceutical Assoc.* for October, 1916.
[12] *Kolloid Z. 28,* 97 (1921).

# Chapter 3.

## The Chemistry, Physical Chemistry, and Colloidal Chemistry of Gelatin and Glue.

Since the chemistry of gelatin is inseparably bound up with its physical chemistry and its behavior as a colloid, confusion only can result if an attempt be made to discuss these aspects separately. We have already considered the position of gelatin among the proteins, and the general nature of the forces holding its constituent atomic groups together (Chapter 2). Let us now consider the more intimate structure of the gelatin "molecule."

### Chemical Structure.

The most important evidence we have regarding the chemical nature of gelatin, is given by the products of its hydrolysis. Skraup and von Biehler[1] found that on hydrolysis with hydrochloric acid, gelatin yields the following substances, all of which had been found by previous workers.

Glycine—α-amino-acetic acid. $CH_2(NH_2).COOH$

Lysine—α-ε-diamino-caproic acid.

$$CH_2.NH_2(CH_2)_3.CH(NH_2)COOH$$

Alanine—α-amino-propionic acid. $CH_3.CH(NH_2).COOH$

Phenylalanine—β-phenyl-α-amino-propionic acid.

$$C_6H_5.CH_2.CH(NH_2).COOH$$

Leucine—α-amino-isocaproic acid.

$$CH_3.(CH_2)_3.CH(NH_2).COOH$$

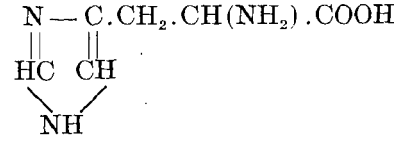
Aspartic acid—amino-succinic acid.

$$COOH.CH_2.CH(NH_2).COOH$$

Glutamic acid—α-amino-glutaric acid.

$$COOH.CH_2.CH_2CH(NH_2).COOH$$

Histidine—β-imino-azole-α-amino-propionic acid.

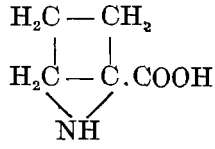


[1] *Monatshefte für Chemie 30,* 476 (1909).

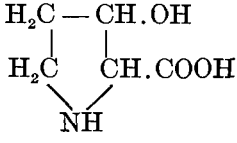
Arginine—α-amino-δ-guanino-*n*-valeric acid.

$$NH{:}C(NH_2).NH(CH_2)_3.CH(NH_2).COOH$$

Proline—α-pyrrolidine-carboxylic acid.



Oxyproline—β-oxy-α-pyrrolidine-carboxylic acid.



Whether these amino-acids exist in the gelatin "molecule" as such, or are formed from the disintegration of larger molecules, cannot with certainty be decided at present.

To see how the degradation products of gelatin compare with those of other albuminoids or scleroproteins, there is given the following table prepared mainly from "The Chemical Constitution of the Proteins," by R. H. Alders Plimmer:

| | *Gelatin* Dakin | *Gelatin* Fischer, Levene and Aders; Hart, Kossel and Kutscher | *Gelatin* Levene and Beatty | *Gelatin* Skraup and von Bethler | *Silk-Fibroin* Fischer and Skita; Fischer | *Spider-silk Fibroin* Fischer | *Elastin* Abderhalden and Schittenhelm, Schwartz; Kossel and Kutscher | *Keratin from Ox Horn* Fischer and Dorpinghaus; Morner |
|---|---|---|---|---|---|---|---|---|
| Glycine ..... | 25.5 | 16.5 | 19.25 | 12.4 | 36.0 | 35.2 | 25.8 | 0.4 |
| Alanine ..... | 8.7 | 0.8 | 3.0 | 0.6 | 21.0 | 23.4 | 6.6 | 1.2 |
| Valine ..... | 0.0 | 1.0 | — | — | 0.0 | — | 1.0 | 5.7 |
| Leucine ..... | 7.1 | 2.1 | 6.75 | 9.2 | 1.5 | 1.8 | 21.4 | 18.3 |
| Isoleucine ... | 0.0 | 0.0 | — | — | — | — | — | — |
| Phenylalanine | 1.4 | 0.4 | — | 1.0 | 1.5 | — | 3.9 | 3.0 |
| Tyrosine .... | 0.01 | 0.0 | — | — | 10.5 | 8.2 | 0.4 | 4.6 |
| Serine ..... | 0.4 | 0.4 | — | — | 1.6 | — | — | 0.7 |
| Cystine ..... | — | — | — | — | — | — | — | 6.8 |
| Proline ..... | 9.5 | 5.2 | 6.25 | 10.4 | + | 3.7 | 1.7 | 3.6 |
| Oxyproline .. | 14.1 | 3.0 | 6.4 | 3.0 | — | — | — | — |
| Aspartic Acid | 3.4 | 0.6 | — | 1.2 | + | — | — | 2.5 |
| Glutamic Acid | 5.8 | 0.9 | 1.75 | 16.8 | 0.0 | 11.7 | 0.8 | 3.0 |
| Lysine ..... | 5.9 | 2.8 | — | 6.0 | + | 5.24 | — | — |
| Arginine .... | 8.2 | 7.6 | — | 9.3 | 1.0 | — | 0.3 | 2.3 |
| Histidine ... | 0.9 | 0.4 | — | 0.4 | + | — | — | — |
| Ammonia ... | 0.4 | 0.4 | — | 0.4 | — | 1.2 | — | — |
| Total per cent. | 91.31 | 42.1 | 43.4 | 70.7 | 73.1 | 90.44 | 61.9 | 52.1 |

Y. Okuda [2] hydrolyzed the gelatin from shark skin with hydrochloric acid, baryta, and sulphuric acid, and found that the fish gelatin gave somewhat more monoamino acid, and much more glycocoll, alanine, leucine, phenylalanine, glutamic and aspartic acids than does bone gelatin. The diamino acid content was about the same in both gelatins, but the proline and serine content of fish gelatin was low, perhaps because of experimental error.

Many theories have been advanced to explain the amphoteric nature of the proteins, i.e. their ability to combine with either acids or bases. H. Bechhold [3] gives the following abstract of the theory, proposed by G. Bredig and extended by W. Pauli [3a] as to the amphoteric nature of albumin; and as these apply equally to gelatin, they are given here at length.

"Let us think of albumin as being built according to the structure of a cyclic [4] ammonium salt:

$$R < \begin{matrix} NH_3 \\ | \\ COO \end{matrix}$$

in which R represents a complicated organic complex, and the absorption of water follows according to the scheme:

$$R < \begin{matrix} NH_3 \\ | \\ COO \end{matrix} + H_2O \rightleftarrows R < \begin{matrix} NH_3OH \\ | \\ COOH \end{matrix}$$

"This is an amphoteric electrolyte which unites with bases and acids, which splits off H as well as OH ions, and in which the $K_A$ (acid dissociation) $> K_B$ (basic dissociation); in these words, it behaves like a very weak acid. Pure albumin consists

[2] *J. Coll. Agric. Tokyo* 5, 355 (1916).

[3] "Colloids in Biology and Medicine," p. 154.

[3a] In a recent paper (*Kolloid Z.* 28, 49 [1921]) entitled "Der Allgemeine Bauplan der Kolloide," Pauli expresses the view that with albumins the amino-acids chemically combined with each other, form neutral particles which receive their charge from one or a few ionizing amino-acids. Wo. Ostwald, J. Loeb, and many others, including the author, do not agree with all of these views of Pauli, which are given in some detail as they represent one of the purely "chemical" views of the behavior of gelatin.

[4] This is what Winkelblech terms an internal salt, whence the anhydride $R < \begin{matrix} NH \\ | \\ CO \end{matrix}$ is formed by the elimination of water.

principally of electrically neutral particles, but forms acid and alkali salts which are strongly ionized.

There exist

| $R<^{NH_3OH}_{COOH}$ | $R<^{NH_3Cl}_{COOH}$ | $R<^{NH_3OH}_{COONa}$ |
|---|---|---|
| neutral albumin | acid albumin | alkali albumin |

"That the *albumin ions* are responsible for the *great internal friction* is to be assumed from the investigations of E. Laqueur and O. Sackur on alkali-caseinates. The cause of this phenomenon is found in the strong hydration (water fixation, swelling) of the albumin ions.[5] According to Wo. Pauli and M. Samec the existence of polyvalent ions must be assumed in the case of acid and alkali albumin. Even assuming the smallest values for the molecular weight of albumin, the quantities of acid or alkali found are so large that they indicate the fixation of several acid or alkali molecules. This offers a further explanation of the marked increase in hydration produced by acids and alkalis. The stability of an albumin solution and its precipitability, e.g. by alcohol, are directly proportional to the number of albumin *ions* it contains. The circumstances here are quite analogous to those with crystalloids. Ions tend to go into solution and to form hydrates; the saturation concentration of neutral particles is always less than that of ions.

"In this way we may explain the properties of strongly ionized pure acid and alkali albumin as contrasted with the slightly dissociated neutral albumin.

"How does this theory agree with the effect of neutral salts? Wo. Pauli explains it in the following way:

$$R<^{NH_3Cl}_{COOH} + NaNO_3 \rightleftarrows R<^{NH_3Cl}_{COONa} + HNO_3$$

| acid albumin | neutral salt | Na salt of acid albumin |
|---|---|---|

In this way was explained not only the increased number of free H ions, which he demonstrated, but also the marked diminution in the internal friction; because an amphoteric salt, in which

[5] That the hydration rather than the size of the protein ions is the cause of their non-filterable character, is the view expressed by T. B. Robertson ("The Physical Chemistry of the Proteins," p. 143, footnote), J. A.

both anions and cations tend to ionize about equally, is but slightly dissociated.

"The action of neutral salts on *alkali albumin* is different; it follows the following scheme:

$$R\begin{cases}NH_3OH\\COONa\end{cases} + KCl \rightleftarrows R\begin{cases}NH_2KCl\\COONa\end{cases} + H_2O$$

alkali albumin — neutral salt — complex albumin salt — water

"Accordingly, a complex albumin salt is formed to which a les amount of ionization may be ascribed than to alkali albumir The action of salts of the alkaline earths follows this scheme:

$$R\begin{cases}NH_3OH\\COONa\end{cases} + \frac{Ca}{2}NO_3 \rightleftarrows R\begin{cases}NH_2NaNO_3\\COO\frac{Ca}{2}\end{cases} + H_2O$$

The replacement of the alkali ion in the hydroxyl of the amin group results in a weakly ionized complex salt. The effect o albumin of organic bases, which are often highly toxic, and o amphoteric electrolytes, have also been studied by H. Handovsky and the results agree with the above scheme.

"The conditions governing the action of neutral salts upon aci albumin are not sufficiently understood to warrant proposing simple scheme."

In a footnote Bechhold remarks that it should not be assumed that only free terminal $NH_2$ groups are to be considered, since the work of Blasel and Matula on deaminized gelatin make it probable that interior $NH_2$ groups are involved.

The method of Blasel and Matula [5a] for *deaminizing* gelatin is as follows:

To a solution of 200 grams of the purest commercial gelatin in 1 liter of warm water, is added 200 grams of sodium nitrite also dissolved in 1 liter of water. After cooling 140 grams of glacial acetic acid is carefully added, and after standing 12 hours, the mixture is heated for two hours on a water-bath. The deaminized gelatin is then salted out by saturation with ammonium sulphate and purified by prolonged dialysis (2 weeks) against running distilled water.

[5a] *Biochem. Z.* **58**, 417 (1914).

The deaminized gelatin, although its free amino groups are all destroyed, still has almost the same acid-combining capacity as ordinary gelatin. This certainly indicates that something other than the chemical attraction of the free $NH_2$ groups is responsible for acid fixation.

T. B. Robertson [6] believes that the —CONH— groups within the molecule are responsible for the acid-and-base-combining capacity of the proteins. The —CONH— group may exist in the keto form, —CO—NH—, or in the enol form, —C(OH)=N—, but neither analytic or synthetic methods are able to distinguish between the two forms. Robertson thinks that the enol form is most probable, since it can attach either acids or bases.

D. Jordan Lloyd [7] thinks that "a more probable explanation seems to be that under the action of acids gelatin goes into the keto-form, and under the action of bases to the enol-form. This view would conform with the observation that the free acid from sodium gelatinate differs in properties from the free base of gelatin hydrochloride. It can also be harmonized with Dakin's theory [8] that the non-terminal groups in proteins go from the keto-form (1) to the enol-form (2)

(1) NH.CO—
R.C(H)—C(=O)—NH.CHR.COOH

(2) NH.CO—
R.C(OH)=C—NH.CHR.COOH

with loss of optical activity under the action of bases at low temperatures."

Wintgren and Krüger [9] believe that since proteins have more than one $NH_2$ group, it is only in dilute solutions that we find the type

$$[GE]\ NH_3^+ + H_2O \leftrightarrows [GE]\ NH_3OH + H^+$$

corresponding to

$$NH_4^+ + H_2O = NH_4OH + H^+$$

[6] "Physical Chemistry of the Proteins," p. 24; "Principles of Biochemistry," p. 156.

[7] *Biochem. J. 14*, 154 (1920).

[8] *J. Biol. Chem. 13*, 357 (1913).

[9] *Kolloid Z. 28*, 81 (1921).

In higher acid concentration several amino groups take part in salt formation, so that "proteins have not *one* but *several* dissociation constants whose values decrease at varying speeds."

**Loeb's Theory of Colloidal Behavior.**

Jacques Loeb [10] concludes from the results of a series of careful and ingenious experiments that it is the H-ion concentration of protein solutions which controls their behavior. "Proteins exist in three states, defined by their hydrogen ion concentration, namely (a) as non-iongenic or isoelectric protein, (b) metal proteinate (e.g. Na or Ca proteinate), and (c) protein-acid salts (e.g. protein chloride, protein sulphate, etc.). We will use gelatin as an illustration. At one definite hydrogen ion concentration, namely $10^{-4.7}$ N, or in Sörensen's logarithmic symbol at $p_H$ 4.7,[11] gelatin can combine practically with neither anion nor cation of an electrolyte. At $p_H > 4.7$ it can combine only with cations (forming metal gelatinate, e.g. sodium gelatinate), at $p_H < 4.7$ it combines with anions (forming gelatin chloride, etc.)."

Loeb then describes experiments with powdered gelatin swollen in ice-cold water, showing that only those gelatins having $p_H > 4.7$ can fix Ag from $AgNO_3$ or Ni from $NiCl_3$; and only those having $p_H < 4.7$ can fix $Fe(CN)_6$. "In this way it can be shown," says Loeb, "that when the $p_H$ is $>4.7$ gelatin can combine only with cations; when $p_H$ is $<4.7$ it can combine only with anions, while at $p_H$ 4.7 (the isoelectric point) it can combine neither with anion or cation. The idea that both ions influence a protein simultaneously is no longer tenable.

"It follows also that a protein solution is not adequately defined by its concentration of protein but that the hydrogen ion concentration must also be known since each protein occurs in three different forms—possibly isomers according to its hydrogen ion concentration."

Loeb claims that the direct chemical union of acids with

[10] *Science*, N. S., Vol. 52, p. 449 (1920).

[11] An H + concentration of $2 \times 10^{-5}$ is expressed according to Sörensen's notation as follows: $p_H$ (the H-ion concentration) $= -\log (2 \times 10^{-5}) = -(0.3-5) = 4.7$ (J. A.).

latin is demonstrated by his experiments, which show that imes as many cc. of 0.1 *N* $H_3PO_4$ are required to bring 100 cc. 1 per cent. gelatin solution to a given $p_H$, as are required in e case of HCl or $HNO_3$, twice the number of cc. of 0.1 *N* alic acid, and the same number of cc. of 0.1 *N* $H_2SO_4$ (. . . in strong dibasic acid, like $H_2SO_4$, both hydrogen ions are held th a sufficiently small force to be easily removed"). Bases ow analogous results, and in conclusion Loeb makes the rather eeping assertion—"The behavior of the proteins, therefore, ntradicts the idea that the chemistry of colloids differs from e chemistry of crystalloids."

If all molecular forces are to be regarded as "chemical," then eb's case is proved at the outset by definition.

W. D. Bancroft [12] takes Loeb to task for drawing general conisions on the basis of experiments made with dilute solutions. ncroft says: "Under the conditions of the experiments Loeb nd that on the acid side of the isoelectric point only anions neutral salts are taken up and the alkaline side of the isoctric point only cations. Since the Hofmeister series calls for effect due to both ions of a neutral salt on the swelling of latin, Loeb concludes that the Hofmeister series is a delusion d a snare. This does not follow at all. Loeb is working at ch extreme dilutions that the specific effects of all ions but drogen and hydroxyl ions are practically negligible. In acid lutions only anions are taken up and in alkaline solutions only tions. Loeb recognizes the specific effect of iodine ions over lorine ions in causing the liquefaction of gelatin, but he conlers that liquefaction stands in no necessary relation to swell-g, an assumption which will be shared by few. With higher ncentrations Loeb will undoubtedly get entirely different relts. His conclusions as to the existence of definite compounds pend on the assumption that he is dealing with true solutions d will fall with that assumption."

In reply to Bancroft, Loeb [13] points out that "salt solutions to grammolecular concentration were used without any inditon of validity of the Hofmeister series being found. Banoft will surely not maintain that solutions of neutral salts up molecular concentration are so dilute that the effects of all

[12] "Applied Colloid Chemistry," p. 255 (1920).
[13] "Proteins," p. 110.

ions except the hydrogen and hydroxyl ions are practically negligible.

"The writer's (Loeb's) statement that the liquefaction of solid gelatin stands in no necessary relation to swelling is correct, since higher concentrations of acids or of salts like $CaCl_2$ diminish the swelling of gelatin while they increase its solubility. This is due to the fact that swelling and solution of gelatin in the presence of acid are functions of different variables, swelling in acid depending on the Donnan equilibrium, while the solution of gelatin depends on the same forces which are responsible for the solution of ordinary crystalloids in water (probably secondary valency forces)."

Because of the facts pointed out in Chapter 4, it seems to the writer that Bancroft's criticism is well founded. The Donnan equilibrium is based on the assumption that there is *complete* ionization of the colloid salt ("gelatin chloride") and the crystalloid (HCl). As concentrations increase, the degree of dissociation and ionization diminish. Another assumption is that the jelly cation of "gelatin chloride" is not diffusible; but as D. Jordan Lloyd points out (*loc. cit.*, p. 164) the "colloidal ion" is diffusible to some extent and must exert an osmotic pressure.

C. R. Smith [14] has shown that traces of impurities, especially Ca salts, exercise a potent influence on the water-absorbing capacity of gelatin, and that this fact may vitiate many of Loeb's conclusions which were based on experiments made with ash-containing gelatin. Smith points out that the increased swelling observed by Loeb in gelatin treated with sodium chlorid, etc. (all excess being washed out), is due to the fact that "these electrolytes remove the repressing lime salts and leave a gelatin combined with sodium cations. . . . It is not surprising that calcium, magnesium, strontium, barium chloride, or magnesium sulfate produce no increased swelling, for they do not remove the ash, and they also leave combined bivalent cations which do not increase swelling as much as univalent cations. Loeb continued to treat gelatin with various salts, under the impression that they were reacting with the gelatin. Only when using oxalates does he mention the formation of a white precipitate (obviously from the lime). He (January 20, 1919) ascribes the increase in osmotic pressure of gelatin to an increase in the

[14] *J. Am. Chem. Soc.* *43*, 1350 (1921).

number of particles, ionization not considered, but later stated that free hydrobromic acid represses the ionization of gelatin bromide and again that the physical properties of gelatin are dependent only on the number of gelatin bromide molecules formed. . . . Loeb's figures for osmotic pressure obtained on incompletely purified gelatin are from 25 to 50 per cent. too low. The results of this paper, however, confirm many of his conclusions."

Loeb in his reply to Smith [15] publishes the analysis by Dr. Hitchcock of his laboratory, of two random samples of the kind of gelatin he used. (Cooper's gelatin purified by treatment with 0.0078 M acetic acid and washing with distilled water of $p_H$ a little above 5.0). The results showed 0.001 per cent. of ash, with qualitive tests for Fe, Ca, and $PO_4$, but negative tests for Cl and $SO_4$. From this Loeb concludes that the ash content of the gelatin he had been using for experiments on swelling, osmotic pressure, and viscosity, "might have been about 1 mg." (per gram). "It was shown by the writer's (Loeb's) experiments that that amount of ash (which equals roughly a 0.000033 M solution of tricalcium phosphate) has no influence on the physical properties of the proteins, such as osmotic pressure swelling, viscosity, or potential difference."

It would be more convincing if Loeb had determined the ash of the particular specimens of gelatin that he actually used. He says that Smith's criticism, that his results on the osmotic pressure and swelling of gelatin are vitiated by the use of ash-containing gelatin, does not apply to his more recent papers published during the last three years. Loeb states that the correct values for the osmotic pressure (of solutions containing 1 g. of originally isoelectric gelatin in 100 cc.) are given in the May (1921) number of the *Journal of General Physiology*. "Former values were lower since the solutions contained less than 1 g. in 100 cc., usually 0.8 g., as was pointed out in a paper published in January, 1921, in the same Journal."

**Ash-free Gelatin.**

C. R. Smith applied for a public service patent (Serial No. 390,253 dated June 19, 1920), and points out [15a] that J. Loeb

[15] *J. Am. Chem. Soc. 44*, 214 (1922).

[15a] *J. Am. Leather Chemists' Assoc.*, Oct., 1922.

erred in crediting Miss Field as the discoverer of ash-free gelatin. Ash-free gelatin looks like the ordinary kind, but a one per cent. solution soon becomes turbid.

C. R. Smith's method of preparing *ash-free* gelatin is here epitomized:

Gelatin of the highest jelly strength (maximum mutarotation ratio 2.2), ground to about 16 mesh, is washed on a filter with cold (0° to 10°) 10 per cent. sodium chloride solution containing 5 cc. of concentrated hydrochloric acid per liter, until the washings are free from lime. The acid is washed out with cold 1 per cent. sodium chloride solution, and then gradually weaker salt solutions are used. Distilled or conductivity water is finally used to wash the gelatin until the washings show no chlorine. After dehydrating with cold 90 per cent. alcohol, the gelatin is then dried.

"When powdered gelatin is washed with cold water alone, the readily diffusible calcium salts soon pass away until further washing becomes ineffective. If it is now washed with a solution of sodium chloride, ammonium chloride, potassium bromide, or presumably any uni-univalent electrolyte, dialysis of the remaining lime salts takes place immediately, probably because certain slowly diffusible, possibly colloidal, salts of calcium react with them to form readily diffusible salts. If the added electrolyte is now washed out, any alkali combined with the gelatin is almost invariably left. Using sodium chloride, sodium carbonate is found in the ash. In order to insure the removal of this alkali as well as iron, heavy metals, etc., acidulated salt solution must be used. The removal of all calcium salts can be accomplished in an hour, but the removal of the hydrochloric acid requires several hours and the use of dilute salt solution until the remaining acid can be removed by water alone without excessive swelling. It is almost impossible to wash gelatin swollen to 40 or 50 volumes. As the last traces of acid are being removed, the gelatin (at 15°) shrinks to particles swollen to about 7 volumes. The removal of the last traces of acid is probably facilitated by the fact that the isoelectric point of gelatin is on the acid side [16] $p_H$ 4.7.

"Ash-free gelatin thus obtained when incinerated leaves no ash

[16] Michaelis, "Die Wasserstoffion Konzentration"; Patten and Kellems, *J. Biol. Chem.* *42*, 363 (1920).

other than the traces of sand when the original glue or gelatin contains such. When ashed with pure sodium carbonate, chlorides, sulphates, or phosphates cannot be detected.

"Ash-free gelatin swells in water at 15° to about 7 or 8 volumes. If such a gelatin be melted and cooled, a clear, stable jelly is produced. If, however, a weaker jelly be prepared, syneresis takes place, with the production of a cloudy jelly. A 0.5 per cent. jelly will flocculate into jelly particles (probably swollen to 7 volumes) and can be filtered off completely from the extruded water, which shows no trace of gelatin.

"Ash-free gelatin forms sols or gels with a minimum tendency to remain dispersed. It is readily precipitated by alcohol without the presence of electrolytes. Traces of acids or alkalis increase the osmotic pressure and prevent its precipitation by alcohol. Gelatin thus peptized by traces of alkalis or acids in the presence of a large percentage of alcohol exhibits a marked resemblance to the metal suspensoids. Traces of electrolytes, for example those present in a drop of tap water, cause immediate precipitation. (This indicates the protective action of hydrolysis products or "impurities" usually present in gelatin. J. A.) Bivalent and trivalent ions are most effective in bringing about precipitation.

"Ultimate analysis of this gelatin gave the following results:[17]

| *Carbon* | *Hydrogen* | *Nitrogen* | *Oxygen* |
|---|---|---|---|
| 50.47 | 6.75 | 17.53 | 25.25 |
| 50.56 | 6.87 | 17.53 | 25.04 |
| 50.52 | 6.81 | 17.53 | 25.15 |

"Moisture was determined by drying at room temperature over sulphuric acid to constant weight for several weeks. Heating at 100° caused no further loss in weight. Moisture correction was applied to all figures. The carbon content was from 0.5 to 1.1 per cent. higher than that in published analyses made on ash-containing material, probably because the latter retained carbon or carbon dioxide which was not considered."

Sheppard, Elliot and Benedict[17a] report that gelatin free from ash and hydrolytic products may be prepared by electrolyzing a 5 per cent. solution of commercial gelatin in a cell of electrofiltros for three to four weeks, the salts passing through the cell

[17] Carbon and hydrogen determinations were made by Dr. D. H. Brauns.

[17a] S. E. Sheppard, Felix A. Elliot, and Miss A. J. Benedict, *Science 46*, 550 (1922).

into the electrode chambers, and reducing the ash to about 0.10 per cent. This partially de-ashed solution is then precipitated by acetone, thus removing the hydrolysis products and still further reducing the ash to about 0.01 per cent. The gelatin thus purified is dissolved in conductivity water, chilled in sheets, and dried. They recommend it for all research work on gelatin, as well as for providing culture media which can be brought to any particular reaction with complete knowledge of the salts present.

**Fischer's Views.**

Martin H. Fischer[18] says: "The measurable hydrogen and hydroxyl ion contents of different protein-water systems upon which such emphasis has been laid for the explanation of their stability are only observable in relatively dilute systems; the ion contents are not inherent to, or necessary for, the stabilization; they are accidental accompaniments incident to the solution of some of the acidic and basic proteins in the excess of water and their hydrolysis with the production secondarily of an overplus of hydrogen or hydroxyl ions."[19]

While recognizing the formation of chemical compounds in protein-acid and protein-alkali systems, Fischer says (*loc. cit.*): "How inadequate for the understanding of the colloid-chemical behavior of such systems are the overplayed 'stoichiometrical,' 'chemical,' 'electrical,' hydrogen and hydroxyl ion notions, usually called upon to explain in some exclusive fashion all the changes observed, must be self-evident.

"Stoichiometrical views cover only those parts of the whole problem which have to do with the quantities produced of differently hydrateable or soluble compounds; 'chemical' notions are no more adequate for the explanation of the problem than they are, at present, for the understanding of the whole problem of solution; electrical and ionic notions are hardly of service when it is remembered that the most stabile of these hydrated colloid systems are such as are composed of *chemically produced,*

[18] "Soaps and Proteins," p. 214.

[19] This recalls the behavior of ferric chloride, dilute aqueous solutions of which slowly hydrolyze and deposit $Fe(OH)_3$ from a weak solution of HCl. By pouring a few drops of strong ferric chloride solution into boiling water, the decomposition takes place instantaneously, and is evidenced by the intense color of the colloidal $Fe(OH)_3$, which however soon precipitates. J. A.

*really neutral compounds of protein with base or acid,* provided only that not more water is present in the system than can be absorbed by the hydration capacities of the protein derivatives. Yet these colloid systems contain no quantities of either hydrogen or hydroxyl ions measurable by ordinary laboratory means."

Taking up the case of gelatin specifically, Fischer [20] says:

"Dry gelatin absorbs water (to yield the system water-dissolved-in-gelatin) and has a limited solubility in water (to yield the system gelatin-dissolved-in-water). Between these extremes and depending merely upon the relative amounts of gelatin and water present there lie the systems gelatin-solution dispersed in hydrated-gelatin (gel) or, with more water, hydrated-gelatin dispersed in gelatin-solution (sol).

"What is the action of alkalis (or acids) upon these systems?

"Under variously worded headings this problem has received much study. The effects of alkalis (and acids) upon the lowermost of the four systems may be found under the caption 'swelling' of gelatin in the presence of acids and alkalis; [21] their effects upon the system gelatin-solution-in-hydrated-gelatin under the heading liquefaction and 'solution' of gelatin; [22] their effects upon the system hydrated-gelatin-in-gelatin-solution under studies in viscosity; [23] their effects upon the system true solution of gelatin-in-water as studies on the 'solubility' of gelatin.[24] What is the relationship between all these?

"It is well to begin by inquiring into the relationship between the swelling of 'soluble' 'neutral' protein and its 'solution.' The notion that solution is but a continuation of swelling persists to this day.[25] Investigation [26] of the problem, however, has

[20] *Loc. cit.,* p. 218 et. seq.

[21] See for example K. Spiro, *Hofmeister's Beiträge 5,* 276 (1904); Wolfgang Ostwald, *Pflüger's Arch. 108,* 563 (1905); M. H. Fischer, "Edema and Nephritis," 3d ed., p. 75, New York, 1920, where references to earlier studies may be found.

[22] M. H. Fischer, *Science 42,* 223 (1915); *Kolloid Z. 17,* 1 (1915).

[23] See for example the work of Hofmeister, Pauli, Hardy, von Schroeder, Handovsky, Schorr, etc., on the viscosity of liquid proteins ("sols").

[24] M. H. Fischer, "Edema and Nephritis," 3d ed., p. 513. As of similar impart but upon other proteins may be cited some studies on wheat gluten. T. B. Wood and W. B. Hardy (*Proc. Roy. Soc.* London, Series B, *81,* 38 (1908), the influence of acids, while F. W. Upson and J. W. Calvin (*J. Am. Chem. Soc. 37,* 1295 [1915]) studied its swelling under similar circumstances.

[25] See for example Wolfgang Pauli, "Kolloidchemie der Eiweiss Korper," p. 63, Dresden (1920).

[26] M. H. Fischer, *Science 42,* 223 (1915); *Kolloid Z. 17,* 1 (1915).

shown that this is not the case. The matter is easily proved by working with gelatin at concentrations and temperatures near its gelation or melting point. Since alkalis and acids increase hydration (increase swelling) the addition of these substances to a barely liquid gelatin-water mixture ought to stiffen it. As a matter of fact just the reverse occurs. By working with a stiff gelatin, a previously solid mixture is made to liquefy upon the addition of these substances.

"*The phenomena of swelling (hydration) and of 'solution'* [27] *in such soluble protein gels as gelatin, while frequently associated, are therefore essentially different. Swelling is best understood as a change whereby the protein enters into physico-chemical combination with more of the solvent (water), as a change in the direction of greater solubility of the solvent in the protein; 'solution' is best conceived of as a change in the direction of greater solubility (an increased degree of dispersion) of the colloid in the solvent. . . .* (p. 220) under the influence of added alkali or acid the 'neutral' gelatin is converted into a basic gelatinate or gelatin chloride. These compounds, at the same concentration, are more soluble in water than the neutral gelatin and hence the liquefaction of these systems."

Fischer [28] then describes experiments showing that the addition of a neutral salt in increasing concentration to a previously liquid gelatin at first increases its viscosity to an optimum point (gelation) and then decreases it. Just as in the case of soaps, the salt becomes hydrated and, as salt-water, becomes emulsified in the hydrated basic (or acidic) gelatin. When salt is added beyond the optimum point, the salt-water becomes the external phase and the viscosity of the system falls. When enough salt is added the whole of the gelatin (as sodium gelatinate or as gelatin chloride and not as "neutral" gelatin) separates off in practically anhydrous form.

Substantially Fischer's views agree with those of Wo. Ostwald. However, Ostwald [29] believes that beyond a certain critical point swelling passes over into solution, the spatial continuity of the two phases relative to each other being then destroyed.

[27] Since there are many opinions regarding the nature of "solution," accurate definition of the term is not easy. We are here using the term in its broadest sense as covering everything, in the case of colloids, from their liquefaction point upwards to the accepted "true" solution of the physical chemists.

[28] *Loc. cit.*, p. 221.

[29] "Handbook of Colloid Chemistry," 2d ed., M. H. Fischer's translation, p. 261.

Based upon ultramicroscopic evidence the writer agrees with Ostwald's view. When the Brownian motion of particles becomes sufficiently violent to carry them beyond each other's range of molecular attraction, then the dispersion due to swelling begins to pass over into solution. With the proteins there seems to be no sharp line between swelling and solution, for slight thermal changes or mechanical action may produce sufficient dispersion of part of the swollen protein to produce a colloidal solution, which may later aggregate once more to a gel with larger motionless particles. Indeed with some, if not all salts, colloidal dispersion precedes true solution. Thus Alexander and Bullowa [30] observed that sodium citrate, on going into solution, gave off streams of actively moving ultramicrons.

Solution, then, results when the intramolecular adsorption of the solvent is powerful enough to force molecules or molecular groups beyond a certain critical distance from each other. The greater the degree of dispersion of the particles, the more rapid the Brownian motion and the nearer the approach to true or molecular solution; and this is conditioned by temperature, pressure, protective substances, coagulators, etc. As dispersed particles aggregate, the Brownian motion decreases sharply, groups about 1.1 μ being nearly motionless. The closer particles are the less water they tend to adsorb. Therefore dilute jellies, when dried, take up more water than do concentrated jellies. Heating to 40° annihilates these differences for the time being, as the complexes are then broken down. The molecular groups constituting gelatin are so large and possess such a powerful idio-attraction, that it is not easy to separate them. Furthermore the degree of separation, that is, the size of the gelatin "molecule," seems to depend upon circumstances, as may be seen by considering work on the molecular weight of gelatin.

## Molecular Weight of Gelatin.

Wide differences of opinion exist regarding the molecular weight of gelatin. Various methods have yielded the following:

| | |
|---|---|
| Schützenberger and Bourgeois | — 1,836 |
| Paal | — 900 |
| Wintgren and Krüger | — 839 |

[30] J. Alexander and J. G. M. Bullowa, *Arch. of Pediatrics* 27, 18 (1910).

| | |
|---|---|
| Proctor and Wilson | — 768 |
| Berrar | — 823 |
| Biltz, Bugge and Mehler | — 5,500-31,000 for different kinds |
| D. Jordan Lloyd | — 10,300 |
| Dakin | — 11,800 |
| J. Loeb | — 12,000 to 25,000 |
| C. R. Smith | about — 96,000 |

D. Jordan Lloyd [31] estimates that the molecular weight of gelatin is about 10,000 or some multiple of this figure. Her evidence, based on chemical grounds, is given below:

Van Slyke's [32] analysis shows the following distribution of nitrogen in gelatin:

| | *Per cent. of total nitrogen* |
|---|---|
| Ammonia nitrogen | 2.25 |
| Melanine " | 0.07 |
| Cystine " | 0.00 |
| Arginine " | 14.7 |
| Histidine " | 4.48 |
| Lysine | 6.32 |
| Mono-amino nitrogen | 56.3 |
| Non-amino " = proline + oxyproline | 14.9 |

Assuming 1 histidine grouping in the gelatin molecules there must be 3 histidine nitrogens. The percentage histidine value, 4.48, can be reduced to 3 by multiplying by the arbitrary factor 0.665; but this would yield figures corresponding to fractional (half) molecules for both ammonia and arginine. Assuming 2 histidine groupings, the factor becomes 1.33 and we have

| | | | |
|---|---|---|---|
| Ammonia nitrogen | 2.9 | approximately | 1 × 3 |
| Arginine " | 19.7 | " | 4 × 5 |
| Histidine " | 6.0 | " | 3 × 2 |
| Lysine " | 8.4 | " | 2 × 4 |
| Mono-amino " | 75.2 | " | 1 × 76 |
| Proline + oxyproline nitrogen | 20.0 | " | 1 × 20 |

These figures indicate that 1 gelatin molecule contains 133x nitrogen atoms distributed thus:

| | |
|---|---|
| 3x Ammonia (amide) groupings | 4x Lysine groupings |
| 2x Histidine " | 76x Mono-amino " |
| 5x Arginine " | 20x Proline + oxyproline " |

In the absence of other evidence, x may be taken as unity. But the total nitrogen in dry gelatin is 18.0 per cent. of the total dry weight, as is evident from the following analysis:

[31] *Biochem. J. 14,* 166 (1920).
[32] *J. Biol. Chem. 10,* 15 (1912).

| | Per cent. N | |
|---|---|---|
| Mulder | 18.3 | —*Annalen, 45,* 63 (1843). |
| Chittenden and Solley | 18.0 | —*J. Physiol. 12,* 33 (1891). |
| Paal | 18.12 | —*Berichte, 25,* 1202 (1892). |
| van Name | 17.81 | —*J. Exp. Med. 2,* 117 (1897). |
| Schützenberger and Bourgeois | 18.3 | —*Jahresbericht Thier-Chem.* 1876, 30. |
| Sadikoff (Kjeldahl method) | 17.47 | —*Z. physiol. Chem. 37,* 397 (1903). |
| " (Dumas' " ) | 18.18 | —" " " " " " |
| | 18.0 ± 2% | |

"If 133 atoms of nitrogen form 18.0 per cent. of the weight of the gelatin molecule, then the lowest weight of gelatin which *can act as a chemical individual must be* $\frac{133 \times 14 \times 100}{18}$ *10,344,* or approximately 10,300. The error in the mean of the analyses given above falls within 2 per cent.; the error in van Slyke's analyses is of the order of 1 per cent.; the total error in the computed value is therefore of the order of 3 per cent."

C. R. Smith [33] working with highly purified ash-free gelatin, found at 35° an osmotic pressure of approximately 48 mm. of water for a concentration of 2 grams (1.78 dry) gelatin per 100 cc. water, and 95 mm. for 4 grams (3.56 dry) per 100 cc. On the *assumption* that the gas laws apply, this indicates for gelatin molecular weight of about 96,000.

**The Crystallization of Gelatin.**

P. P. von Weimarn [34] claims to have crystallized both gelatin and agar. He maintained a very dilute solution of gelatin in aqueous alcohol at 60°–70° in a dessicator containing dry potassium carbonate which absorbs water vapor but not alcohol vapor. As the concentration of alcohol slowly increases, the gelatin separates out in "crystals." C. R. Smith [34a] reports that this method failed in his hands.

It is to be noted that this method, similar to that by which Hofmeister crystallized egg albumen, involves an extremely slow aggregation of the constituent particles of the gelatin, during which their aggregation tendencies may have opportunity to establish themselves. In this respect it is analogous to the

[33] *J. Am. Chem. Soc. 43,* 1350 (1921).
[34] P. P. von Weimarn, "Grundzüge der Dispersoid Chemie," 1911, p. 106.
[34a] *J. Am. Leather Chemists' Assoc.,* Oct., 1922.

deposit of quartz crystals from silicious waters, and is free from the criticism that must attach to "crystallization" of colloids in the presence of electrolytes. For just as colloids exercise a powerful influence on crystallization[35] so too do crystalloids tend to give colloidal gels a definite form or orientation.[36] Thus the results of S. C. Bradford,[37] who claims to have crystallized gelatin in the presence of mercury salts, are open to doubt. So also are von Weimarn's results, for he did not use ash-free gelatin.

Even when gelatin slowly dries, between a slide and a cover glass for example, there seems to be registered an attempt towards orientation; dendritic forms appear which have been described by Liesegang.[38]

Contrary to what is commonly believed, colloids do diffuse, albeit but slowly. Too little detailed reference is made to the classic work of Graham,[39] who clearly brought out this feature. Thus he says [40] that tannic acid passes through parchment paper about 200 times slower than sodium chloride; gum arabic 400 times slower. "The separation of colloids from crystalloids by dialysis is, in consequence, generally more complete than might be expected from the relative diffusibility of the two classes of substances." At the outset of his paper, Graham says: "The range also in the degree of diffusive mobility exhibited by different substances appears to be as wide as the scale of vapor tensions. Thus hydrate of potash may be said to possess double the velocity of sulphate of potash, and sulphate of potash again double the velocity of sugar, alcohol, and sulphate of magnesia. But the substances named, belong all, as regards diffusion, to the more 'volatile' class. The comparatively 'fixed' class, as regards diffusion, is represented by a different order of chemical substances, marked out by the absence of the power to crystallize, which are slow in the extreme. Among the latter are hydrated silicic acid, hydrated alumina, and other metallic peroxides of the aluminous class, when they exist in the soluble form; with starch, dextrine, and the gums, caramel, tannin, gela-

[35] J. Alexander, *Kolloid Z. 4,* 86 (1909).
[36] R. E. Liesegang, *Kolloid Z. 7,* 96 (1910).
[37] *Biochem J. 14,* 91 (1920).
[38] R. E. Liesegang, *Kolloid Z. 7,* 306 (1910).
[39] *Phil. Trans. Roy. Soc. London 151,* 183-224 (1861).
[40] *Ibid.,* pp. 213-217.

tine, vegetable, and animal extractive matters. Low diffusibility is not the only property which the bodies last enumerated possess in common. They are distinguished by the gelatinous character of their hydrates. Although often largely soluble in water, they are held in solution by a most feeble force. They appear singularly inert in the capacity of acids and bases, and in all ordinary chemical relations. But, on the other hand, their peculiar physical aggregation with the chemical indifference referred to, appears to be required in substances that can intervene in the organic processes of life. The plastic elements of the body are found in this class. As gelatine appears to be its type, it is proposed to designate substances of the class as *colloids,* and to speak of their peculiar form of aggregation as the *colloidal condition* of *matter.* Opposed to the colloidal is the crystalline condition. Substances affecting the latter form will be classed as *crystalloids.* The distinction is no doubt one of intimate molecular constitution."

We should not be surprised that colloids can be crystallized, for we now know that all substances may exist in either the colloidal or the crystalloidal state, depending upon conditions. Where molecular mobility is great and capable of self-expression, visible crystals are formed, whereas where crystallization is inhibited, as with glass, soaps, chilled metals, etc., the colloidal state tends to appear and persist. E. Hatschek [41] has described the peculiar properties of camphorylphenylthiosemicarbazide whose suddenly chilled 5 per cent. alcoholic solutions form colloidal gels which gradually become crystalline. W. B. Hardy [42] had similar results with azomethin.

The effect of temperature on the aggregation of gelatin particles is shown by C. R. Smith,[43] whose work indicates a difference between gelatin dried at above 35° and that dried at below 15°.

Some idea as to the relative size of the groups in the case of 1 per cent. gelatin solution may be gained from the following table taken from H. Bechhold,[44] which shows in decreasing order, the sizes indicated by ultrafiltration experiments:

[41] *Kolloid Z. 11,* 158 (1912).
[42] *Proc. Roy. Soc. 87,* 29 (1912).
[43] *J. Am. Chem. Soc. 41,* 135 (1919).
[44] "Colloids in Biology and Medicine," p. 99.

SUSPENSIONS
Prussian Blue
Platinum-sol (Bredig)
Ferric oxide hydrosol
Casein, in milk
Arsenic sulphide hydrosol
Gold solution, Zsigmondy's No. 4, about 40 μμ
Bismon (Colloidal bismuth oxide), Paal
Collargol (colloidal silver), von Heyden, 20 μμ
Gold solution, Zsigmondy's No. 0, about 1–4 μμ
1 per cent. gelatin solution.
1 per cent. hemoglobin solution mol. wt. about 16,000.
Serum albumin.
Diphtheria toxin.
Protalbumoses.
Colloidal silicic acid.
Lysalbinic acid.
Deutero-albumose A.
Deutero-albumose B, mol. wt. about 2,400.
Deutero-albumose C.
Litmus.
Dextrin, mol. wt. about 965.
CRYSTALLOIDS.

Gelatin lies in the heart of the colloidal zone, and it is interesting to compare its superior water-taking capacity with that of dextrin which has much smaller particles.

# Chapter 4.

## The Chemistry, Physical Chemistry and Colloidal Chemistry of Gelatin and Glue (*Continued*).

### Is Gelatin a Distinct Chemical Entity?

The great variation in the analyses of gelatin, and the diversity in the results of experimenters, naturally raises the question as to whether gelatin is a distinct chemical entity. Most experiments have been made on gelatins which have been very loosely described, if indeed any description is given at all. Thus D. Jordan Lloyd used "Coignet's Gold Label Gelatin" and Jacques Loeb used "Cooper's Gelatin." These descriptions, even though fortified by determinations of ash, and hydrogen ion concentration, give no idea as to the chemical nature of the gelatin experimented with.

Gelatin always contains considerable gelatoses and even some gelatones which are products of its own hydrolysis, but practically no one reports what per cent. these are or even gives the jelly strength, or optical rotation (mutarotation) from which a rough idea as to their percentage might be figured out. It is safe to assert that no one has ever prepared and experimented with "chemically pure gelatin," assuming that such a thing could be made. From the evidence at present available it seems that gelatin is not a definite chemical entity.

W. M. Bayliss,[1] while making experiments on the action of trypsin on "Coignet's Gold Label Gelatin," made some pertinent observations. To a solution of gelatin, trypsin was added. Part was heated to 100° at once to prevent digestion and the other part was digested at 39° for some days. An equal amount of $\frac{n}{10}$ KCl was added to both parts, and the change in conductivity noted—

| | |
|---|---|
| Undigested gelatin ..... | 6,760 gemmhos |
| Digested gelatin ..... | 1,266 " |

(A gemmho is a reciprocal megohm.)

[1] *Arch. des Scien. Biologiques,* Vol. XI, Supplt., p. 261, St. Petersburg, 1904.

Commenting on this Bayliss says: "Gelatin behaves differently (from caseinogen); it has been mentioned already that the presence of gelatin in a solution does not, to any degree worth consideration, effect its electrical conductivity. The products of its digestion, on the contrary, diminish the conductivity of a solution containing electrolytes. In the undigested mixture, in fact, the conductivity was practically the sum of that of the gelatin mixture and that of the KCl; in the digested, on the contrary, it was far less, owing to the influence of the non-electrolyte now showing itself in the usual way. So that, therefore, changes of some kind take place in gelatin during digestion by trypsin which tend to diminish, instead of increasing, any conductivity due to electrolytes.

Amino-acids are conductors to a certain degree,[2] but their properties as such will not suffice to account for the great increase in conductivity observed. . . . It appears, however, that in the case of gelatin, amino-acids are not produced in any appreciable quantity, at all events not within the first two hours of the action of trypsin. We must look elsewhere then for the causes. One of these, viz., the effect of change of physical state, has already been mentioned. The point next suggesting itself is that concerning the inorganic constituents of these proteid and related bodies. The balance of evidence seems to be decidedly in favor of the view that these constituents are, if not actually in chemical combination with the proteid molecule, in such a close state of association as to be incapable of ionization. It has not been found possible hitherto to prepare an unaltered proteid free from ash.[3] No doubt a considerable amount is usually present as impurity which can be separated by prolonged dialysis."

It seems that besides being a body of variable constitution, gelatin carries impurities which are likewise variable in kind and amount, and which may exercise a potent influence on experiments made with it. Most experimenters are not sufficiently careful in defining the moisture content of the gelatin they use, so that doubt exists as to the true strength of the solutions they

[2] Kohlrausch and Holborn, "Leitvermögen der Electrolyte," 1898.

[3] C. R. Smith has prepared ash-free gelatin, which is quite a different thing from isoelectric gelatin, for the latter may contain neutral ash-producing salts. Dhéré and Gorgolewski (*Compt. rend. 150*, 434, 1910), made a demineralized gelatin which was almost ash free. J. A.

worked with. Thus J. Loeb [4] made no mention of the ash or moisture content of his gelatin; his calculations were based on 1 per cent. solutions while in reality he was probably using solutions of about 0.8 per cent.—an error of about 20 per cent. from this cause alone. Even in his recent papers and book, while he reports ash and moisture, he does not report the quality or strength of the gelatin he used.

C. R. Smith [4a] states that "working with an indefinite product of varying jelly strength and ash content, it is not surprising to find few reliable measurements of its physical and chemical properties." Smith, however, states that recent work points to the combination of acids and bases with gelatin, although he does not exclude the possibility of adsorption, and concludes that "we are justified either by reason of correctness or convenience in referring to gelatin chloride, sodium gelatinate, etc."

The mere fact that satisfactory "stoichiometric" compounds have been produced with gelatins containing all sorts of ash, degradation and other impurities, would seem to indicate that approximately uniform free or active surfaces or electrostatic fields lie at the basis of the experimental phenomena.

Such considerations as those strike at the very root of the experiments and especially of the conclusions of those who like H. R. Procter, J. A. Wilson and J. Loeb [5] insist on the formation of definite salts of gelatin. If gelatin is not a definite chemical entity, it is not justifiable to speak of "gelatin chloride" and "sodium gelatinate."

By referring to Chapter 2, it will be seen how various runs from different kinds of glue stock may appear in the market as "gelatin." The analytical results of R. H. Bogue [6] show that while gelatins and glues have roughly the same general degradation products, considerable differences exist between those derived from hide stock and those derived from bone stock, and there are wide variations in gelatins derived from the same class of stock. The analyses are given herewith, together with analyses of several glue proteins which Bogue purified by fourfold precipitation with 95 per cent. alcohol.

[4] See e.g. his address before the Harvey Society, *Science, N. S. 52,* 451 (1920); *J. Gen. Physiol. 3,* 89 (1920).

[4a] *J. Am. Leather Chemists' Assoc.,* Oct., 1922.

[5] See "Proteins and the Theory of Colloidal Behavior."

[6] *Chem. Met. Eng. 23,* 61 (1920).

It should be pointed out that even these analyses do not show the full extent of the variations to be expected in gelatin, for we must also take into account differences in the degree of hydrolysis, which begins the moment the gelatin forms, and continues during its manufacture and may even continue while experiments are being made with it. (See Chapter VIII.)

HIDE GLUE ANALYSES (BOGUE)

Figures show per cent. of total nitrogen in each fraction

| | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ | $H_6$ | *Average* |
|---|---|---|---|---|---|---|---|
| Ammonia N | 1.63 | 1.89 | 3.20 | 2.15 | 2.44 | 2.49 | 2.90 |
| Melanin N | 0.53 | 0.50 | 0.74 | 0.53 | 0.60 | 0.63 | 0.59 |
| Cystine N | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | Trace | 0.00 |
| Arginine N | 13.27 | 16.28 | 13.76 | 13.72 | 13.50 | 12.87 | 13.90 |
| Histidine N | 1.31 | 1.30 | 3.19 | 3.31 | 2.45 | 1.59 | 2.19 |
| Lysine N | 8.17 | 8.50 | 8.58 | 7.40 | 8.00 | 7.22 | 7.97 |
| Amino N in filtrate | 58.87 | 55.17 | 55.00 | 57.90 | 58.02 | 56.10 | 56.84 |
| Non-amino N in filtrate | 17.00 | 15.53 | 15.58 | 15.26 | 15.24 | 15.20 | 15.63 |
| Total regained | 100.78 | 99.17 | 100.05 | 100.27 | 100.25 | 96.10 | 100.02 |

BONE GLUE ANALYSES

| | $B_1$ | $B_2$ | $B_3$ | $B_4$ | $B_6$ | $B_9$ | *Average* |
|---|---|---|---|---|---|---|---|
| Ammonia N | 4.43 | 4.49 | 4.57 | 4.49 | 4.48 | 5.04 | 4.55 |
| Melanin N | 0.74 | 1.18 | 1.03 | 0.82 | 0.76 | 0.95 | 0.91 |
| Cystine N | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | Trace | 0.00 |
| Arginine N | 13.32 | 12.82 | 13.28 | 12.74 | 13.56 | 13.32 | 13.17 |
| Histidine N | 1.60 | 0.54 | 1.52 | 1.44 | 1.58 | 4.02 | 1.78 |
| Lysine N | 7.18 | 8.23 | 7.18 | 8.57 | 9.42 | 9.13 | 8.28 |
| Amino N in filtrate | 56.90 | 58.15 | 57.30 | 57.58 | 54.30 | 53.40 | 56.27 |
| Non-amino N in filtrate | 16.21 | 15.18 | 15.32 | 14.36 | 15.90 | 14.54 | 15.25 |
| Total regained | 100.38 | 100.59 | 100.20 | 99.80 | 100.00 | 100.40 | 100.21 |

PURIFIED PROTEIN, FISH GLUE AND ISINGLASS ANALYSES

| | $H_1$ *Protein* | $B_9$ *Protein* | *Fish Glue* | *Isinglass* |
|---|---|---|---|---|
| Ammonia N | 1.33 | 3.57 | 5.15 | 3.98 |
| Melanin N | 0.78 | 0.74 | 1.12 | 0.68 |
| Cystine N | 0.00 | Trace | Trace | 0.00 |
| Arginine N | 12.61 | 10.96 | 13.80 | 14.20 |
| Histidine N | 0.82 | 2.24 | 2.04 | 2.33 |
| Lysine N | 8.34 | 8.60 | 8.58 | 6.06 |
| Amino N in filtrate | 60.00 | 58.05 | 60.20 | 58.65 |
| Non-amino N in filtrate | 15.49 | 15.47 | 9.66 | 13.59 |
| Total regained | 99.37 | 99.63 | 100.55 | 99.49 |

Bogue summarizes his conclusions as follows:

"Hide and bone glues vary slightly in their chemical constitution on passing from grade to grade. This is interpreted to

signify that as the boiling of a glue progresses some 'foreign substances' as chondridin, keratin, mucin, etc., become hydrolyzed and enter the solution. These have no value in glue, and by adulteration lower the value of the product.

"Hide and bone glues differ from each other in their chemical constitution. This is taken to signify that the protein complexes from which the glues are derived are different in the two cases, or that the ratio of the several constituents is different.

"Glues of different stock within both hide and bone series show a difference in constitution, which is attributed to variations in the protein complexes of the several stocks.

"The differences between hide and bone glues are found in the protein fraction to a lesser extent, and in the proteose-peptone fraction to a greater extent than obtained in the whole glues.

"If the purified protein from the highest grade animal glues may be considered as pure gelatin, then it follows that isinglass is not a pure gelatin, or if the assumption be made that isinglass consists only of gelatin, then the purified animal glue protein contains impurities.

"The lower the grade of a glue, the further is it removed in constitution from that of the purified protein, and, if this protein be assumed to consist only of gelatin, then the gelatin content of glues diminishes with the grade, and substance from which the hydrolytic products are obtained consists of gelatin in decreasing amounts, as the grade decreases.

"Fish glue corresponds more closely in its composition to low-grade bone glue than to any other.

"Fish glue and isinglass show a fundamental difference from animal glues in their low 'non-amino nitrogen of the filtrate' (proline, oxyproline, and tryptophane)."

S. E. Sheppard and S. S. Sweet [7] have shown that impurities exert a powerful influence on gelatin. They brought ash-free gelatin to different $p_H$ values, and found that it showed maximum rigidity (jelly strength) at $p_H$ 8, at all concentrations. The curves show a "shoulder near the isoelectric point (which they say is $p_H$ 4.8) but no definite maximum or minimum. The curves were greatly altered by traces of aluminum salts, enough to give as little as 0.01 per cent. of $Al_2O_3$ on the dry gelatin

[7] *Science* 46, 28 (1922).

displacing the maximum on the alkaline side and producing a secondary maximum at $p_H$ 5.

S. E. Sheppard has just published [7a] an interesting discussion of gelatin in photographic processes, and is actively investigating many collateral questions. He reports that experiments with F. A. Elliot show that with high and low $p_H$ values gelatin shows perceptible hydrolysis even at 50° C. He also observes that "on washing out strong electrolytes from gelatin, it will tend to approach the isoelectric point, but if hydrolyzable substances are present, the gelatin will retain unequal amounts of the basic or acidic constituents, the excess depending upon conditions."

**The Significance of Hydrogen Ion Concentration.**

The importance of H ion concentration ($p_H$ value) is so stressed to-day, that a brief consideration of the principles involved in its determination will not be amiss.

Pure water is slightly dissociated according to the equation

$$H_2O \rightleftarrows H^+ + OH^-$$

This is a reversible reaction, and therefore at any temperature an equilibrium is reached where the rate at which water molecules split into ions, equals the rate at which these ions recombine to form water. The Law of Mass Action [8] demands that the rate of ion formation depends upon the concentration of undissociated water ($C_{H_2O}$), while the rate of ion recombination is proportional to the product of the concentrations of positive and negative ions ($C_{H^+} \times C_{OH^-}$).

$$\text{Therefore } C_{H_2O} = C_{H^+} \times C_{OH^-}$$

$$\text{or } \frac{C_H \times C_{OH}}{C_{H_2O}} = \text{a constant value called } k.$$

But with water under ordinary conditions the dissociation is so small that the amount of undissociated water may be assumed to remain constant. That is

$$C_H \times C_{OH} = k \times (C_{H_2O}) = K_W,$$

the constant of dissociation for water.

[7a] *J. Ind. & Eng. Chem.* *14*, 1025 (1922).

[8] How far the Law of Mass Action may be applied to colloidal solutions is still an open question.

By several methods the value of $K_W$ has been to be $10^{-14}$ at 22°; that is $C_H = C_{OH} = 10^{-7}$, which means that in pure water there is a concentration of 1/10,000,000 for both H and OH ions.

Assuming that the Law of Mass Action continues to hold, if $C_H$ is increased by addition of an acid or acid salt which dissociates in water, the value of $C_{OH}$ must diminish proportionately, since $C_H \times C_{OH}$ must remain constant. Correspondingly, the addition of an alkali or an alkaline salt diminishes the number of H ions.[9]

Therefore a solution is acid when $C_H > 10^{-7}$, or $C_{OH} < 10^{-7}$, and alkaline when $C_H < 10^{-7}$ or $C_{OH} > 10^{-7}$. It must be remembered, however, that the deviation of H or OH ion concentration from the value $10^{-7}$ depends upon the extent to which the added acid or basic substance is dissociated, and represents therefore *not* the *total* acidity or alkalinity but rather the *effective reaction,* i.e. the *degree* of acidity or alkalinity. Thus while $\frac{n}{10}$ HCl and $\frac{n}{10}$ acetic acid will each neutralize equivalent quantities of $\frac{n}{10}$ NaOH, their respective H ion concentrations at 18° are given in the following table from Michaelis.[10]

| *Degree of Normality* | | $C_{H^+}$ | *Equivalent* $p_H$ *value* |
|---|---|---|---|
| HCl ..... | 1.0 | $8.0 \times 10^{-1}$ | 0.10 |
| | 0.1 | $8.4 \times 10^{-2}$ | 1.07 |
| | 0.01 | $9.5 \times 10^{-3}$ | 2.02 |
| | 0.001 | $9.7 \times 10^{-4}$ | 3.01 |
| | 0.0001 | $9.8 \times 10^{-5}$ | 4.01 |
| Acetic Acid ..... | 1.0 | $4.3 \times 10^{-3}$ | 2.37 |
| | 0.1 | $1.36 \times 10^{-3}$ | 2.87 |
| | 0.01 | $4.3 \times 10^{-4}$ | 3.37 |
| | 0.001 | $1.36 \times 10^{-4}$ | 3.87 |
| Na OH ..... | 1.0 | $0.90 \times 10^{-14}$ | 14.05 |
| | 0.1 | $0.86 \times 10^{-13}$ | 13.07 |
| | 0.01 | $0.76 \times 10^{-12}$ | 12.12 |
| | 0.001 | $0.74 \times 10^{-11}$ | 11.13 |

[9] "The discrepancies observed, especially in strong acids, between the ionic concentrations as measured by conductivity methods on the one hand and with the hydrogen electrode on the other, suggest that the quantity which we call hydrogen ion concentration may not actually represent the degree of normality of hydrogen ions in the solutions under test. Some have preferred to call this quantity 'activity.'" Leeds and Northrup Co. Catalog 75 (1921), p. 6.

[10] "Wasserstoffionen Konzentration."

As the table shows, HCl is a "strong" acid and acetic a "weak" acid, as measured by effective reaction. But since the $H^+$ and $Cl^-$ part company and ionize more readily than do $H^+$ and $CHCOO^-$, it is evident that the latter bond is less readily relaxed in aqueous solution; so that acetic acid is really stronger from this point of view. This means that *the anion largely* if not entirely controls the $p_H$ value of an acid. Therefore even if as J. Loeb claims, the $p_H$ value be the main factor controlling the swelling and general colloidal behavior of gelatin through the Donnan equilibrium, the *ultimate* cause is to be found in the *specific nature of the anions* of acids *or the cations* of bases, because they control the H ion concentration ($p_H$). They thus form the *raison d'être* of the Hofmeister series.

Thomas and Baldwin [10a] showed that salts change the $p_H$. In the case of hydrochloric and sulphuric acids, sodium chloride increases the $p_H$, whereas sodium sulphate decreases it. These measurements were made after waiting two days.

Since it is inconvenient to express and read H ion concentrations by products of the character given in the table above, Sørensen proposed to perform the multiplication logarithmically and use the logarithm of the product after dropping the minus sign.

Thus an acidity 10 times as great as that of pure water (that is $C_H = 10 \times 10^{-7}$) would be represented by $\log 10 + \log 10^{-7} = 1 - 7 = -6$; and dropping the minus sign, $p_H$ (Sørensen's expression) $= 6$. In like manner an acidity 100 times greater than that of water would be $C_H = 100 \times 10^{-7} = 2 - 7 = -5$, or $_H = 5$.

| *$p_H$ value* | *Number of times H (or OH) ion concentration exceeds that of pure water* | |
|---|---|---|
| 1 | 1,000,000 | |
| 2 | 100,000 | |
| 3 | 10,000 | |
| 4 | 1,000 | |
| 5 | 100 | acid side ↑ |
| 6 | 10 | |
| 7 | 0 | pure water |
| 8 | 10 | |
| 9 | 100 | ↓ alkaline side |
| 10 | 1,000 | |
| 11 | 10,000 | |
| 12 | 100,000 | |

[10a] *J. Am. Chem. Soc. 41,* 1981 (1919).

This table shows two facts which must not be forgotten; first that an *increase* in $p_H$ means a *decrease* in H ion concentration, and second that the acidity *does not decrease numerically* with the $p_H$ value but *decreases in logarithmic ratio.* It is obvious, therefore, in plotting experimental results, that the $p_H$ values should be reconverted into true values, i.e. H-ion concentration, or else logarithmic paper should be used. If the $p_H$ values are laid off *numerically* as abcissas or as ordinates, the resulting curve will be logarithmically compressed, and the presence of inflections or of cusps in the true curve may thereby be overlooked. In any event such curves give a wrong idea of the relative hydrogen ion concentrations.[11]

The table shows another point of great interest which is often overlooked, which is that $p_H = 4.7$ (the isoelectric point of gelatin), represents an *extremely slight* acidity. Thus ordinary distilled water prepared in the laboratory still, has a $p_H$ of about 5.5, but when boiled to expel the $CO_2$ absorbed, the reaction drops to $p_H = 7$ (neutrality). On the other hand $\frac{n}{100}$HCl has a $p_H =$ only 2.02. The most striking changes with gelatin occur between about $p_H = 2$ and $p_H = 4$ on the acid side, and $p_H = 9$ and $p_H = 11$ on the alkaline side.[12] These represent comparatively weak acidity or alkalinity; but with a slightly ionized acid like acetic, so much acid must be used to produce a $p_H =$ about 2, that the specific solubilizing action of the acid on gelatin becomes very marked.[12a]

[11] Many of J. Loeb's experimental results are given solely in erroneous curves of this character, and should be given in tabular form or the curves redrawn. For full details regarding apparatus for determination of H ion concentration, the formulas whereby the electrical readings are converted into $C_{H+}$, and the precautions to be observed, the reader is referred to standard texts, and especially to W. M. Clark's book, "The Determination of Hydrogen Ions," Baltimore, 1920.

[12] Thus C. R. Smith found that 1 gram of air dry ash free isoelectric gelatin swelled in pure water to 7-8 cc., while the maximum acid swelling was 48 cc. and the maximum alkaline swelling was 30 cc. Low jelly strength gelatins give decreased swelling.—Smith, *J. Am. Chem. Soc. 43,* 1360 (1921).

[12a] See e.g. J. Loeb, "Proteins," p. 80.

**The Titration Curve of Gelatin.**

Dorothy Jordan Lloyd and C. Mayes,[13] in order to determine the amount of HCl or NaOH which would combine with a certain weight of gelatin, determined potentiometrically the differences between the H ion concentrations of 1 per cent. gelatin solutions containing different percentages of acid and alkali. The gelatin was Coignet's Gold Label, reduced to $p_H = 4.6$, ash between 0.00 and 0.06 per cent. Knowing the H ion concentration of equally concentrated systems containing no gelatin, they calculated by the formula of Blasel and Matula[14] the concentrations of HCl removed by the gelatin from independent solution.

The Blasel and Matula formula is based on the erroneous assumption that the ionization of HCl is the same whether or not the normality of H and Cl are identical, so they made a correction for this, using the Cl ion concentration as a factor. But the value of the Cl concentration is based on the further assumption, which was also made by Procter and Wilson[15] that "gelatin chlorid" is completely dissociated. This latter assumption is not supported by the figures of Bugarsky and Liebermann, on Cl ion concentration.[15a] Jordan Lloyd and Mayes remark that this assumption is liable to lead to an increasing error with higher H ion concentration.

On plotting their results with the *normality* values of H ion concentration as abscissas and the amount of HCl fixed as ordinates, they obtained not a simple smooth curve, but one consisting of two, possibly three distinct regions, indicating that "up to a given concentration of hydrogen ions, a group of hydroxyl ions having approximately equal ionization constants is involved; beyond this concentration, and up to a second fixed value, a second group approximating to a second constant is involved; and beyond this again there is slight evidence of a third group. The factors required in order to bring the second and possible third groups into conformity with the generalized statement of the law of mass action are not yet fully known."

[13] *Proc. Roy. Soc. B, 93,* 69 (1922).
[14] *Biochem. Z. 58,* 417 (1914).
[15] *Trans. Chem. Soc. 109,* 307 (1916).
[15a] *Pflüger's Arch. 72,* 51 (1898).

Their results for the amount of NaOH fixed had greater experimental errors than did the determination of acid fixation, for the gelatin was probably attacked by the alkali in the presence of the spongy platinum of the electrode. The curve was made by using *normality* values of NaOH as abscissas and the amounts of NaOH fixed as ordinates. The curve rises abruptly, apparently seeking a maximum when $OH^-$ = about 0.005 N, but then begins to rise sharply again, giving a very steep curve. "Hence it is obvious that in alkaline solution gelatin does not behave simply as a weak acid dissociating in accordance with the law of mass action. It is possible that this abrupt rise accompanies some structural change of the protein molecule such as Dakin had shown to occur in strong alkaline solution." [16]

Jordan Lloyd and Mayes then discuss the mechanism for the fixation of acid and alkali. While in solutions of HCl less than 0.02 N it is *possible* that gelatin may bind the acid by its free amino-groups, "with increasing concentration of acid, more acid is bound than can be accounted for on this hypothesis, and it is therefore necessary to consider what part the imino-nitrogen of the peptide linkage (— COHN —) could play. Robertson [17] states that the acid binding properties of the proteins are not much increased by hydrolysis, and we have found that the reaction of a 1 per cent. solution of gelatin, which was found to be $p_H = 1.3$, had only changed to $p_H = 1.12$ after 12 hours at 100° C. This change is of the same order as the experimental error of the method, nevertheless hydrolysis of the gelatin had occurred during the heating in the strong acid solution, as was shown by the fact that the gelling power had been destroyed. It seems, therefore, that the peptide linkage can function as an acid-binding group. . . . It seems clear that some of the — COHN — groups can act as basic groups combining with acids. What rôle, if any, other groups (such as the hydroxyl groups of the hydroxy acids) in the molecule play in acid fixation is still unknown. It will be necessary to follow experimentally the fate of the chlorine ion before final decisions are possible. . . . The theory that proteins fix bases by means of their free carboxyl groups has given way on accumulation of evidence that there are not enough of the latter to explain the quantitative reactions.

[16] *J. Biol. Chem. 13*, 357 (1912-13).

[17] "The Physical Chemistry of the Proteins."

. . . The possibility of linkage at some of the hydroxy groups of the substituted amino-acids, serine and hydroxy-proline, is not to be ignored. Hydrolysis of gelatin by caustic soda has been shown to increase slightly its basic binding power, a fact which suggests that not all the —COHN— linkages are as potent as base fixers as the free —COOH— groups. Loeb [18] has shown that bases react with gelatin at the same hydroxyl ion concentration in equivalent proportions. This fact shows that the reaction is ionic, and that the compounds formed are of the nature of ionizable salts. Loeb only worked with solutions whose alkalinity is less than $p_H = 9$. His experimental values correspond very closely to our values over the same range. . . . There is both qualitative and quantitative evidence to show that in the same protein (gelatin) the mechanism of fixing acids is different from that of fixing bases."

D. I. Hitchcock [18a] determined the combination of gelatin with hydrochloric acid. Using 1, 2½, and 5 per cent. gelatin solutions with varying acid content, he subtracted the $p_H$ values of the acid-gelatin solutions from those of solutions containing equal amounts of pure acid, the difference being considered as indicating the amount of acid combined with the gelatin. He reports that about 0.00092 mol. of hydrochloric acid combine with 1 gram of gelatin between $p_H$ 1 and 2, but found no evidence of a discontinuous section in the titration curve, as did Lloyd and Mayes (*vide supra*).

Oakes and Davis [19] found a definite relationship between the grade of a gelatin and the amount of acid required to titrate it over the range $p_H$ 4.7 to $p_H$ 3.5. To make this change in reaction they report as follows:

| *Gelatin* | *Jelly Strength* $p_H$ *42; 21° C.* | *cc. 0.2 M H Cl* | *"Molecular Weight" of Gelatin* |
|---|---|---|---|
| x | 59 | 3.79 | 1,319 |
| 2 | 430 | 2.85 | 1,753 |
| 2E | 565 | 2.70 | 1,852 |
| 3 | 800 | 2.48 | 2,016 |
| 3B | 1,025 | 2.40 | 2,083 |

[18] *J. Gen. Physiol. 1,* 379, 487 (1919).
[18a] *J. Gen. Physiol. 4,* 733-9 (1922).
[19] *J. Ind. Eng. Chem. 14,* 706 (1922).

They think these figures "indicate the order of magnitude of the molecular weight, and its progressive increase with the grade of gelatin," although they state elsewhere in the same paper: "Since there is probably no gelatin that is not made up of a series of the products of hydrolysis of the original tissue, no gelatin can have what may be called molecular weight. What is determined is the mean molecular weight of all the various fractions making up the gelatin sample." Nevertheless they believe that a (presumably chemical) compound is formed at the maximum point of the viscosity—$p_H$ curve.

Since Oakes and Davis do not give the ash content of these gelatins and since lower grade gelatins often contain more ash than higher grades, it is probable that the variations in the above table are to some extent due to the ash. T. B. Robertson found that profound hydrolysis did not materially change the acid fixing capacity of a gelatin. Furthermore the increase in viscosity of isoelectric gelatin by the addition of acid, is readily accounted for by swelling of its complexes without assuming the formation of a gelatin "salt." Oakes and Davis also state: "The difference in ash content of gelatin is, then, the main cause for the lack of agreement between classifying gelatins by viscosity and jelly strength measurements, and for a given ash content viscosity measurements may be substituted for jelly strength measurements." The importance of ash depends not only on its *amount*, but also upon its *composition;* in any event, as above indicated, it cannot be left out of consideration.

The fact that profound hydrolysis as well as deaminization do not materially alter the acid and base fixing power of gelatin, is an indication that definite gelatin salts and metal gelatinates do not exist, a view further strengthened by the irregularities of acid and base fixation. While the theory that such compounds exist may be made to fit many of the experimental facts by assuming conveniently variable dissociation values, it seems more likely to the writer that the fixation of acid and alkali should be regarded as due to adsorption, the extent of which varies as the total free or effective adsorbing surfaces of the molecular complexes are changed by varying conditions, one of which is hydrogen ion concentration.

How do $H^+$ ions or $OH^-$ ions function when they produce

initially an increased swelling of isoelectric gelatin which has already been swollen to its limit in isoelectric water of $p_H = 7$? Even ash-free isoelectric gelatin swells considerably in pure water $p_H = 7$, proving that its "molecules" or molecular groups have considerable residual attraction, which enables a high-class air-dry gelatin to hold about 20 per cent. of water that can be driven off by drying at 110°. Obviously with an acid or alkali the ions are concentrated within the gelatin particles, for the concentration of the external solution (unless in too great an excess) is diminished.

The original view of W. B. Hardy [20] (also accepted by J. Perrin) was that "as the H and OH ions have by far the highest specific velocity the colloidal particle will entangle an excess of H ions in acid and thereby acquire a + charge and of OH ions in alkali and thereby acquire a — charge. These charges will decrease the surface energy of the particles and thereby lead to changes in their average size.[21] This would mean that, in the kinetic equilibrium existing, H (or OH) ions would accumulate within the "molecule" of gelatin, and cause its distension by electric repulsion along the lines suggested by Tolman and Stearns.

Hardy [22] later regarded the H and OH ions as being held by chemical attraction, but pointed out that "though one may speak of the colloid particles as being ionic in nature they are sharply distinct from true ions in the fact that they are not of the same order of magnitude as are the molecules of the solvent, the electric charge which they carry is not a definite multiple of a fixed quantity and one cannot ascribe to them a valency, and their electrical relations are those which underlie the phenomena of electrical endosmose."

Even though Hardy [23] express the view that proteins form salts with acid and alkalis, he expressly points out that "the reactions are not precise, an indefinite number of salts of the form $(B)n$ BHA being formed where the value of $n$ is deter-

[20] *J. Physiol. 29,* 29 (1903).

[21] Very likely the small size as well as the speed of these ions is also a factor and as $H^-$ is smaller and speedier than $OH^-$ we should not be surprised if we find that the minimum swelling of gelatin is not in pure water, but *very* slightly on the alkaline side of the isoelectric point. J. A.

[22] *J. Physiol. 22,* 251 (1905); *Proc. Roy. Soc. 79,* 413 (1907).

[23] T. B. Wood and W. B. Hardy, *Proc. Roy. Soc. 81,* 38 (1909).

mined by conditions of temperature and concentration, and of inertia due to electrification of internal surfaces within the solution.[24]

It seems, as Tolman and Stearns suggest, that the H (or OH) ions, adsorbed at the free surfaces of the micellular groups, disturb whatever balance exists at the isoelectric point, and the resulting + (or —) charges at these free surfaces cause repulsions which still further distend the gelatin.

But how shall we account for the fact that after reaching a maximum at about $p_H = 3$, further additions of acid or alkali cause *contraction* again? In higher concentrations the "salting out" action of the acid or alkali dehydrates the gelatin and causes shrinking. A. Kuhn,[25] who investigated the swelling of gelatin in over fifty aliphatic and aromatic acids, found that the swelling is controlled by four factors:

A 1. Individual Swelling or Hydration.

B 2. Incidental Peptization (Sol formation)
B 3. Hydrolysis

↓ increasing concentration of acid.

C 4. Dehydration, or Flocculation.

The maximum is determined by groups A and B, and is defined as the point where the increasing swelling or hydration due to rising acid concentration is overbalanced by sol formation and hydrolysis, which also increase at the same time.[26]

Since the forces which govern adsorption are molecular, i.e. due to residual atomic fields of force (see Chapter II, p. 28), it is natural that they should be greatly influenced by the chemical constitution of the molecular groups which form the gelatin "molecule." Part of these residual fields are balanced in holding the molecular groups together as the gelatin "molecule," but the remaining moieties are free to attract and hold ions or groups

[24] According to R. Keller (*Kolloid Z. 27,* 255 [1920]), the degree of dispersion exerts a vital influence on the chemical and electrical properties of colloids. Thus very dilute solutions of methylene blue move to the cathode despite addition of alkali; but in somewhat coarser dispersion it moves partially or entirely to the anode. The reversal of charge of colloids is not to be expressed in terms of H ion concentration, for chemical combination of stoichiometric character hardly exists between colloids and ions whose weight and volume are roughly as 1,000,000,000 to 1. J. A.

[25] *Kolloidchem. Beihefte 14,* 147 (1921).

[26] Kuhn's experiments must be repeated for they were made with gelatin containing 3.13 per cent. of ash.

having an opposite charge, and thus "fix" acid, alkali, etc., in *proportion to the effective free fields of the acid and alkali,* which of course vary stoichiometrically; that is, according to their respective effective valencies. Therefore the fixation of acids and bases in *their* stoichiometrical ratios is no proof that a definite chemical compound has been formed, i.e. that *the gelatin* has combined stoichiometrically. Nor is it surprising that on the acid side of the isoelectric point, when gelatin has a net positive charge it should act as an acid and vice-versa.

As Wo. Ostwald [27] puts it, in adsorption there comes into play *not* the stoichiometric mass, but the *active* mass, which means the sum of the chemically active surface layers. When true molecular or "crystalloid" dispersion exists, the ratio between these two is unity; but in colloidal aggregation the active mass is only a fraction of the stoichiometric mass, the value of the fraction depending on the size of the particles of the colloid, i.e. on its specific surface. Furthermore according to N. Schilow,[28] adsorption of electrolytes depends not only on the sign of the adsorbent, but on the nature of the electrolyte and solvent, that is on the collective properties of the system. He was able to reverse some adsorption series merely by small additions to the solvent.

Furthermore the Donnan equilibrium and its consequences which J. Loeb [29] relies upon to prove the formation of definite chemical compounds, are just as well explainable on the basis of a kinetically balanced adsorption, as on the basis of "chemical compounds" which hydrolyze. Loeb says (*loc. cit.,* p. 63): "It can be stated as a result of all these titration experiments, that the ratios in which acids and bases combine with proteins are identical with the ratios in which acids and bases combine with crystalloids. Or, in other words, the forces by which gelatin, egg albumen, and casein (and probably proteins in general) combine with acids and alkalis are the purely chemical forces of primary valency."

Now while the first statement is justified by the fact that simple ions like $Cl^-$, and $Na^+$ always combine according to *their* valence fields of force, the second statement is unwarranted, and

[27] *Kolloid Z. 30,* 254 (1922).
[28] *Z. physik. Chem. 100,* 425 (1922).
[29] "Proteins."

is not putting the first statement in other words; for the second statement *assumes that the gelatin* also combines with primary valence forces, which is not the case. The gelatin compounds lack the precise and definite character connoted by the present meaning of the expression "chemical compound."

# Chapter 5.

## The Structure of Gelatin Solutions and of Gelatin Jellies.

The structure of jellies has long been a moot question, and is not yet settled. In an historical review Zsigmondy [1] says that the oldest theory assumed a porous structure for distensible bodies; water penetrating the pores, was held by capillary or by molecular attraction, and thus produced swelling.

In 1858 Nägeli [2] advanced his *micellular theory* in which distensible bodies were assumed to be made up of tiny anisotropic crystal-like aggregations of molecules called *micells*, which retain their identity in solution. The micells are surrounded by a layer of water whose thickness is limited by the fact that the attraction of the micells for each other finally dominates the attraction of the micells for water. The swelling caused by the penetration of the water into the micellular mass thus reaches an equilibrium which may be shifted by changes in temperature, pressure, etc. Frankenheim [3] had also expressed similar views. O. Bütschli [4] advanced what is known as the honey-comb theory. His first experiments [5] included soap solutions and emulsions of oils. By hardening gelatin jellies with alcohol or chromic acid, Bütschli was able to demonstrate microscopically a honey-comb structure; but it is probable the structures demonstrated are in this case artifacts produced by the action of the hardening agents [6] used with the intention of rendering visible a structure

[1] R. Zsigmondy, "The Chemistry of Colloids," p. 68, trans. by E. B. Spear, 1917.

[2] C. von Nägeli and S. Schwendener, "Das Mikroscop" (2d ed.), Leipzig, 1877; C. von Nägeli, "Theorie der Gärung," Munich, 1879.

[3] "Die Lehre von der Kohäsion," Breslau, 1835.

[4] O. Bütschli, "Ueber den Bau quellbarer Körper usw.," Göttingen, 1896; "Untersuchnngen über Structuren," Leipzig, 1898; "Untersuchungen über die Mikrostruktur künstlicher und natürlicher Kieselsäuregallerten," Heidelberg, 1900.

[5] "Untersuchungen über mikroscopische Schäume und das Protoplasma," Leipzig, 1892.

[6] See H. Bechhold, "Colloids in Biology and Medicine," trans. by J. G. M. Bullowa, Ch. XXIII, New York, 1919; also W. Pauli, "Der Kolloidale Zustand und die Vorgänge in der lebendigen Substanz," Brunswick, 1902.

already existing. Bütschli's view was supported by G. Quincke,[7] who stresses the effect of the surface tension existing between the "oleaginous" phase and a second phase richer in water. Quite similar is the view of W. B. Hardy,[8] who considered gelatin jellies to consist of two phases, one a solution of gelatin in water, the other a solution of water in gelatin.

Van Bemmelen, though first leaning to the micellular theory of Nägeli, later agreed with Bütschli, whose work indicated the following dimensions for the diameters (d) of the tiny cavities in silica-gels, and for the major limits of thickness of their cell walls (m):

| *Substance* | *d* | *m* |
|---|---|---|
| Tabischir (from bamboo nodes) | 1.45μ | 0.152–0.187μ |
| van Bemmelen's silica gel | 1.00μ | 0.27μ |
| Bütschli's silica gel | 1.50μ | 0.30μ |

Zsigmondy [9] and his pupil, W. Bachmann, hold what may be termed the fine grained theory, which does not materially differ from that of Nägeli.[10] Zsigmondy believes (*loc. cit.*, p. 70) that upon the relatively gross heterogeneity of Bütschli there is superimposed a much finer discontinuity. Bachmann (*loc. cit.*, p. 99) found that gelatin benzolgels and alcogels show the same type of curves as van Bemmelen found in silicic acid gels, in the course and hysteresis cycle of their vapor pressure isotherm. "The application of the theory of capillarity to solidified gelatin jellies permits an approximate calculation of their vacant spaces. On the average they are from 30 to 100 times smaller than the honey-comb spaces made visible by Bütschli in such jellies by coagulators. Bütschli's honey-comb structure, whose spaces of 700 to 800 μμ are enormous when compared with the truly amicroscopic dimensions here concerned, can play no part as factors depressing the vapor tension, and are therefore not responsible for the hysteresis cycle of a gel, which is one of its special characteristics." Bachmann therefore concludes that the real gel structure is much finer than Bütschli's "honey-comb."

N. Sutherland [10a] advanced what may be termed the semplar

[7] *Drude's Annalen*, 1902 and 1903.

[8] *Z. f. phys. Chem. 33*, 326 (1900); *Proc. Roy. Soc. 66*, 95 (1900).

[9] R. Zsigmondy, *Physik. Z. 14*, 1098 (1913).

[10] See W. Bachmann, *Kolloid Z. 23*, 85 (1918); also Zsigmondy, "The Chemisty of Colloids," p. 127 et seq.; p. 224 et seq.

[10a] *Proc. Roy. Soc. 79B*, 130 (1907).

theory. The molecules link up by their atomic electric charges, forming a three-dimensional pattern or semplar, which is repeated many times in each particle.

W. Moeller [11] considers gelatinization as a kind of crystallization, in which thread-like crystals traverse the jelly in every direction and thus form a net-like lattice of thread-like crystals. Based largely on microscopic and ultramicroscopic evidence, many investigators advance a similar view. Thus Bogue [12] considers gelatin made up of "streptococcal threads" of molecules. It must be remembered, however, that the ultramicrons seen in the ultramicroscope, are only diffraction images of particles smaller than a wave length of light; they can therefore never be microscopically resolved. The cocci-like appearance is no criterion of the actual shape of the particles; and as Wo. Ostwald [13] points out, according to the Frauenhofer-Babinet principle, "holes" reflect the same as discs, i.e. both show as "grains" in the ultramicroscope.

Bogue [13a] has elaborated his views on the structure of gels, besides reviewing several other theories, as well as studying the influence of electrolytes of varying H ion concentration, and of the valence of the combining ion upon gel strength, viscosity, swelling, foam, alcohol number and turbidity. He reports that the greatest opacity results from the largest aggregates of least swollen particles, which coincides with the view advanced on p. 78 as to the complex nature of gelatin particles, and with Alexander's zone of maximum degree of colloidality.

D. Jordan Lloyd [14] adopts and extends the general view of Hardy, and believes that a gelatin gel consists "of two phases, solid and liquid, and two chemical states of gelatin, *viz.* gelatin *per se* and gelatin in the form of soluble salts. Such gels therefore are three component systems, the components being water, gelatin, and an acid (or base). . . . The process of gelation is therefore pictured as follows: gelation will only occur on the cooling of a sol which contains in solution isoelectric gelatin, and gelatin salts in equilibrium with free electrolytes. As the sol

[11] *Kolloid Z. 23,* 11 (1918).
[12] R. H. Bogue, *Chem. Met. Eng. 23,* 61 (1920). See also *J. Am. Chem. Soc. 44,* 1343 (1922).
[13] *Kolloid Z. 22,* 80 (1917).
[13a] *J. Am. Chem. Soc. 44,* 1342 (1922).
[14] *Biochem. J. 14,* 165 (1920).

is cooled the insoluble isoelectric gelatin is precipitated in a state of suspended crystallization and forms a solid framework throughout the system. The more soluble gelatin salts remain in solution, and by their osmotic pressure keep the framework extended. Gels therefore are two-phase systems, the solid phase consisting of isoelectric gelatin, the liquid of gelatin in the salt form." Isoelectric gelatin, therefore, where the contractile forces of the framework are unopposed, should be unstable and should squeeze out the liquid phase. Jordan Lloyd[15] has produced such a contracting clot of isoelectric gelatin and finds that it shows numerous small spheres about 0.5 μ in diameter, like the spherites described by Bradford.[16]

What Jordan Lloyd calls "suspended crystallization" is an indication of the protective or crystal-inhibitive action of a portion of the gelatin solution, for isoelectric gelatin as Loeb[17] has shown is inert and insoluble in cold water. The facts support the general view of E. Jordis,[18] that electrolyte impurities are essential to the *stability* of gels. In the case of gelatin, owing to its very high protective action (gold number), surprisingly minute percentages of hydrogen- or hydroxyl-ions are able to produce sufficient "gelatin salt" to stabilize the essentially unstable isoelectric gelatin; and, as Jordan Lloyd observes, the purest water obtainable is still an electrolyte, and is alkaline to gelatin, and therefore will react with it to produce "salts." The highly purified gelatin produced by Field[19] was evidently not absolutely free from stabilizing substances, since its jelly was stable; it gave, however, an opaque jelly which was made transparent by traces of acid or alkali, and even by carbon dioxide absorbed from the air.

The formation of a gel by Jordan Lloyd's isoelectric gelatin would seem to indicate that, contrary to her contention, the formation of a gelatin salt is not necessary to the *formation* of a gel, even although the salt may be necessary to *stabilize* the gel when once formed. Hence only the dispersed substance and water are really essential to gel formation with gelatin, a typical

[15] *Biochem. J. 14,* 584 (1920).
[16] *Biochem. J. 14,* 91 (1920).
[17] J. Loeb, *J. Gen. Physiol. 1,* 41 (1918).
[18] *Z. Electrochem. 8,* 677 (1902).
[19] Ada M. Field, *J. Am. Chem. Soc. 43,* 667 (1921).

"emulsoid," as is the case with ferric hydroxide and other so-called suspensoids.

H. G. Bennett [20] believes that a jelly contains a continuous network of water under a great compression due to the contractile forces of surface tension. The higher the degree of dispersion of the particles, and the greater the concentration, the greater the proportion of the water present in the gel will be in the compressed state, and therefore the greater the viscosity, finally culminating in rigidity. Swelling is caused by the electrostatic repulsion of similarly charged adsorbed ions, which repulsion, however, diminishes more rapidly as swelling proceeds than does the contractile force above referred to. A balance is consequently reached, which is influenced by the "lyotrope" influence of dissolved substances that affect the compressibility of the water.

Procter [21] criticizes Bennett's theory, and points out that his own theory of acid swelling (see p. 93) accounts quantitatively for the phenomena observed. He also makes the justified objection that surface tension itself causes compression.[22] Procter further observes that any existing compression cannot cause a large increase in viscosity, and that electrostatic repulsion is out of the question because the colloid particles are neutralized by the formation of an electrical double layer. The reason charged particles move in electrophoresis is that the layer is continuously displaced in a direction opposite to that of the motion of the particle.

H. R. Procter [22a] in speaking of the structure of gelatin jellies said: "True homogeneity can only be postulated of a hypothetical fluid, and certainly not of any atomic structure, or even of the atoms themselves. The dilute solution of any substance must have considerable spaces of solvent between the molecules," whereas "large organic chains may cohere without separation from the solvent, where the smaller and more definitely polar molecules of a crystalline substance would form rigid crystals in which only a definite proportion of solvent would be included

[20] *J. Soc. Leather Trades Chem.* **2**, 40 (1918).

[21] *J. Soc. Leather Trades Chem.* **2**, 73 (1918).

[22] The compression nevertheless does exist, although it is due not to surface tension, but to the very forces that produce surface tension, namely the specific attractions or residual fields of force of the atoms or molecules involved.

[22a] *Seventh Int. Cong. of Appl. Chem.*, 1909.

as water of crystallization. The true issue is therefore not whether jellies have a discontinuous structure, but whether the network is so fine that the constituents are within range of each other's molecular forces, or so coarse that these forces may be wholly or mainly neglected."

Procter does not here consider that both conditions may coexist, and that the "solvent" may in addition contain selected portions of a complex mixture.

F. C. Thompson [22b] believes that gelatin solutions "consist of a network of solid gelatin, molecular, or at least extremely fine, with pure water in the interstices." To this view a similar criticism applies as to that of Procter.

An indication that the water in gelatin jellies is "available," is found in Graham's observation that diffusion occurs in jellies almost as freely as in pure water, and in Dumanski's observations [22c] that the conductivity of electrolytes shows the same effect. Thompson, however, regards gelatin solutions stronger than 0.18 per cent. as solids because they resist indefinitely a small shearing strain.

Wo. Ostwald [23] believes that the process of swelling represents the reverse of syneresis. "The coarser structure of the solid is, as it were, broken up; in other words, coarse aggregates are divided into the primary particles of which they are composed. As N. Gaidukow [24] has found the ultramicroscopic particles of a gel become smaller in the process of swelling, or at least lose their highly refractive character. But in the process of swelling there occurs another change which may, under certain circumstances, actually run counter to the increase in dispersion. The individual particles absorb the medium in which they are swelling; they become *solvated.* This *increases* the size of the particles and so fluid droplets may be formed. The two changes, in other words, the combination of increase in degree of dispersion with a change in a type of the dispersed substance from the side of the solid to that of the fluid, seem most characteristic of the process of swelling."

[22b] *J. Leather Trades Chemists' 3,* 209 (1919).

[22c] *J. Physic. Chem. 60,* 553 (1907).

[23] "Theoretical and Applied Colloid Chemistry," 1917, trans. by M. H. Fischer, p. 100.

[24] See N. Gaidukow, "Dunkelfeldbeleuchtung und Ultra-mikroskopie in der Biologie und in der Medizin," Jena, 1910; *Kolloid Z. 6,* 260 (1910).

Here Ostwald tacitly assumes the existence of a secondary structure in the larger particles of gels, which is also the view held by Zsigmondy.[25] Experiments made by J. Alexander[26] with karaya gum reduced to various degrees of fineness support this view; for increase in viscosity and, later on, gel formation, accompany the hydration and swelling of the gum fragments. "In fact, with hydrophile or emulsoid colloids, as the dispersed phase becomes *less* viscous by swelling, the colloids as a whole becomes *more* viscous. Viscosity may also increase as ultramicrons condense or aggregate, as is the case in cooling gelatin." (Alexander, *loc. cit.*)

Ostwald believes that gelatin gel is a two-phase liquid system. He says (*loc. cit.*, p. 103): "In gels produced by swelling, I do not know of an instance in which the dispersed elements are solid or crystalline in character. They are, apparently, always liquid." E. Hatschek[27] has shown mathematically that this theory is untenable.

It should be borne in mind that no substance is inherently gaseous, liquid, or solid. These three classic states of matter depend upon the proximity to each other of the constituent atoms or molecules of the substance in question. This proximity controls the degree of their attraction, their state of aggregation or dispersion, and the extent or practical cessation of their kinetic motion. It in turn is controlled mainly by temperature, pressure, and atomic or molecular forces ("chemical" and "physical" forces), especially of solvents, which may render the state of aggregation permanent within certain limits. There is no sharp line to be drawn between liquids and solids, as may be seen in the case of a cooling gelatin solution; and although the transition from the liquid to the gaseous state seems abrupt, we have liquids of all degrees of mobility. Whether we regard a substance as solid or liquid will therefore depend upon the tests or criteria we fix for these states.

Furthermore substances liquid when in mass, need not necessarily act so when dispersed in or adsorbed by another substance. Small mercury globules act like a soft "solid" metal, and colloidal mercury acts like a suspensoid. Bridgman[28] has shown

[25] *Z. physik. Chem. 98*, 14 (1921).
[26] *J. Am. Chem. Soc. 43*, 434 (1921).
[27] E. Hatschek, *Trans. Faraday Soc. 12*, 17 (1916).
[28] P. W. Bridgman, *J. Franklin Inst.*, March, 1914.

that water under high pressures is solid even at ordinary temperatures, and there are many reasons for believing that the water adsorbed by gelatin and other colloids no longer *acts* as a mobile liquid.

In considering gel formation we cannot neglect the kinetic factor. The ultramicroscope shows that as particles grow smaller their Brownian motion increases rapidly; so that there comes a point where a small increase in temperature will change the gel into a fluid. Stirring, rubbing or other mechanical separation will suffice, as may readily be demonstrated ultramicroscopically with agar gel, from which a sliding cover glass dislodges ultramicrons that assume active motion.

**The Ultramicroscopic Evidence.**

Let us now consider the ultramicroscopic evidence in the case of gelatin solutions and jellies.

When warm, pure gelatin solutions appear practically homogeneous, but on cooling there forms a submicroscopic or amicroscopic heterogeneity, depending on concentration. The largest particles are seen in 0.5 to 1 per cent. solutions which set to weak jellies and show flocks of microns and submicrons. Below 0.1 per cent. and over 6 per cent., no ultramicrons are visible, although the polarization of the Tyndall beam proves the presence of amicrons.

W. Menz [29] followed ultramicroscopically the formation of gelatin gels. As a 0.5 per cent. gelatin solution cools, numerous submicrons appear and join to form flocks. Shortly before the submicrons appear the field becomes luminous, and the new phase appears suddenly in the form of tiny drops.[30]

Zsigmondy, under whose direction much of this work was done, states: [31] "On warming, a motion on the surface of these drops can be seen and they either become invisibly small, or the contours gradually fade and the place where the drops were is now characterized for some little time by a glimmering zone. Evi-

[29] *Z. physik. Chem. 66,* 129 (1909); P. P. von Weimarn, *Kolloid Z. 4,* 133 (1909); *6,* 277 (1910); "Grundzüge der Dispersoidchemie," Dresden, 1911; W. Bachmann, Inaug. Diss. Göttingen, 1910; and von Lepkowski, *Z. physik. Chem.* 75, 608-614 (1911).

[30] The converse of this is seen in the case of the digestion of partly coagulated egg albumen by pepsin. See J. Alexander, *J. Am. Chem. Soc. 32,* 680 (1910).

[31] "The Chemistry of Colloids," p. 226.

dence of the small diffusion in the liquid is afforded by the fact that on cooling, the drops may be obtained on the spot where they disappeared. In fact, two particles that were prevented from uniting by warming, may be so far restored that they may still unite after the cooling process has been carried out. In contradistinction to the case of gelatin, submicrons from critical systems unite to form a homogeneous phase even after the cooling. The drops are circular, large, and have no such variations in form as are so prominent in the case of gelatin particles."

The actual facts are probably by no means as simple as most of these explanations of gel formation assume. The structure of the dispersed phase of gels seems to be at least duplex, if not even more complicated, and seems to be controlled largely by an equilibrium between two opposing forces—(1) the attraction of the molecules or ultimate particles for each other, (2) the attraction of the molecules or molecular groups for the dispersion medium, which is water in the case of hydrosols. Molecular aggregation proceeds to a certain point dependent mainly on temperature, pressure and chemical nature, and the *primary particles* thus formed unite to make larger aggregates or *secondary particles*.[32] The adjustment of this equilibrium takes time, which accounts for the fact observed by F. Stoffel [33] that quickly chilled gelatin and slowly chilled gelatin exhibit different permeability to the same diffusing substance, but become equalized upon standing several days at room temperature. This slow annealing or hysteresis is evidently the consequence of a progressive aggregation.

It is only to be expected that gelatin solutions of varying concentration will yield gels of different interior structure, and therefore dry gelatins of different water absorbing capacity. Thus L. Arisz (*loc. cit.*, p. 88) found that 10 and 20 per cent. jellies swell much more than 50 and 80 per cent. jellies, and W. D. Bancroft [34] mentions unpublished experiments of Cartledge showing that 8, 16, 24 and 32 per cent. gelatin jellies, when dried to 96 per cent., each took up water at a different rate.

[32] See W. Mecklenberg, *Mitt. K. Materialprüfungsamt. 37*, 110 (1919); The Svedberg, *Proc. Faraday Soc.* (1920); *Chem. Met. Eng. 24*, 26 (1921).

[33] Inaug. Diss. Zürich, 1908.

[34] W. D. Bancroft, "Applied Colloid Chemistry," p. 250.

Gelatins made from dilute solutions are more bibulous,[35] and swell more than those made from more concentrated solutions. Probably their "secondary" particles are smaller and more numerous, which means that they possess a greater "free" surface.

**Polariscopic Evidence.**

C. R. Smith [36] has shown that at 35° gelatin in 3 g. per 100 cc. solution exhibits a specific rotatory power $(\alpha)_D$ of $-120.6°$ to $-123.5°$, or of $-141°$ figured on a moisture- and ash-free basis. When cooled at 15° or below, $(\alpha)_D$ is found to be about $-272°$, or $-313°$ on a moisture- and ash-free basis. The *ratio* between $(\alpha)_D$ at 35° and 15° is thus 2.21 to 1, and holds for the best grades of commercial gelatin whether made from bones, hides or Russian isinglass.

Smith attributes this *mutarotation* to a thermo-reversible equilibrium

$$\text{Sol form A} \rightleftarrows \text{Gel form B},$$

which to him appears to be a bimolecular reaction, disturbed to some extent by some other reaction, possibly of a monomolecular nature, which takes place at the same time. Between 35° and 15° both forms co-exist, and levorotation, signifying increasing formation of the gel form B, closely parallels increase in viscosity.

Davis and Oakes [36a] find that the transition point Sol form A $\rightleftarrows$ Gel form B lies between 38° and 38.1°, and by interpolation fix it at 38.03°.

M. H. Fischer (private communication) considers this the transition realm of water in gelatin to gelatin in water.

C. R. Smith [37] has studied polariscopically and by jelly strength tests, the effect of salts and acids on the sol $\leftrightarrows$ gel equilibrium. They change both the final state and the velocity with which it is reached. Sulphates displace the equilibrium toward the gel side between 15° and 35° C. and the maximum jelly strength is rapidly reached at low temperatures. Chlo-

[35] This may be one reason why some people prefer "thin cut" glues, another reason being ease of solution.

[36] *J. Am. Chem. Soc. 41,* 135 (1919).

[36a] Clarke E. Davis and Earle T. Oakes, *J. Am. Chem. Soc. 44,* 464 (1922).

[37] Private communication of unpublished work.

rides, bromides, and iodides lower the viscosity of gelatin and shift the equilibrium toward the sol side. Salts and even weak acids do not, full strength being reached if a low temperature (about 5° C.) is maintained for a sufficient time. Therefore equilibrium rotation with sulphates is larger than with pure water, whereas with iodides it is less.

The facts resemble those met with in the dynamic allotropy of sulphur,[38] and indicate the formation, with decreasing temperature, of larger molecular aggregates, which, however, need not be chemical compounds in the ordinary acceptation of the term. It is interesting also to note that L. Arisz [39] found that the Tyndall phenomenon in gelatin increases in intensity with falling temperature, and is also dependent on the previous history of the gelatin, which controls the size of its particles.

In the case of colloidal metals, the attraction of the metal molecules for each other is so powerful that unless an adsorbed layer of protector or ions intervenes, the aqueous films are squeezed out, and there results a more or less water-free metal sponge, bathed in practically metal-free water. With most oxides and sulphides the mutual attraction of the molecules is not so great, probably because the main attractive forces have been satisfied in the formation of the chemical compounds in question, or the attraction for water is greater; and therefore the tendency to gel formation is greater. G. Varga [40] estimated that particles of stannic acid gel 12.7 μμ in diameter contain only ⅛ their volume of massive stannic oxide, the remaining ⅞ being mainly water. The micellular complexes even of dry gelatin, contain a very large amount of water, the higher grades holding most.

**Complex Structure of Gelatin.**

With gelatin we have a mixture of large and highly polar molecules containing $NH_2$ and COOH groups, which probably form adsorption [41] aggregates that constitute the primary particles of gelatin. These primary particles have an interior structure with extremely fine capillaries, because the residual attrac-

[38] W. E. S. Turner, "Molecular Aggregation," p. 92.
[39] L. Arisz, *Kolloidchem. Beihefte* 7, 1 (1915).
[40] *Kolloidchem. Beihefte 11,* 1 (1919).
[41] The larger and more complex the reacting masses the more "physical" rather than "chemical" does the reaction appear to be.

tions of the constituent molecules are relatively weak and are unable to displace the adsorbed water films which make the colloid hydrous and hydrophile. It is furthermore probable that the "fluid" surrounding the primary particles is not pure water, but is an aqueous solution containing a larger portion of the more soluble constituents or those with smallest molecules.[42]

The primary particles form secondary groups in this "fluid," which fills the larger capillary spaces produced thereby. Increase in temperature, or the addition of certain salts (e.g. $CaCl_2$, $NaNO_3$), cause dispersion again into smaller or primary particles which is mainly reversible; but continued or high temperature or the presence of much acid and especially alkali, seems to attack the primary groups and bring about what we term hydrolysis, a splitting up of the adsorption complexes into their constituent polypeptides, and ultimately degeneration into amino-acids.[43]

S. E. Sheppard and F. A. Elliott [44] see no need of postulating a sub-microscopic but supermolecular structure in gelatin, attributing any "structure" to an *environment impress.*

Since P. W. Bridgman by mere pressure produced a new black allotropic form of phosphorus, and demonstrated that the same very high pressures produced in water molecular aggregates which persisted for days, it is reasonable to assume the existence of a sub-microscopic but supermolecular structure in gelatin, at the surfaces of which great compression is known to occur.

In a technical paper on "Colloidal Fuels" S. E. Sheppard [44a] made a suggestion as to the emulsoid colloid state, which was developed more fully in a letter to "Nature." [44b] The essential feature of the hypothesis put forward was that the micelles, or plurimolecular units of such colloid systems, are formed, and their growth and aggregation determined by "the orientation of

[42] Segregations of this kind are common, especially in soaps and alloys. See Jerome Alexander, "Colloidal State in Metals and Alloys," *Trans. Am. Inst. Min. and Met. Eng.*, Vol. 64 (1920); *Chem. Met. Eng.*, January, 1922.

[43] W. Mecklenberg attributes the differences in the nature of stannic acid gels to variations in the size of their primary particles.

[44] *J. Am. Chem. Soc. 44*, 373 (1922).

[44a] *J. Ind. Eng. Chem. 13*, 37 (1921). These remarks are taken from an advance copy of a paper entitled "The Interfacial Tension between Gelatin and Toluol," by S. E. Sheppard and S. S. Sweet, to appear in *J. Am. Chem. Soc.* My thanks are due to Dr. Sheppard for his courtesy.

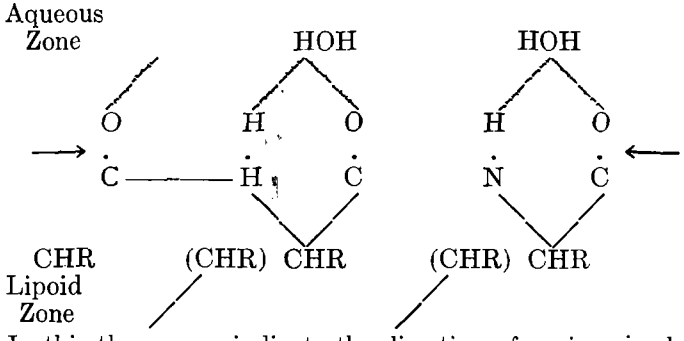
[44b] "The Nature of the Emulsoid Colloid State," *Nature*, March 17 (1921), p. 73.

definite atom groups, entirely in the sense of the theory of molecular orientation due to structure proposed for surface and interfacial tension phenomena by W. B. Hardy,[44c] W. Harkins [44d] and J. Langmuir.[44e]

"The genesis of a micelle, as plurimolecular unit of a colloid system, may be regarded as a consequence of equilibrium, usually incomplete, between homochemical solution forces and heterochemical forces, the former tending to dissociate and decompose the chemical molecule, the latter resisting decomposition. In the case of proteins the most probable general type of linkage, according to R. H. A. Plimmer [44f] is of the form

$$NH_2.CHR.CO.(NH.CHR.CO)_n.NH.CHR$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad COOH$$

when n refers to the degree of polypeptide condensation and R is an alkyl or other substituent group. On the hypothesis suggested here we may, imperfectly, represent the redistribution of this in the presence of water for the polypeptide chain by




In this the arrows indicate the direction of an imagined plane or intra-molecular interface *i* separating the hydrophile groups $\begin{matrix} C & - & N \\ \cdot & & \cdot \\ O & & H \end{matrix}$, which are consolute with water (in virtue of residual affinities tending to complete the amino and carboxyl groups), from the hydrophobe or hydrocarbon groups—CHR. Not only in one and the same protein molecule, but also to a variable extent between molecules, we may admit that this primary orientation leads to mutual attraction between water-soluble and

[44c] *Proc. Roy. Soc. 81A,* 610 (1912).
[44d] *J. Am. Chem. Soc. 39,* 354 and 541 (1917).
[44e] *J. Am. Chem. Soc. 38,* 2221 (1916).
[44f] "Chemical Constitution of the Proteins," II. p. 2.

water-insoluble groups respectively. Without any actual cleavage of the molecule, we have orientation and a stratichemical field of force which is of a similar character, in essence, to crystallization, but results in incomplete instead of complete equilibrium. The hydrocarbon or lipoid atom groups will approach the fluid on the solid state according to molecular weights and constitution; hence the system may be likened, in one aspect, to a sub-molecular emulsion, the lipoid groups tending to form interconnected sheets of atom-groups necessarily permeable to water and water solutes, although mechanically developing a stress resisting rupture in virtue of the fields of attraction and repulsion induced. The micelles are the smallest plurimolecular units thus built up.

"The following brief survey indicates the present status of the question. Somewhat destructive criticism of the foregoing hypothesis by J. W. McBain [44g] was shortly followed, in the same journal [44h] by N. K. Adam's observations on mono-molecular films of palmitic acid on water and aqueous alkali solutions. They confirmed the theory of the orientation of soap molecules in surfaces and micelles, suggested by Harkins, Davies and Clark.[44i] Further, the structure of the soap micelle proposed by Adams was quite in accord with Sheppard's suggestion that orientation determined the growth of the micelle. More recently, J. Loeb [44j] has explained the stability of protein solutions and the difference between gel formation and precipitation by reference to an orientation hypothesis of the protein molecule. Loeb's "watery" groups and "oily" groups correspond respectively to the "hydrophile" and "hydrophobe" or "hydrocarbon" groups of Sheppard's note. Finally, it may be noted that E. J. Witzemann, in an interesting paper [44k] considers that orientation at surfaces, as shown by soaps, is of less importance for proteins and polysaccharides. Generally, however, his argument supports a chemical view of the biocolloids.

"Whatever the increased consideration gained for the hypothesis by these contributions, it remains actually a working hypothesis, to be tested by definite consequences capable of experimental

[44g] *Nature, loc. cit.*, p. 74.
[44h] *Ibid.*, April 28, 1921.
[44i] *Loc. cit.*
[44j] "Proteins and the Theory of Colloid Behavior," p. 283 (1922).
[44k] *J. Phys. Chem. 26*, 201 (1922).

verification. These consequences reach in two directions,—on the one hand, the behavior of emulsoid colloids to their thermodynamic environment,[441] on the other, fundamental chemical changes (oxidation-reductions, substitution, etc.). On the first count, the surface and interfacial tensions of emulsoid colloids are of particular interest. It is known that on shaking weak solutions of gelatin, with immiscible solvents such as benzole, gasolene, toluene, etc., gelatin, still considerably hydrated, tends to be thrown out and aggregated as an interfacial layer.[44m]

"It appeared desirable to investigate this more fully, in particular as a function of hydrogen ion concentration. The properties of gelatin as an emulsifying agent for kerosene have been studied by H. N. Holmes and W. C. Child [44n] in relation to (a) the surface tension of the gel-oil interface, (b) determination of whether or not gelatin is adsorbed to form a concentration layer around the oil droplets and (c) viscosity of the solution. The present investigation, while not at variance with their results, shows that in such studies the hydrogen-ion concentration may be a determining factor. This is to be expected, but the relation of the property to $p_H$ in the present case is somewhat different from those instanced by Loeb and others. It will be remembered that in the cell protoplasm we have a complex lipoid-protein interface, so that the property in question is physiologically important, as also industrially, in relation to certain processes for preparing glue and gelatin."

Although the original polypeptides or amino-acids composing gelatin may have a limited power to crystallize, *in mixtures we are confronted with what appears to be a general tendency on the part of substances of different crystallization speed to interfere with each other's normal crystallization.* This tendency seems to be due to the fact that in crystallizing, all substances must pass into or through the colloidal zone where they are apt to be adsorbed by larger particles; it is exhibited by glasses and metals, by soaps, and by mixtures of fatty acids, and tends to keep the mixture in a state of fine aggregation.

Some substances, assuming an iso-colloidal state, are able to

[441] On the orientation theory, their electromagnetic environment.

[44m] Winkelblech, *Zeit. f. angew Chem. 13,* 1753 (1900); *cf.* also W. Bancroft, "Applied Colloid Chemistry," p. 260.

[44n] *J. Am. Chem. Soc. 42,* 2049 (1920).

interfere with their own crystallization (auto-protection).[45] Hardy found that 5-dimethylaminoanilo-3, 4-diphenylcyclo-1, 2 dione, upon cooling its solutions in organic solvents, gives gels which gradually become crystalline. (See also p. 49.) Frequently substances whose crystallization is interfered with, assume the form of tiny globulites, as is the case with lactose for example. Concentrated solutions of sucrose crystallize with difficulty and act somewhat like a "glue." The tendency toward crystallization is markedly inhibited by colloidal protectors such as glue, gum arabic, etc.[46]

From the evidence at present available, the following picture may be drawn of the formation of a gelatin gel from any ordinary warm solution of gelatin:

(1) The hot solution contains adsorption complexes of polypeptides ("gelatin molecules") which possess residual unsatisfied free fields of force, and a powerful idioattraction. These complexes with their adsorbed ions, are dispersed in a "fluid" containing, in still finer dispersion, hydrolysis products of the original gelatin complexes, and ions from the dissociation of salts (including "salts" of gelatin), acids, or alkalis; and also containing the undissociated salts, etc.

(2) As the temperature drops, and thermal agitation diminishes, the gelatin "molecules" begin to aggregate. As these aggregations increase in size, their Brownian motion diminishes, until they finally form clustered, almost motionless, masses, which, if the concentration is not too small, are on all sides within the range of each other's molecular attraction. That is, although they are actually separated by adsorbed aqueous films, they practically "link arms" to form what D. Jordan Lloyd calls a continuous solid phase.

(3) The size of these molecular aggregations and the size of their tiny pores, will vary with the nature of what is adsorbed at their interfaces; that is, will vary with the nature of the "impurities" present. Speed of chilling and tempering also exert a syneretic influence, just as they do with metals. Viscosity and jelly strength will vary with particle size, there being a zone of maximum colloidal effect.[46a]

[45] See e.g. W. B. Hardy, *Proc. Roy. Soc. London* (A), *87*, 29 (1913); J. Alexander, *J. Ind. & Eng. Chem.* (1923).

[46] J. Alexander, *J. Soc. Chem. Ind. 28*, 280 (1909).

[46a] See Jerome Alexander, *J. Am. Chem. Soc.* 434 (1921).

(4) The remaining "fluid" or dispersing phase, which surrounds the molecular complexes, will be in a state of kinetic equilibrium with the molecular complexes, so far as concerns particles or ions diffusible into the pores of the latter. This dispersing phase will contain most of the water, the highly soluble products, and those ions which are too large to enter the pores of the "solid" phase or which are unadsorbed.[47]

(5) The adsorption of ions (especially hydrogen or hydroxyl ions) tends up to a certain point to separate the gelatin "molecules" constituting the molecular groups[48] and thus enables the groups to take up more water. Beyond this point, "salting-out," sol formation and hydrolysis predominate.[48a] The same effect may also be accounted for on the basis of the Donnan theory.[48b]

In a heterogeneous mixture of complex groups such as are found in gelatin solutions or jellies, it is very unlikely that there is any definite arrangement of molecules into threads, chains, or strings. Since molecular groups adjoin each other in every direction, it is only natural that microscopic or even ultramicroscopic examination reveals what seem to be spheres, which, according to the focus of the instrument, may appear to form elongated groupings. Those familiar with the limitations of optical instruments will understand that these apparitions are only diffraction images of irresolvable particles. In fact, Scherrer has shown with the X ray spectrometer that gelatin is truly amorphous.[49]

Comparing gel structure to a "pile of shot," [50] "anastomosing threads," [51] "thread-like crystals," [52] "streptococcal threads," [53] hardly gives one a correct picture; for while the imagination may isolate such groupings from the mixture of molecular groups, they have no real existence. It is true that the polar nature of the molecules may tend to produce some kind of orientation, and that some chain-like structures may be formed; but in general the tendency does not establish itself, and the incidental

[47] Some (e.g. H. R. Procter, J. A. Wilson and J. Loeb) believe that gelatin forms definite salts and that a Donnan membrane equilibrium exists.

[48] Tolman and Stearns, *J. Am. Chem. Soc. 40,* 264 (1918).

[48a] A. Kuhn, *Kolloidchem. Beihefte 14* (1921).

[48b] J. Loeb, "Proteins and the Theory of Colloid Behavior."

[49] P. Scherrer, *Nach. Ges. Wiss. Göttingen,* 1918.

[50] Bradford, *Biochem. J. 12,* 382 (1918).

[51] T. B. Robertson, "The Physical Chemistry of the Proteins," 1918, p. 302.

[52] W. Moeller, *Kolloid Z. 23,* 11 (1918).

[53] R. H. Bogue, *Chem. Met. Eng. 23,* 61 (1920).

formation of chains or threads is not an *essential* of gel formation. Jelly formation occurs even in emulsions where there is no evidence of chain structure. In the welter of conflicting attractive forces and closely packed molecules, the weak residual attractive forces remain unsatisfied, so that there may be a state of stress which is a cause of elasticity of the jelly. With very dilute solutions of gelatin, however, polar grouping in chains probably takes place to a considerable extent, as ultramicrographs of such solutions would indicate.[54]

D. Jordan Lloyd[54a] has followed the action of hydrochloric acid, sodium hydroxide and sodium chloride on the gelling power of gelatin purified by dialysis at the isoelectric point. Taking the minimum quantity of gelatin required to produce a gel after standing at 15° for 48 hours, she found the minimum concentration of pure gelatin to be 0.8 per cent. Hydrochloric acid lessens the gelling power, showing maximum reduction at $p_H$ 2-3, and again at higher acidity than $p_H$ 0.7. Sodium hydroxide causes slight decrease between $p_H$ 10-12, and above this prevents gelatinization. Though no simple relation between sodium chloride content and gel power was evident, neutral salts oppose the action of H ions.

The fact that gelatin may be altered by "molecular bombardment" was shown by E. Mühlenstein,[55] who exposed a gelatin layer 46 μ thick to X rays from polonium, and found, after soaking in water and drying, a permanent depression of about 22 μ in the exposed portion of the gelatin. As the depression is invisible prior to the soaking, it is not purely mechanical, but is evidently due to some change in molecular structure.

A. Tian[56] found that wave lengths of quartz-mercury ultraviolet light of 3,000 Å which coagulate albumin, did not affect dry gelatin and only fluidified the jelly.

Gelatin jellies, upon being strained, show double refraction, an evidence of anisotropic structure.

[54] J. S. van der Lingen, *J. Franklin Inst. 191,* 651 (1921), finds that the pseudoisotropic layers in such anisotropic liquids as *p*-azoxyanisole, *p*-azoxyphenetol, anisaldazine, and ammonium *p*-cyanobenzalaminocinnamate (Stumpf's ester), do not possess a space-lattice, and show no evidence of being microcrystalline.

[54a] *Biochem. J. 16,* 530-45 (1922).

[55] *Arch. sci. phys. nat. 2,* 423 (1920).

[56] *Compt. rend. 151,* 219 (1910).

P. W. Bridgman[57] subjected gelatin jelly to a pressure of 9,000 kilos per sq. cm., and found no visible change except that the gelatin was cracked into rather large lumps, doubtless because at this pressure water freezes into one of the four varieties of pressure-ice, making the gelatin jelly so rigid that it could not accommodate itself to the shape of the containing vessel.

The addition of alcohol to gelatin solutions dehydrates the micellular groups, converting the "emulsoid" gelatin into a "suspensoid" opalescent solution which shows ultramicrons.[58] W. O. Fenn[59] has studied the effect of electrolytes upon this change.

[57] Private communication.

[58] See e.g. O. Scarpa, *Kolloid Z. 15*, 8 (1914).

[59] *Proc. Nat. Acad. Sci. 2*, 534 (1916); *J. Biol. Chem. 22*, 279, *34*, 141 and 415 (1918).

# Chapter 6.

## The Influence of Various Factors on the Swelling of Gelatin.

Many factors influence the amount of water absorbed by dry gelatin, and also the speed with which the water is taken up. Among these are: *The ratio of the free surface area to the volume;* [1] *the hydrogen ion concentration;* [2] *the temperature of the system;* [3] *the elastic modulus of the gelatin;* [4] *the ratio of the mass of the gelatin in the system to the mass of dissolved electrolyte;* [5] *the previous history of the gelatin,* upon which depends its internal structure; [6] and the effect of unclassified substances like urea, pyridine and the amines.

When gelatin swells in water the volume of the swollen gelatin is less than the combined volumes of the original gelatin and the absorbed water.[7] Heat is developed by the absorption of water,[8] indicating that there is a compression or condensation of the water coincident with its entrance within the capillary and molecular spaces of the gelatin. This "heat of swelling" is analogous to the heat developed when moisture is absorbed by superdried peas, starch or dextrin.

H. G. Bennett [9] attributes the heat liberated by swelling gelatin

[1] F. Hofmeister, *Arch. exp. Path. Pharm. 27,* 395 (1890); Wo. Pauli, *Pflüger's Arch. 67,* 219 (1897); Spiro, "van Bemmelen Festschrift," 1910, p. 261; M. H. Fischer, "Das Oedem," Dresden, 1910.

[2] Chiari, *Biochem. Z. 38,* 167 (1911); H. R. Procter, *J. Chem. Soc. 105,* 313 (1914); J. Loeb, *J. Gen. Physiol. 1,* 41 (1918).

[3] Procter and Burton, *J. Soc. Chem. Ind. 35,* 404.

[4] Procter and Wilson, *J. Chem. Soc. 109,* 307 (1916).

[5] D. Jordan Lloyd, *Biochem. J. 14,* 149 (1920). This seems to be an illustration of the Donnan equilibrium. J. A.

[6] W. R. Hardy, *Proc. Roy. Soc. 66,* 95 (1900); Arisz, *Kolloidchem. Beihefte 7,* 1 (1915); see also W. D. Bancroft, "Applied Colloid Chemistry," p. 251, 1921.

[7] G. Quincke, *Arch. f. d. ges. Physiol. 3,* 332 (1870).

[8] E. Wiedemann and C. Lüdeking, *Wied. Ann. 25,* 145 (1885). See also E. Hatschek, "An Introduction to the Physics and Chemistry of Colloids," London, 1913, p. 55.

[9] H. G. Bennett, "Animal Proteins," p. 204, 1921.

(5.7 calories per gram of gelatin) to the compression of the absorbed water, and from the LeChatelier theorem predicts what is actually found—that gelatin swells best in *cold* water. "The fact of water compression determines the rigidity of the gel, and the changes in this compression of the continuous phase [10] determines the surface tension resultant which hinders swelling, and which is one of the two main factors [11] fixing both the rate at which gelatin swells in water, and the final volume attained by the gel" (p. 209).

The stiffening, shrinking and "salting out" action of sulphates, tartrates, etc., Bennett considers to be examples of "lyotrope" compression, while the contrary effect is exhibited by iodides, thiocyanates and urea, which may entirely inhibit gelatinization. In this latter respect Bennett's "lyotrope series" is marred, for calcium, magnesium and zinc chlorides, as well as sodium and calcium nitrates also inhibit gelatinization.

Increase in temperature causes an increased heat of swelling, which leads Wilson [12] to the conclusion that the heat is due, not to swelling, but to chemical combination between the gelatin and a small portion of the absorbed water. The more likely explanation is that in warmer water gelatin swells more rapidly, thus producing more heat per unit of time. For as Hofmeister [13] observed, the swelling of gelatin plates has a higher initial velocity, after which it proceeds more slowly to a maximum. Furthermore at higher temperatures the gel particles probably undergo a further dispersion resulting in more free surface.

Thus Arisz [14] observed the following differences in water absorption by gelatin with variation in temperature, the figures given representing the weight of one gram of swollen gelatin at the time indicated.

[10] Bennett here means the water is the continuous phase. But in a jelly it is probable that the dispersed phase is also "continuous." Bancroft gives as an illustration of such condition a roll of wire fencing standing in the air—the wire is continuous but so also is the air. Strictly speaking, all matter consists of discrete particles. J. A.

[11] The other factor, according to Bennett, is the "lyotrope" (Hofmeister series) effect of salts, etc., upon the compressibility of water. J. A.

[12] J. A. Wilson, 3d Report on Colloid Chemistry, British Association A. S. (1920), p. 51.

[13] F. Hofmeister, *Arch. f. Exper. Path. und Pharm.* 27, 395 (1890); 28, 210 (1891).

[14] L. Arisz, *Kolloidchem. Beihefte* 7, 49 (1915).

| *Temperature* | *1st day* | *2nd day* | *3rd day* |
|---|---|---|---|
| 2° | 10— | 10— | 10— |
| 12° | 10 | 10 | 10 |
| 20° | 16 | 18 | 19 |
| 25° | 33 | 40 | 46 |
| 30° | 35 within the first few hours followed by solution. | | |

The figures tabulated are approximate, as Arisz' results are given in curves which show that most of the water is taken up within a few hours.

Hofmeister and Wo. Ostwald have also pointed out the influence of the shape of the piece of gelatin on the degree of swelling. Thin sheets not only swell *more rapidly*, but they also swell *more* than thick sheets.[15] The amount of water absorbed by a piece of gelatin depends very materially upon the *previous history* of the gelatin which influences its internal structure. (See p. 83, Chapter 5). Procter dried out (presumably at low temperatures) three jellies containing respectively 5, 10 and 20 per cent. of gelatin. Upon soaking these in cold water for seven days he found that they absorbed respectively 14.6, 7.7, and 5.8 times their weight of water.

Arisz [16] gives curves showing the influence of the *age* of a block of gelatin jelly upon its capacity to absorb water. Freshly prepared jellies absorb most water, which is an indication of the progressive aggregation of the gelatin particles in aging jellies (syneresis), with a concomitant diminution of free surface. Curiously enough, Arisz found, contrary to expectation, that a block of gelatin jelly swollen at 10° *loses* water when warmed to 20°; and a jelly first swollen at 20° actually swells faster when the temperature is reduced to 10°. These variations in water absorption are influenced by the previous gel history. Apparently two opposing factors are at work: 1st, heat tends to produce finer dispersion with greater water absorption; but, 2nd, the heat seems to relax the attraction of the gelatin for the water, probably because of increased kinetic activity.[17]

[15] Therefore a ground glue or gelatin would appear to absorb more water than the same product in flake form. Incidently this indicates a source of error in grading on the basis of "water-absorption," and a possible reason why some users who test on this basis, prefer thin cut flakes of glue or gelatin.

[16] *Loc. cit.*, p. 57.

[17] For further details and experiments on the *intermittent swelling and drying* of gelatin jellies, the reader is referred to the original paper of Arisz. See also A. G. Brotman, *J. Soc. Leather Trades Chem. 5*, 226 (1921).

In general gelatin swells more in the solution of any acid or any alkali than it does in pure water.[18] With very minute quantities of acid there is a slight diminution of swelling, the minimum with HCl being at a concentration of $\frac{n}{210.1}$, after which the curve rapidly rises, so that at $\frac{n}{90.9}$ there is already a swelling beyond that which occurs in pure water. Wo. Ostwald's results show that both with HCl and KOH the swelling reaches a maximum about $\frac{n}{38.6}$, after which it slowly diminishes.

According to Fischer (*loc. cit.*, p. 29), if two like gelatin discs are simultaneously immersed, the one in pure water and the other in $\frac{n}{20}$ HCl, the superior degree of swelling of the latter is plainly visible at the end of six hours, and is still more marked after a day or two. Then the disc in the pure water is still somewhat yellow and cloudy, whereas the gelatin in the dilute acid is swollen so clear and hyaline, that it can hardly be seen at the bottom of the dish.

**Lyotrope or Hofmeister Series.**

Wo. Ostwald inclined to the belief that the swelling was exclusively a function of the H-ion concentration of the acid solution, whereas Fischer believes that it is determined by the concentration of the H-ions *minus* the effect of the particular anion concerned. M. H. Fischer's experiments show that the order of acids in increasing the swelling of gelatin is as follows:

$$HCl > HNO_3 > CH_3COOH > H_2SO_4 > H_3BO_3$$

"The position of the 'weak' acetic acid between the 'strong' nitric and sulphuric acids (which two are about equally dissociated, and yield a higher concentration of hydrogen ions than the equinormal acetic acid) is by itself an argument against the explanation which considers *only* the concentration of hydrogen ions." [19]

[18] K. Spiro, *Beiträge zur chem. Physiol. 5*, 276 (1904); Wolfgang Ostwald, *Pflüger's Archiv. 180*, 563 (1905); M. H. Fischer, "Edema," New York, 1910.

[19] Fischer, *loc. cit.*, p. 31. These series of anions and cations are known as the *lyotrope* (or solution changing) series. They are also termed the *Hofmeister series* in honor of their discoverer.

Gelatin is so sensitive to the presence of acid, that highly purified gelatin will swell less in conductivity water than in ordinary distilled water which contains $CO_2$.[20]

With alkalis there is no initial decrease in swelling. Their order in increasing swelling is as follows:

$$KOH > NaOH > Ca(OH)_2 > NH_4OH.$$

Since at the concentrations employed, the dissociation of the first three of these alkalis is about the same, Fischer concludes that the swelling of gelatin in various alkalis is dependent upon the OH-ion concentration *minus* the effect of the cation. Thus calcium is more active in inhibiting swelling than is sodium, while potassium permits the greatest swelling.[21]

It is well known biologically that bivalent ions counteract the injurious effect of monovalent ions, which often act as poisons. The antitoxic action of polyvalent ions has been demonstrated by Jacques Loeb on the fertilized eggs of *fundulus heroclitus,* a small fish, by R. S. Lillie on the larval forms of *arenicola,* a sea annelid, and by Wo. Ostwald on *gammarus pulex,* the sand flea. Many animals which live in sea-water are killed by sodium chloride solutions isotonic with sea-water. Ostwald has also shown that the swelling of gelatin is much more powerfully depressed by polyvalent ions than by monovalent ions (Mg<Ca<Ba<Sr<Cu<Fe), and M. H. Fischer has had like results with fibrin. It therefore seems that the "impurities" of sea-water keep the biocolloids at a certain optimum degree of swelling or turgidity.

R. S. Bracewell[22] believes that the amount of acid adsorbed by proteins is determined mainly by their content of the two diamino acids, lysine and arginine; and his experimental data show that the acid adsorbed per gram of protein (gelatin, fibrin, casein, gliadin, edestin) is roughly proportional to the number of free $NH_2$ groups per gram of protein.

Tolman and Stearn[23] attribute the swelling of gelatin, fibrin

[20] T. Oryng, *Kolloid. Z. 17,* 14 (1915). Conductivity water has $p_H = 7$, while ordinary distilled water has $p_H = 5.5$ because of dissolved $CO_2$. Wo. Pauli first pointed this out about 1905.

[21] This difference may be of importance in the functioning of muscle, especially heart-muscle. C. R. Smith (*J. Am. Chem. Soc. 43,* 1360 [1921]) gives figures on the swelling of ash-free gelatin in various alkalis.

[22] *J. Am. Chem. Soc. 41,* 1511 (1919).

[23] R. C. Tolman and A. E. Stern, *J. Am. Chem. Soc. 40,* 264 (1918).

and similar colloids in acids and alkalis to the selective adsorption of H or OH ions respectively at the surface of pores or pockets within the gel. Owing to electrostatic repulsion these pores increase in size, the increase being accompanied by imbibition of the solution. When a neutral salt is added to the solution, its ions arrange themselves in such a way as to neutralize the original electrostatic repulsion, thus producing shrinking. The fact that salts with polyvalent ions are most effective in producing dehydration is consequent upon the superior power of the latter to neutralize the existing electric field, although they take up no more room than monovalent ions.

Gelatin swells somewhat *less* in a solution of dilute alkali than it does in an equinormal solution of acid. Fischer believes that this is due to the depressing action on swelling of a salt formed by the alkali in the gelatin which is generally acid in reaction. The results of C. R. Smith support this view. He believes that they drive back the ionization of the acid.[24] Let us then consider the effect of salts on the swelling of gelatin.

Neutral salts possess the power of inhibiting, to variable extent, the degree of swelling of gelatin in acids or alkalis. In reducing swelling the initial small percentages of salts have a relatively more potent influence.

The effect of salts in depressing swelling seems to be for the most part the result of the combined action of its anions and cations. The following series shows the relative effectiveness in producing a depression of swelling in acids or alkalis:

*For cations*

$$Fe(ic) > Cu(ic) > Sr > Ba > Ca > Mg > NH_4 > Na > K.^{24a}$$

*For anions*

$$\text{citrate} > \text{tartrate} > \text{phosphate} > SO_4 > \text{acetate} > I > CNS > NO_3 > Br > Cl.$$

Thus according to Bechhold [25] 0.78 gram gelatin in 100 cc. of 0.05 *n* HCl swelled until it weighed 14.61 grams. In the presence of $\frac{m}{2}$ potassium citrate it weighed only 2.84 grams, and in

[24] *J. Am. Chem. Soc. 43*, 1350 (1921).

[24a] M. H. Fischer (private communication) believes that the position of $NH_4$ is uncertain, and that it probably is the last member of the series.

[25] H. Bechhold, "Colloids in Biology and Medicine" (J. G. M. Bullowa's translation), 1920.

the presence of $\frac{m}{2}$ KCl it weighed about 7 grams. Bancroft [26] points out that a large part of the decrease in swelling may be due to the diminished H-ion concentration of the solution, and not exclusively to the citrate ion.

In determining the H-ion concentration of gelatin colorimetrically, use may be made of the well-known series of indicators described by Clark, Lubs and Acree.[27] The limitations of such indicators must be borne in mind.

## Donnan's Theory.

H. R. Procter [28] gives the following abstract of the theory of the swelling of gelatin in acids, evolved by himself and his pupils, chief among whom is J. A. Wilson: [29]

"In equilibrium between a jelly and its external solution not only must all osmotic pressures be equally balanced, but as has been shown by Donnan,[30] the electro-chemical condition must be fulfilled that the *products* of any pair of diffusible anions and cations common to both phases, must be equal. Thus with gelatin chloride and free acid the chloridions multiplied by the hydrions must be equal in the jelly and the external acid.[31] On the other hand, the osmotic pressures depend not on the *products* but simply on the *sum* of diffusible particles present. In the external acid the numbers of hydrions and chloridions are obviously equal, while in the jelly the chloridion of the gelatin chloride is added to the equal hydrion and chloridion concentration of the free acid present, thus making the final concentrations of these ions in the jelly unequal.

"Now, as the sum of two unequal factors is always greater than that of two equals giving the same product, or, geometrically the perimeter of a square is always less than that of any other

[26] W. D. Bancroft, "Applied Colloid Chemistry," p. 254.

[27] *J. Am. Chem. Soc. 41,* 1190 (1919).

[28] First Report on Colloid Chemistry, Brit. Assoc. Adv. Sci. (1917), p. 8.

[29] H. R. Procter and J. A. Wilson, *J. Chem. Soc. 109,* 305 (1916). M. H. Fischer (private communication) points out that rubber swells in benzol, nitrocellulose in ether-alcohol, and soaps in many organic solvents, although in such cases the existence of a Donnan equilibrium is precluded, for there is no dissociation. J. A.

[30] *Z. Elektrochem. 17,* 572 (1917); Donnan and Harris, *Trans. Chem. Soc. 99,* 1575 (1911).

[31] This is known as a "Donnan equilibrium." J. A.

rectangle of equal area, and as the sides represent the osmotic pressure, while the area represents the product, it is clear that the two inequalities cannot at once be completely fulfilled, but in electro-chemical equilibrium the osmotic pressure must be in excess and the jelly must tend to swell unlimitedly and finally to dissolve. That it does not do so is a consequence of its colloid nature, which depends upon cohesive attractions drawing the colloid particles together to polymerized masses or to a continuous network, and which consequently opposes swelling and solution, while the diffusible ions are held to the colloid ions by electro-chemical attractions, and, as they cannot escape from the jelly, tend to drag it apart and dilute it by absorption of the external acid, from which they expel a part of its acid concentration.[32]

"The equilibrium is therefore a very complex one, but finally depends on the excess of internal osmotic pressure being balanced against the internal attraction or cohesion of the colloid particles, both ions and molecules. For mathematical discussion the reader must be referred to original papers by Procter and his pupils. It will, however, be obvious that as the external solution becomes more concentrated, the proportion of absorbed acid (or salt) is increased, while that of gelatin chloride is limited to the quantity of gelatin present. The difference of concentration of hydrion and chloridion in the jelly is therefore diminished, and it contracts under the influence of its own internal attractions.

"Precisely similar considerations apply to the action of alkalis on gelatin. Ionizable salts are formed by combination of the base with the carboxyl group of the proteid, and the osmotic equilibrium is with the cation and OH instead of with the anion and H. Neutral gelatin, as an amphoteric body, of course ionizes to a limited extent with water alone, and its dissociation constants are of the same order of quantity as those of the water with which it is in equilibrium. It is, however, slightly stronger as a base than as an acid, and consequently its neutral point of minimum swelling is slightly on the alkaline side. This

[32] The distinction between this view and that of Tolman and Stearn, *J. Am. Chem. Soc. 40,* 264 (1918), is rather finer than claimed by J. A. and W. H. Wilson, *J. Am. Chem. Soc. 40,* 886 (1918). J. A.

has important bearings on manufacturing practice,[33] the greatest flaccidity of the raw skin, which is required for the softest leather, being obtained in weakly alkaline liquids.

"It has been pointed out by Donnan [34] that in consequence of the unequal distribution of positive and negative diffusible ions which has just been described, the surface of an acid or alkaline jelly in equilibrium has necessarily an electrical charge or potential, greatest at the maximum swelling, and such charges seem an essential of the colloid state.[34a] The surface is positive or negative according to whether the diffusible anion or cation is retained in the colloid. Thus gelatin and hide fiber are negative in alkaline and positive in acid solutions, and it will be shown later that this has an important bearing on the theory of leather manufacture.

"Wilson [35] has extended these facts to a general theory of colloids and adsorption, showing that all surfaces must possess a potential due to unbalanced chemical forces on the surface; and therefore in a liquid containing electrolytes, must condense ions or particles of the one sign on its surface, and repel those of the opposite sign; and also showing that surfaces must therefore be surrounded with a film of liquid of different concentration to the bulk, to which the same considerations and equations are applicable as to the adsorbed solution of colloid jellies."

Wilson and Kern [35a] report that "gelatin, like collagen, shows two points of minimum swelling with change of hydrogen-ion concentration, one at $p_H$ 4.7 and the other at 7.7." They suggest "that the two points of minimum represent the iso-electric points of the gel and sol forms of gelatin, respectively." Since Wilson and Kern describe the gelatin they used simply as "high-grade gelatin" without mentioning anything about the percentage or composition of the ash, their experiments must be repeated with gelatin of known purity before their results or deductions can be accepted. Their double minimum may be due to the presence of some adsorbed impurity such as alumina; for alum is very commonly used in preparing the stock or in clarifying the

[33] Procter here refers to leather manufacture. J. A.
[34] *Z. Elektrochem. 17,* 579 (1911).
[34a] See note 29 above. J. A.
[35] J. A. Wilson, *J. Am. Chem. Soc. 38,* 1982 (1916).
[35a] J. A. Wilson and E. J. Kern, *J. Am. Chem. Soc. 44,* 2633 (1922).

liquors, and as S. E. Sheppard has shown[35b] the addition of alumina to an ash-free gelatin superimposed upon a maximum of elasticity at about $p_H$ 8. to 9, a second maximum at about $p_H$ 4.

Jacques Loeb[36] has also discussed the Donnan equilibrium in its relation to membrane potentials and osmotic pressure, and found that Procter's formula is the correct expression for the Donnan membrane equilibrium, which he thinks determines swelling and viscosity as well as osmotic pressure and electric charge.[37]

**Thermal Expansion of Gelatin.**

Alan Taffel[38] has shown that gelatin gels expand regularly with increasing temperature. The expansion curves resemble that of water, but are flatter in proportion to the concentration of the gel, but show no sudden inflection as does that of glass below its softening point. The expansion coefficients, as well as the specific volumes for any one temperature, are linear functions of the concentration of the gel. Variation in H-ion concentration does not affect the expansion coefficient.

Irrespective of dilution, one gram of gelatin always exhibits the same contraction at any one temperature. This contraction is 0.073 cc. per gram of gelatin at 15°, and 0.065 cc. at 32°; which indicates that only a fraction of the gel water contracts, the weight percentage being the same for gels up to 25 per cent. Gel contraction is not due to filling up of pores in solid gelatin by water. The curve expressing the relation between concentration and the calculated distance between particles, is an hyperbola, whereas the concentration setting-point curve observed by Sheppard and Sweet[39] shows a double flexure, the rapid rise at 70 per cent. concentration being attributed to the fact that their very large molecular forces begin to come into play.

Gelatin lowers the temperature of maximum density of water

[35b] S. E. Sheppard, S. S. Sweet, and Anber J. Benedict, *J. Am. Chem. Soc. 44,* 1857 (1922).

[36] *J. Gen. Physiol. 3,* 667 and 691 (1921).

[37] Loeb's views are fully set forth in his book, "Proteins and the Theory of Colloidal Behavior," New York, 1922.

[38] *J. Am. Chem. Soc. 121,* 1971-84 (1922).

[39] *J. Ind. Eng. Chem. 13,* 413 (1921).

by an amount directly proportional to its concentration expressed in grams of gelatin per 100 grams of water. This lowering is shown to be due to the ordinary volume changes of dry gelatin with changing temperature, and the variations in contraction on imbibition of gels at various temperatures.

# Chapter 7.

## The Viscosity of Glue and Gelatin Solutions.

The importance of viscosity measurements as a means of following changes occurring in colloidal solutions, has long been recognized. Thus Thomas Graham in his classic paper entitled "On the Properties of Silicic Acid and Other Analogous Colloidal Substances" [1] says:

"The ultimate pectization of silicic acid is preceded by a gradual thickening in the liquid itself. The flow of liquid colloids through a capillary tube is always slow compared with the flow of crystalloid solutions, so that a liquid-transpiration-tube may be employed as a colloidoscope. With a colloidal liquid alterable in viscosity, such as silicic acid, the increased resistance to passage through the colloidoscope is obvious from day to day. Just before gelatinizing, silicic acid flows like an oil."

The instruments used for measuring viscosity must depend upon the degree of accuracy desired and the time and quantity of the solution available. For most scientific investigations the Ostwald viscosimeter [2] or that of Couette,[3, 4] have been used. The hour glass viscosimeter of H. A. Determan [5] is very useful where only small quantities of fluid are available. The well-known Engler viscosimeter is also used, and any simple graduated pipette will serve.[6] All these depend upon the time required for the fluid to flow through a tube.

The MacMichael viscosimeter [7] operates on the principle of measuring by the angular torque of a standardized wire, the force required to cause two surfaces, one cm. apart, to move

[1] *Proc. Roy. Soc. London,* June 16, 1864; also *Pogg. Ann. 123,* 529 (1864).

[2] See Ostwald-Luther-Drucker, "Handbuch für physik. chem. Messungen," Vol. 3, p. 230, Leipzig, 1910.

[3, 4] E. Couette. *Ann. de Chim., Ser.* 6, *8,* 685. A modified form is described by E. Hatschek, *Kolloid Z. 12,* 238 (1913).

[5] H. Bechhold, "Colloids in Biology and Medicine," p. 113.

[6] See J. Alexander, *J. Soc. Chem. Ind. 25,* 158 (1906).

[7] *J. Ind. Eng. Chem. 7,* 961 (1915).

past each other at the rate of one cm. per second, at the same time overcoming the internal friction of the liquid under test, against itself throughout the intervening space. Part of the liquid moves with each surface, and the intervening layers shear past each other.[8]

It would unduly extend the limits of this book to enter into a general theoretical and mathematical discussion of viscosity, for as Hatschek observes,[9] in the present state of theory, all that can be deduced from viscosity measurements is that some change has taken place, the nature of which is either a matter for speculation or for empirical interpretation.

Davis and Oakes [9a] report that "the viscosities of gelatin solutions of various concentrations at 40° conform to Arrhenius' viscosity formula.

A note of warning must be sounded, however, against the automatic acceptance of any formula expressing viscosity, without considering *all* the factors influencing the case in question. Mathematics is essential in solving problems, but we should remember that it is only a tool to work with. Granted certain postulates, it proceeds infallibly to direct or collateral conclusions. The danger in applying mathematics to chemical and physical problems is that, blinded by its logical perfection, we may accept erroneous postulates or neglect influential factors. Frequently, in Nature, unsuspected factors are discovered which compel us to revise our previous conclusions—for example, the recognition of the vitamines has rendered necessary a careful reconsideration of former experiments upon nutrition and a revision of the conclusions based thereon.[10]

Prof. Eugene C. Bingham [10a] points out the fact that viscosity as commonly reported, consists of several factors:

[8] The manufacturers of the instrument, Eimer and Amend, New York, issue a descriptive circular in which an accuracy of within 5 per cent. is claimed, which suffices for commercial work.

[9] First Report of the British Assoc. for the Adv. of Science, on "Colloid Chemistry and its Industrial Applications." 1917, p. 2. A bibliography is appended.

[9a] *J. Am. Chem. Soc. 44,* 464 (1922).

[10] For a critical discussion of the viscosity formulæ of A. Einstein, *Ann. der Phys. 19,* 289 (1906), and E. Hatschek, *Kolloid. Z. 7,* 301 (1910), see M. von Smoluchowski, *Kolloid. Z. 18,* 190 (1916), who points out some of the assumptions, omissions, or errors which limit the application of these formulæ.

[10a] Private communication. For full details, see his book, "Fluidity and Plasticity," p. 215, et seq.

"In measuring the deformation of any viscous material we assume that the deformation $\sigma$ of one plane is directly proportional to the shearing stress $F$ hence $v = \varphi Fr$ where $\varphi$ is the fluidity and $r$ is the distance from another plane supposed to be at rest. If r is unity the fluidity is evidently measured by the slope $\frac{\sigma}{F} = \varphi$. But there are very numerous substances which do not follow this simple law. If the deformation of the material is measured at different shearing stresses, it is found that the points fall on a curve which does not pass through the origin.

"The intercept is known as the yield value f, and it may be roughly defined as the shearing stress required to cause continuous deformation while the slope of the curve defines the mobility $\mu$ according to the equation $\sigma = \mu(F - f)r$. Since the fluidity as ordinarily measured is dependent on the magnitude of the shearing stress, the fluidity is merely an *apparent* fluidity and conclusions drawn from such apparent fluidities may be quite illusory. On the other hand the yield value and mobility are two properties which are independent of the shearing stress or the dimensions of the instrument used. Since these properties also vary over an extremely wide range, they are particularly well suited for the identification of colloids.

"In gelatin it appears that as the temperature is raised, the yield value decreases in a linear manner and becomes zero at a definite temperature indicating that the colloid passed into a true liquid. As the concentration increases the yield value increases very rapidly and the mobility decreases."

The striking difference between solutions of crystalloids like salt and colloids like gelatin, is that whereas wide variations in the concentration of salt in solution affect the viscosity but slightly, the viscosity of gelatin solutions rises sharply with increased concentration of gelatin. The effect of temperature changes is still more striking, for with, say, a 10 per cent. solution of gelatin the difference of a few degrees will change a fluid into a solid.[11]

Viscosity is consequent upon the *internal friction* of the substance in question; in fact, this latter expression is frequently

[11] For a general discussion of the viscosity of colloids, including gelatin, see e.g. Wo. Ostwald, "Handbook of Colloid Chemistry"; T. B. Robertson, "The Physical Chemistry of the Proteins," Ch. 13; and W. D. Bancroft, "Applied Colloid Chemistry," p. 190.

used as being synonymous with viscosity. It is immediately evident, therefore, that viscosity is intimately dependent upon the number, size, electric charge, and degree of hydration and aggregation of the particles of the dispersed substance. Many (e.g. Ostwald and T. B. Robertson) incline to the belief that protein solutions have a net-like structure which is responsible for the high viscosity they exhibit.

This view is in accord with recent experiments of the British Adhesives Research Committee,[11a] who tested the effect of various salts on the jelly strength, surface tension, tensile strength and viscosity of glues, and found that a salt which produces great dispersion will tend to form a glue of lower viscosity than one of small dispersive capacity. Their acceptance of the statement that gelatin itself is not a good adhesive, is, of course, unwarranted.

One factor not generally stressed is the kinetic factor. With increasing aggregation, the Brownian motion of dispersed particles diminishes rapidly, so that although there may be no actual permanent structure in a gelatin solution, there are at any instant a certain number of slowly moving or practically motionless groups, whose number and size increase as gelatinization is approached. These large groups greatly increase the internal friction or viscosity, and anything which fosters their formation will have a like result. With crystalloids the rapid kinetic motion of the dispersed particles tends to prevent them from exercising a material effect on viscosity.

It is interesting to note that there seems to be a zone of maximum colloidality or viscosity,[12] which corresponds roughly with the so-called colloidal zone of dispersion (about 5 μμ to 100 μμ). Thus the experiments of M. H. Fischer [13] show that with *increasing* molecular size soaps make more viscous solutions; while on the other hand when cream is homogenized, the *decrease* in the size of the fat globules is also accompanied by higher viscosity. The experimental facts reviewed by Wo. Ostwald [14] indicate the existence of such a zone in the case of gelatin, on either side of which its viscosity diminishes.

[11a] First Report, p. 24, London, 1922.

[12] See J. Alexander, *J. Am. Chem. Soc.* *43*, 434 (1921).

[13] "Soaps and Proteins"; *Chem. Eng.* *27*, 155 (1919).

[14] "Handbook of Colloid Chemistry," 2d ed., p. 158 et seq. See also Bogue, "Gelatin and Glue," p. 191 et seq., and p. 217.

R. H. Bogue[15] has investigated the relation between the viscosity and concentration of gelatin sols, and finds no constant ratio between the two, but finds that variations in the hydrogen ion concentration cause wide variations in viscosity and in the volume occupied by a unit weight of gelatin. Isoelectric gelatin (H-ion concentration $= 2 \times 10^{-5}$) had the lowest viscosity and the lowest degree of solvation; gelatin chloride (H-ion concentration $= 3.1 \times 10^{-4}$) had the highest, and calcium gelatinate (H-ion concentration $= 2.5 \times 10^{-6}$) is intermediate. Solvation and viscosity appear to be parallel functions, according to Bogue.

Whether the purely "chemical" or the physical explanation be assumed, these facts indicate the potent influence on viscosity, of changes in the size of the particles constituting the dispersed gelatin. Bogue assumes an equilibrium: surface tension (of dispersion medium) $\rightleftarrows$ solvation potential (of dispersed phase), with a possible reversal of phases at higher concentrations. The degree of aggregation of the primary particles seems to be an important factor, there being a balance between the attraction of the solvent for the particles and the attraction of the particles for each other which makes the secondary groups more or less hydrous according to circumstances.

Bogue[15a] after a critical review of several theories of gel structure, including Loeb's occlusion theory,[15b] concludes that many contemporary investigations have been found to support a catenary or fibrillar hypothesis which he epitomizes as follows: "The sol consists of slightly hydrated or swollen molecules united into short chains. When the temperature falls the threads increase in length and number, and their power of water absorption increases, resulting in an increase in viscosity. A solid jelly results when the relative volume occupied by the swollen molecular threads has become so great that freedom of motion is lost, and the adjacent heavily swollen aggregates cohere. The rigidity is dependent upon the relative amount of free solvent in the interstices of the aggregates, and on the amount of solvent that has been taken up by the gelatin in a hydrated or imbibed condition. The resiliency or elasticity is dependent upon the length and number of the catenary threads. Solution is the

[15] *J. Am. Chem. Soc. 43,* 1764 (1921).
[15a] R. H. Bogue, *J. Am. Chem. Soc. 44,* 1343 (1922).
[15b] J. Loeb, *J. Gen. Physiol. 3,* 827 (1921); *4,* 73, 97, 351 (1921-22).

reverse of gelation. Swelling is determined by osmotic forces and the Donnan equilibrium."

By the additions of increasing amounts of alcohol, the micellular groups are progressively dehydrated and shrunken, the gelatin solution becomes opalescent and its viscosity drops.

L. Arisz [16] dissolved 10 per cent. of gelatin in glycerin of sp. gr. 1.176 (containing 32 per cent. of water), and noted the changes in viscosity at different temperatures and over different periods of time. At 65° the viscosity was unchanged after 24 hours, but at higher temperatures it suffers a gradual decrease which amounts to 8 per cent. after 30 minutes at 95°. If the viscosity of water be considered as unity, the viscosities of the 10 per cent. gelatin-glycerin solutions determined with an Ostwald viscosimeter after equilibrium had been reached at lower temperatures were:

| *Temperature* | *Viscosity* |
|---|---|
| 65° | 222 |
| 55° | 415 |
| 50° | about 950 |
| 47° | less than 4,200 |
| 46° | about 5,000 |
| 44° | over 30,000 |

That is, at 44° the solution had practically gelatinized. Very low temperatures *inhibit* gelatinization of dilute gelatin solutions. Thus Arisz found, contrary to expectation, that 1½ per cent. solution which set after 3 days at 20°, was still fluid after 2 weeks at 2°. This is probably because of the reduced kinetic motion of the gelatin particles consequent upon low temperature. He also found that the viscosity as well as the Tyndall phenomenon of gelatin solutions depend upon their previous thermal history. The establishment of the viscosity equilibrium takes time, and there is a time lag observable upon warming or on cooling the solutions. (For full details of Arisz's elaborate experiments, the original must be consulted.)

The viscosity of a gelatin solution is influenced by time; temperature; concentration; mechanical agitation; innoculation or seeding with a more aggregated solution; hydrolysis, enzymic or bacterial decomposition; the addition of acids, alkalis, salts, and of a wide variety of non-electrolytes.

[16] *Kolloidchem. Beihefte* 7, 1 (1915).

The time and temperature effects are familiar to everyone; it is a matter of common knowledge that standing and chilling increase the thickness of solutions of glue and gelatins. These effects may be seen quantitatively by tabulating some of the results of P. von Schroeder [17] and S. J. Levites.[18]

INCREASE IN VISCOSITY OF GELATIN SOLUTIONS WITH TIME

| P. von Schroeder | | | S. J. Levites (det. with Ostwald viscosimeter) | | |
|---|---|---|---|---|---|
| *After elapse of* | *At 21.0°* | *At 24.0°* | *At 31.0°* | *After elapse of* | *At 25°* | *At 26°* |
| 5 min. .... | 1.83 | 1.65 | 1.41 | —— .... | 2.19 | 2.94 |
| 10 " .... | 2.10 | 1.69 | 1.41 | 15 min. .... | 2.39 | 3.00 |
| 15 " .... | 2.45 | 1.74 | 1.42 | 30 " .... | 2.59 | 3.05 |
| 30 " .... | 4.13 | 1.80 | 1.42 | 45 " .... | 2.80 | 3.13 |
| 60 " .... | 13.76 | 1.90 | 1.42 | 60 " .... | 3.00 | 3.19 |
| | | | | 75 " .... | 3.20 | 3.24 |
| | | | | 90 " .... | 3.40 | 3.29 |
| | | | | 11 hours.... | gelatinized | gelat. in 24 hrs. |

R. H. Bogue (*loc. cit., infra.*) also gives a table showing the variation in viscosity of 6 hide glues and 7 bone glues at temperature between 150° and 83° F. (67° and 28° C.).

The well known effects of increase in viscosity due to increasing concentration of gelatin is shown in the following tables taken from S. J. Levites (*loc. cit.*) and R. H. Bogue: [19]

| Gelatin ($\alpha$ Glutin) | | Hydrolyzed Gelatin ($\beta$ Glutin) non-gelatinizing | |
|---|---|---|---|
| *Conc. of Gelatin in per cent.* | *Viscosity at 35°* | *Conc. of $\beta$ Glutin in per cent.* | *Viscosity at 35°* |
| 0.25 | 1.10 | 0.50 | 1.186 |
| 0.50 | 1.22 | 1.00 | 1.362 |
| 0.75 | 1.32 | 1.50 | 1.332 (?) |
| 1.00 | 1.46 | 2.00 | 1.432 |
| 1.50 | 1.75 | 3.00 | 1.603 |
| 2.00 | 2.05 | 4.00 | 1.856 |
| 3.00 | 2.96 | | |

EFFECT OF CONCENTRATION ON THE VISCOSITY OF GLUE (BOGUE)

| *Ratio of Glue to Water* | *Hide Glues* | | | | *Bone Glues* | | | |
|---|---|---|---|---|---|---|---|---|
| | $H_1$ | $H_2$ | $H_3$ | $H_5$ | $B_1$ | $B_2$ | $B_4$ | $B_5$ |
| 10 to 170 | 42.0 | 42.4 | 42.6 | 41.8 | 40.8 | 41.8 | 41.0 | 41.4 |
| 20 to 150 | 46.4 | 46.4 | 44.2 | 42.6 | 43.0 | 44.0 | 42.4 | 41.4 |
| 30 to 160 | 59.8 | 58.4 | 49.4 | 47.0 | 48.4 | 50.6 | 44.6 | 41.6 |
| 40 to 140 | 99.8 | 83.6 | 60.0 | 56.4 | 59.8 | 61.4 | 50.0 | 43.4 |

[17] *Z. Physik. Chem.* **45**, 75 (1903).
[18] *Kolloid Z.* **2**, 210 (1907).
[19] *Chem. Met. Eng.* **23**, 61 et seq. (1920).

Mechanical agitation decreases the viscosity of gelatin solutions, just as it does with many other colloids. Consequently in making or using glue or gelatin unnecessary agitation is to be avoided; for although with slight stirring the dispersing effect is reversible or negligible, where violent agitation is used there may be produced an irreversible disintegration.

The effect of an addition of aged gelatin solution in accelerating the gelatinization of gelatin solutions has been observed by H. Garrett.[20] Wo. Ostwald regards this as due to a chemical change in the gelatin, probably to its hydrolytic cleavage; but the more likely explanation is that the aged gelatin furnishes "nuclei" upon which larger aggregates are formed, thus speeding up the aggregation which finally results in gelatinization.

Any agency which produces hydrolysis or degeneration of gelatin, will reduce the viscosity of the solutions. Enzymes (pepsin, trypsin) and bacteria bring about the result, and bacterial decomposition must be carefully guarded against by antiseptics (chloroform, toluol, phenol), refrigeration, or sterilization or it will vitiate the results of many experiments.

The previous thermal history of gelatin, however, is a most important factor in determining its viscosity, as is evident from the following table from P. von Schroeder:

EFFECT OF HEATING ON VISCOSITY OF GELATIN SOLUTIONS

| *Hours heated to about 100°* | *Viscosity of* | | |
|---|---|---|---|
| | *1 per cent. solu.* | *2 per cent. solu.* | *3 per cent. solu.* |
| 0.5 | 1.29 | 1.75 | — |
| 1.0 | 1.23 | 1.55 | — |
| 1.5 | 1.20 | 1.49 | — |
| 2.0 | 1.17 | 1.47 | 1.76 |
| 2.5 | 1.15 | — | — |
| 3.0 | 1.14 | 1.37 | 1.68 |
| 3.5 | 1.13 | — | — |
| 4.5 | 1.11 | — | — |
| 5.0 | — | 1.30 | 1.54 |
| 8.0 | — | 1.25 | 1.47 |
| 10.0 | — | 1.24 | 1.42 |
| 12.0 | — | 1.23 | 1.40 |
| 14.0 | — | 1.22 | 1.39 |
| 16.0 | — | 1.22 | 1.39 |

Practically, this means that continued heating rapidly reduces the viscosity of glue and gelatin solutions, or makes them "run thin." The longer a glue solution is heated the lower becomes its adhesive value.

[20] Diss. Heidelberg, 1903; *Phil. Mag.* (6), *6*, 374 (1903).

**Influence of Added Substances on Viscosity.**

The influence of added substances on the viscosity of gelatin varies widely, depending on the kind and quantity of the substance added, and also the time. In summarizing the results of P. von Schroeder,[21] S. J. Levites [22] and Gokun,[23] Wo. Ostwald [24] states that with gelatin the initial value of the viscosity following the addition of a salt follows the general rule of mixtures: salts which raise the internal friction of water affect the gelatin similarly and vice versa. A very different final value is approached asymptotically.

The following is a tabular result of some of von Schroeder's work:

INCREASE (+) OR DECREASE (—) IN VISCOSITY OF 1 PER CENT. GELATIN SOLUTION 1 HOUR AFTER THE ADDITION OF SALTS

| | *Na* | *K* | *NH*$_4$ | *Mg* | *Li* |
|---|---|---|---|---|---|
| $SO_4$ — 1/16 *n* .... | — | — | — | + 0.22 | + 0.17 |
| $SO_4$ — 1/8 n ..... | + 0.33 | + 0.09 | + 0.17 | + 0.44 | + 0.24 |
| $SO_4$ — 1/4 n ..... | + 1.01 | — | + 0.48 | + 0.94 | + 0.47 |
| $SO_4$ — 1/2 n ..... | + 7.63 | — | + 1.64 | — | — |
| Cl — 1/8 n ..... | — | — | — 0.15 | + 0.10 | + 0.05 |
| Cl — 1/4 n ..... | — 0.12 | — 0.08 | — 0.01 | + 0.20 | — 0.02 |
| Cl — 1/2 n ..... | + 0.01 | — 0.03 | — 0.23 | + 0.32 | + 0.20 |
| Cl — 1 n ..... | — 0.09 | — 0.20 | — 0.20 | — | — |
| $NO_3$ — 1/8 n .... | — 0.12 | — 0.00 | — 0.04 | — | — |
| $NO_3$ — 1/4 n .... | — 0.02 | — 0.15 | — 0.16 | — | — |
| $NO_3$ — 1/2 n .... | — 0.11 | — 0.24 | — 0.27 | — | — |
| $NO_3$ — 1 n ..... | — 0.20 | — 0.32 | — 0.25 | — | — |

H. Bechhold and J. Zeigler [25] report the following results on melting points:

| | *Melting Point °C.* |
|---|---|
| 10 per cent. gelatin + 2 mol. $Na_2SO_4$ ..... | 34.2 |
| 10 " " " ..... | 31.6 |
| 10 " " " + 1 mol. NaCl ..... | 28.5 |
| 10 " " " + 1 mol. Na I. ..... | 10.0 |

With salts in rather high concentrations, the effect on solidification time, and on jelly strength or melting point, runs as follows: *for anions*, sulphate > citrate > tartrate > acetate > chloride > nitrate > bromide > iodide > sulphocyanate > benzoate >

[21] *Z. physik. Chem. 45*, 75 (1903).
[22] *Kolloid Z. 2*, 210 (1907).
[23] *Kolloid Z. 3*, 84 (1908).
[24] "Handbook of Colloid Chemistry," 2d ed., p. 169.
[25] "Colloids in Biology and Medicine," p. 162.

salicylate. According to Bechhold the action of cations is of smaller importance.

The results of R. H. Bogue [26] on glues are summarized by him as follows:

Practically all the substances added lowered the gel strength.

Strong (9N) sodium hydrate had the greatest effect, followed by potassium iodide, strong (9N) sulphuric acid, sodium sulphate, acetic acid and magnesium chloride. The effect of the others was small.

The viscosity was raised constantly by magnesium chloride, chloral hydrate, and sodium silicate.

The viscosity was raised to a maximum, after which it fell more or less rapidly, by sodium hydrate, disodium phosphate, and acetic acid.

The viscosity was lowered constantly by potassium iodide, sulphuric acid, phosphoric acid, and sodium sulphate.

There was no appreciable effect on the viscosity due to sodium chloride and magnesium sulphate.

Monosodium phosphate produced a sharp drop of one second at 0.1 per cent., followed by a sharp rise of 3½ seconds at 0.5 per cent., after which it rose a little further and then dropped again.

The disparities between von Schroeder's results on gelatin and Bogue's results on glue indicate perhaps a difference in experimental procedure (temperature and time of heating before taking viscosity, etc.), or else a material difference in the behavior of glue and gelatin, or perhaps a difference in "impurities" or hydrogen ion concentration. As Ostwald remarks in a footnote, pure gelatin would, perhaps, show totally different results from those of von Schroeder. In any event it is obvious that the experimental facts need to be carefully redetermined, having in mind all the variable factors which recent investigations have shown materially effect the properties of gelatin. Thus the experiments of D. Jordan Lloyd [27] and J. Loeb [28] were performed with gelatin containing about 0.1 per cent. of ash. Loeb's figures (*loc. cit.*, p. 35) indicate that one sample of his gelatin contained mainly calcium and iron phosphates. As C. R. Smith [29]

[26] *Chem. Met. Eng. 23,* 61 et seq. (1920).
[27] *Biochem. J. 14,* 584 (1920).
[28] "Proteins and the Theory of Colloidal Behavior," New York, 1922.
[29] *J. Am. Chem. Soc. 43,* 1350 (1921).

has shown how to prepare absolutely ash-free gelatin, much of the preceding work must be repeated.

The view of H. R. Procter, J. A. Wilson and J. Loeb [30] is that gelatin forms definite hydrolyzable salts, e.g. gelatin chloride with HCl and sodium gelatinate with NaOH. Loeb believes that the effect of acids, alkalis, and salts on the viscosity, swelling, osmotic pressure, and general behavior of gelatin is explainable on the basis of the Donnan theory of membrane equilibria. Loeb (*loc. cit.*, p. 204) concludes from his experiments that "it seems that the viscosity of the solutions of proteins is primarily a function of the relative volume occupied by the protein in solution," but that "the difference in the viscosity of solutions of gelatin and crystalline egg albumen cannot be ascribed to differences in the degrees of hydration of the individual protein ions since at the isoelectric point the protein is not ionized." (Loeb's measurements were made at or near this point.)

Loeb believes that gelatin solutions contain submicroscopic particles of solid jelly, and that a Donnan equilibrium arises between these and the surrounding solution. This equilibrium regulates the amount of water occluded by the submicroscopic particles of solid jelly floating in the gelatin solution, and the high viscosity of gelatin solutions is due to the presence of these swollen particles which increase the relative volume occupied by the gelatin in solution.

Loeb here criticizes the theory of Wo. Pauli, who holds [31] that the viscosity of protein solutions depends primarily upon hydrated protein *ions*. Wo. Ostwald and M. H. Fischer likewise disagree with Pauli, but uphold the aggregation hypothesis which is condemned by Loeb. Strange to say, Ostwald, Loeb, and Fischer draw about the same picture of what happens in gelatin, although they differ as to the *mechanism by which* the result is brought about.

"The quantities of water which can be occluded in a solid jelly of gelatin are enormous. If we assume the molecular

[30] The views of H. R. Procter and J. A. Wilson are set forth in many journal articles, a good abstract of them being found in the "First Report on Colloid Chemistry and its Industrial Applications," London, 1917, pp. 5 et seq. (by Procter), and the Third Report, London, 1920, pp. 48 et seq. The various journal articles of J. Loeb are collected in his book, "Proteins and the Theory of Colloidal Behavior," New York, 1922, in which his views and many experiments which he believes confirm them, are set forth at length.

[31] "Kolloidchemie der Eiweisskörper," Dresden and Liepzig, 1920.

weight of gelatin to be of the order of magnitude of about 12,000, a solid gel of 1 per cent. originally isoelectric gelatin contains over 60,000 molecules of water to 1 molecule of gelatin. It is out of the question that such masses of water could be held by the secondary valency forces of the gelatin and water molecules. . . . All the experiments described agree with the occlusion theory but not with the hydration theory." [32]

Occlusion is believed by most physicists and chemists to be due to residual stray fields of force at the surfaces involved. H. Freundlich [33] would probably call it capillarity, while some would call it absorption, adsorption, or sorption, the latter term being suggested by McBain [34] as being free from theoretical assumption as to its cause.

R. S. Lillie [35] observed that neutral salts depress the osmotic pressure of gelatin solutions, a result explained as due to aggregation consequent upon the precipitating action of the salts. But salts *decrease* the viscosity of gelatin solutions, and as Loeb properly concluded from some of his experiments that aggregation increases the viscosity of gelatin [36] he argues that the salts cannot cause aggregation.

Loeb here entirely overlooks the zone of maximum degree of colloidality referred to on p. 101. Aggregation increases viscosity only up to a certain point or zone, after which further aggregation may *reduce* viscosity. Below this zone the kinetic motion of the particles seems to be a controlling factor, while above it diminution in the free surface of the particles or in the amount of water they hold, tend to reduce viscosity. Thus karaya gum powder whose water-imbibing capacity has been diminished by heating, yields less viscous solutions than the original gum. The heated gum particles show inferior swelling because their constituent submicroscopic molecular groups remain more aggregated (that is less dispersed); more water remains "free" (unadsorbed or unoccluded) and the viscosity is therefore less than is the case with the unheated gum. A similar condition exists with minerals like clay, and emulsions like cream; their viscosity *increases* with subdivision of the dispersed phase.

[32] Loeb, *loc. cit.*, pp. 229-230.
[33] "Kapillarchemie," Leipzig, 1909.
[34] J. W. McBain, *Phil. Mag.* (6), *18*, 916 (1909).
[35] *Am. J. Physiol. 20*, 127 (1907).
[36] *J. Gen. Physiol. 4*, 97 (1921-2); also *loc. cit.*, pp. 16 and 114.

The importance of the hydration (hydration, swelling, water occlusion) of secondary groups or micellular aggregates in increasing viscosity, is obvious, and depends upon the total free surface of these groups. Thus the atoms or molecular groups of *metals* draw together so powerfully, that the water films about their nascent colloidal dispersions are squeezed out; the dispersed phase is dehydrated and the viscosity very low, most of the water being in the *dispersing* phase. When the dispersed phase is highly hydrated as with *gelatin, gum karaya,* etc., the viscosity is high, most of the water being in the *dispersed phase.* With *emulsions,* increasing subdivision of the dispersed phase brings increase in active surface and in viscosity, although presumably the individual particles of dispersed oil are hydrated only at their exterior. Comparing these three types, the fact that the gelatin particles have interior surfaces and are at least duplex, becomes evident.

With cooling gelatin, however, the zone of maximum colloidality or viscosity is approached from the opposite side, and we have an *increase* in viscosity due to *aggregation* of the dispersed phase. Sodium salts of the fatty acids also illustrate this approach, as their solutions become more viscous and hold more water with increase in the molecular complexity of the fatty acid.[37] From Bechhold's table (see Chapter 3, p. 50) it may be seen that the particles in gelatin solution are of the order of 4 μμ, so that there is considerable latitude for increase in viscosity due to aggregation, before passing beyond the colloidal zone. Interesting results should be obtained by taking the viscosity of gelatin heated to 110° for various periods of time, for the results of Bogue[38] indicate that a rise followed by a fall in viscosity may be expected.

M. A. Rakusin[39] in a monograph entitled "The Animal Skin as an Amphoteric and Colloidal Protein" quotes the analytical results of von Schroeder and Pässler[40] showing that animal skins of diverse origin show a remarkable uniformity in ultimate analysis.

[37] For further discussion of this view see J. Alexander, "The Zone of Maximum Colloidality. Its Relation to Viscosity in Hydrophile Colloids, Especially Karaya Gum and Gelatin." *J. Am. Chem. Soc. 43,* 434 (1921).

[38] *Chem. Met. Eng. 23,* 61 et seq. (1920).

[39] Kolloidchemische Beihefte 15, 103-184 (1922).

[40] *Dingl. polytech. J. 287,* Heft 11, 12 and 13 (1893).

| *Skin* | *C* | *H* | *N* |
|---|---|---|---|
| Ox (average) | 50.47 | 6.46 | 17.76 |
| Calf | 50.21 | 6.46 | 17.78 |
| Camel | 50.02 | 6.43 | 17.67 |
| Horse | 50.20 | 6.44 | 17.93 |
| Pig | 49.90 | 6.31 | 17.84 |
| Rhinoceros (average) | 50.19 | 6.37 | 18.04 |
| Goat | 50.31 | 6.35 | 17.48 |
| Deer | 50.34 | 6.38 | 17.42 |
| Sheep | 50.19 | 6.49 | 17.05 |
| Chamois | 50.14 | 6.37 | 17.38 |
| Dog | 50.26 | 6.45 | 16.97 |
| Cat | 51.10 | 6.51 | 17.05 |
| Gelatin | 49.91 | 6.35 | 17.72 |

The figures apply to water-free substance. In the case of the ox and rhinoceros the average refers to pieces of hide from different portions of the animal. The gelatin was one sold by Grübler for bacteriological purposes.

From these figures von Schroeder and Pässler concluded that hide substance and gelatin represent a chemical individual, a conclusion in which Rakusin unwisely concurs, for in the light of our present knowledge of isomerism, stereoisomerism, polymerism, tautomerism, etc., its danger would be obvious even in the case of substances far less complex than the proteins. With ossein and gelatin where we have an as yet undefined complex of polypeptides and amino-acids, the impossibility of such a conclusion becomes immediately manifest.

Here again we have a striking instance of the desirability of refraining from rushing to frame a plausible theory which will fit a certain set of facts "within the limit of experimental error," without considering other known experimental facts and the possibility of unsuspected but potent factors. In the case of the proteins colloidal protection is probably such a factor, and as Bismarck once said the things he most feared were the "imponderables."

Rakusin states (*loc. cit.*, p. 110) that hide powder, in contradistinction to gelatin, contains a small quantity of sulphur that can be split off, for with lead or bismuth salts in the presence of alkali it gives a slight precipitate. Mörner [41] attributed this sulphur to the presence of cystine, but Rakusin claims to have proven that "the sulphur in gelatin is fixed as *chondroitin-*

[41] Oppenheimer, *Handb. d. Biochem. d. Tiere u. des Menchen 1,* 331 and 395 (Jena 1909).

*sulphuric acid* which exhibits no protein reaction whatever, but reacts with barium chloride; its dextro-rotation distinguishes it from sulphuric acid." From this it is evident that Rakusin used a gelatin containing chondrin or some similar impurity, and it leads one to suspect that the sulphur in hide powder may come from some impurity such as keratin, for example.

Herzog and Adler [42] showed that both hide and gelatin exhibit an apparent negative adsorption—that is, they selectively adsorb the solvent leaving the solute more concentrated. Rakusin says that in dyeing hide a positive and negative adsorption occur simultaneously,[43] but this seems to be a rather recondite way of saying that the hide takes up both water and dye. He also asserts with great positiveness that the combination between dye and hide is chemical, but says that crystal violet, which washes out with alcohol, constitutes "a preliminarily inexplicable exception." So too did methylene blue, which was dissolved out by both boiling water and by alcohol; and in this case Rakusin promises to repeat the experiment with the purest dye obtainable.

Hide powder and gelatin were both dyed by methyl orange (dimethylanilin-azo-benzolsulphonic acid), but the adsorption product showed the curious anomaly of being reversible in boiling water but irreversible in 95 per cent. alcohol.

Rakusin also discusses at length the tanning of hide by tannin, formaldehyde, aldoses, phenols of various kinds, and homologous substances, picric acid, naphthols, chinone, "neradol," and alum, iron, and chrome salts. For full details reference must be made to his monograph.

Non-electrolytes in general have slight action on the viscosity of gelatin, although many of them materially influence its jelly strength and melting point. Bechhold and Zeigler report the following:

| | *M. P. in °C.* |
|---|---|
| 10 per cent. gelatin | 31.66 |
| 10 " " " + 1 mol. grape sugar | 32.25 |
| 10 " " " + 2 " glycerin | 32.17 |
| 10 " " " + 2 " alcohol | 30.00 |
| 10 " " " + 1 " urea | 26.30 |

Furfurol, rescorcinol, hydroquinone and pyrogallol also lower the apparent melting point of gelatin.

[42] *Kolloid. Z.* 2, Suppl. II, 3 (1908).

[43] See also M. Rakusin and G. Pekarskaja, *J. Russ. Chem. Ges.* 1917, 1899.

# Chapter 8.

## Collagen or Ossein.

Collagen[1] (literally *glue-former*) is, as its name indicates, the parent substance of glue and gelatin. It is a substance particularly characteristic of mature vertebrates, and is found in all of them with the exception of the border-line *Amphioxus lanceolatus,* according to Hoppe-Seyler. This same investigator reports that jelly-forming tissue is practically never met with in invertebrates, although he found some in two cephalopods, *Octopus* and *Sepiola* (the devil-fish and the cuttle-fish).

From this it is obvious how vitally collagen is involved in bone formation. In fact, bone consists essentially of tricalcium phosphate and calcium carbonate deposited in collagen which acts as a colloidal protector and inhibits their crystallization. H. Bechhold[2] has discussed some of the theories of ossification, and describes experiments of R. E. Liesegang, who simulated bone formation by allowing disodium phosphate and calcium chloride to diffuse toward each other in gelatin jellies.[3] Pauli and Sameč found that serum albumen increases the solubility of calcium carbonate 475 per cent., and of calcium phosphate 90 per cent.; but with the cleavage products of albumin, the figures are reversed. Since human bone ash contains about 85 per cent. of $Ca_3(PO_4)_2$ and 9 per cent. $CaCO_3$ Bechhold believes that the disposition of the bone salts is consequent upon or accompanies the disintegration of cells or tissues, which corresponds with the histological evidence.

In pathological cases bone formation may be inhibited, as in rickets; or bone already formed may be destroyed, as in osteomalacia. The disposition of lime is evidently closely bound up with variation in the protective action of the body colloids, too high a degree of colloidal protection working against deposition.

[1] Ossein is collagen derived from bones.
[2] "Colloids in Biology and Medicine," p. 268.
[3] "Beitrag zur einer Kolloidchem. Theorie des Lebens," Dresden, 1909.

Selective adsorption also seems to be a factor in bone formation, as well as in the allied phenomenon of calcification, of tubercles for example. The presence of a vitamine (possibly certain fatty acids) in foods is a factor, and sunlight also exercises an influence.

W. von Gaza [4] in dealing with the changes of tissue colloids in the healing of wounds, says that connective tissue cells have the specific property of forming collagen from simpler albuminous substances. This collagen is held in colloidal solution initially because of the presence of the acid oxidation products of life (especially $CO_2$). As the colloidal solution of collagen accumulates in the cell, oxidation diminishes and finally a reversal of reaction occurs—neutrality instead of acidity. The formation of collagen is thus analogous to lignification, the formation of lignin from cambial sap, a phenomenon which has been investigated by Wislicenus.[5] Collagen and lignin both function as supporters and protectors, and are marked by great stability under normal conditions of life. Even after death this stability persists as is evidenced by fossil bones and wood.

After growth, collagen remains unchanged apart from colloidal syneresis. In order that healing of a wound (i.e. in the skin) may take place, it is necessary that the paraplastic collagen be brought into a fluid or semi-fluid condition, for it is an old maxim that *corpora non agunt, nisi fluida.* Fluidification seems to be brought about by local accumulation of acid due to interruption of the normal circulation consequent upon the wound. Similarly collagen must first be swollen to a certain extent by hydrochloric acid before pepsin will disintegrate it. It is not, however, attacked by leucocytes or by the tryptases of the tissues.

R. H. A. Plimmer [6] points out that pepsin attacks both gelatin and ossein (collagen), while trypsin attacks only gelatin. This is held to indicate that ossein has a "closed ring" or anhydride

[4] *Kolloid Z.* **23**, 1 (1918).

[5] *Kolloid Z.* **6**, 19 (1910).

[6] "Chemical Constitution of the Proteins," 2d ed., Part II, p. 11. A. W. Thomas and F. L. Seymour-Jones (article in press) have been able to attack collagen with trypsin under certain conditions. The action is most rapid at $p_H$ 5.9 and is not materially accelerated by soaking. Collagen without trypsin, slowly hydrolyzed at 40°, as was observed in a blank determination. Fine hide powder was attacked by trypsin much more rapidly than coarse hide powder, showing the great effect of surface.

form, which only pepsin can open. A more likely explanation that in ossein the constituent molecular groups are more highly dehydrated and closer together than in gelatin, and this condition is relieved to some extent by the acidity essential to the activation of the pepsin. This is not inconsistent with the fact that, as Emil Fischer and Abderhalden have shown, the action of enzymes on the comparatively simpler polypeptides depends upon the configuration of the latter, as is shown in the case with sugars.[7]

A. Ewald,[8] working with collagen derived from the tendon of the mouse, studied the shortening of its fibrils on heating in water, which is very marked if the collagen is first purified by tryptic digestion. The behavior of collagen treated with formaldehyde is so characteristic that F. C. Thompson thinks [9] it may serve as a new qualitative test. At 93° C. such fibers shrink to one third of their original length, but regain half the loss upon soaking in cold water. On treating again at 69° C. they once more contract to one third; but their original length is completely regained by prolonged soaking in cold water.

Hofmeister observed that on heating dry gelatin to about 130° it became insoluble, being reconverted into ossein; and he held that this indicates that ossein is an anhydride of gelatin. J. Alexander [10] believes the insolubility consequent upon the removal of the protective aqueous films, the constituent molecules or particles of the gelatin approaching so close as to form an irreversible gel. An analogous condition exists with silica and with clay, where dehydration up to a certain point is reversible, after which the material will not hydrate or redisperse again within a reasonable time.

Thomas and Kelly [10a] report the isoelectric point of collagen as $p_H = 5$, whereas Porter [10b] reports it as 4.8.

While the main sources of collagen are hide or skin and bones, it is found also in tendons or sinews, connective tissue, in the cornea and sclerotic coat of the eye, and in the scales of fishes.

[7] See S. B. Schryver, "Allen's Commercial Organic Analysis," 4th ed., Vol. 8, p. 469.

[8] *Z. Physiol. Chem. 105,* 115 (1919).

[9] "S. C. I. Annual Report on Appl. Chem.," 1919, p. 361.

[10] "Allen's Commercial Organic Analysis," 4th ed., Vol. 8, p. 586.

[10a] *J. Am. Chem. Soc. 44,* 195 (1922).

[10b] *J. Soc. Leather Trades Chem. 5,* 259 (1921); *6,* 83 (1922).

W. S. Ssadikow [11] has made experiments with native tendocollagen, which is a compact mass of fibrous structure. If dehydrated by alcohol and carefully dried over sulphuric acid at room temperature and then up to 105°–180°, the fibrous structure is not lost. The collagen does not become brittle and cannot be ground. Above 180° it browns and becomes brittle, the "intracolloidal" water being driven off. If dried in the presence of its adsorption water, tendocollagen loses its white color and opacity, and assumes the hyaline form, which may also be produced by heating it for some time with hot water on a hot water bath, or by treatment in the cold with weak caustic alkali or strong alkali sulphide.

Resoaking the hyaline tendocollagen in water restores the fibrous condition, unless the hydrolytic influence has exceeded a certain limit, when the change becomes irreversible, i.e. soaking one month in 2 per cent. KOH, or treatment with a saturated solution of $Na_2S$. On treatment with $CS_2$, thionyltendocollagen is formed, which shows characteristic reactions and is quite resistant to hydrolysis. The amount of sulphur bound depends upon the previous treatment of the collagen, and varies from 0.12 to 3.05 per cent.

Ssadikow also reported [12] on the action of carbon bisulphide on gelatin and on ossein. To a hot solution of gelatin he added 1 per cent. of powdered caustic soda or calcium hydroxide, followed by a few cc. of carbon bisulphide. The mixture was then allowed to set. The reaction was evidenced by the development of a brown color, the evolution of ammonia and hydrogen sulfide, and the precipitation of thiogelatin (thioglutin). On slowly drying thioglutin at ordinary temperatures, an intensely red skin develops which is not soluble in hot water.

When $CS_2$ acts on collagen in the presence of alkali, the alkali first causes an hydrolysis and the $CS_2$ then is taken up by the resulting products of hydrolytic splitting. This process Ssadikow calls "thiohydratation." The amount of sulphur taken up depends upon concentration and time of action of the alkali, i.e. upon the degree of hydrolytic splitting.

Glutin (gelatin), thionylized by 5–10 per cent. solutions of $CS_2$ in alcohol, ether, benzene, etc., takes up from 0.32 to 0.40 per cent.

[11] *Kolloidchem. Beihefte,* Vol. 1 (1911).
[12] *Loc. cit.;* also *Kolloid Z. 1,* 193 (1907).

sulphur (average = 0.39 per cent.). The addition product *usually* shows a dark color with alkaline lead acetate, but always exhibits a characteristic "erythrin reaction" as follows: Thionylglutin dried at 110° is heated on a water bath for ten minutes with 1 per cent. chloracetic acid. The solution is filtered, cooled, and mixed with three volumes of strong alcohol and then neutralized with ammonia. A precipitate forms immediately or after a while, depending on the concentration of the thionylglutin; and on standing from two to twelve hours this precipitate develops a fine pink color, which starts at the top (probably because it is due to oxidation) and gradually deepens to brown.

When $CS_2$ acts on highly degenerated gelatin the product is yellow and has a characteristic odor of mustard oil. The solution of this thionylxanthoglutin (thionylξglutin) in chloracetic acid is yellow, and shows the erythrin reaction markedly. This erythrin reaction is also shown to some extent by collagen which has not been thionylized. "Glutein" made from the nasal septum cartilage of the pig bound from 0.61 to 1.89 per cent. sulphur depending on the degree of hydrolysis.

Glutin brominated in ethereal solution adds as much bromine whether thionylized or not. Tanning with tannin or formaldehyde does not interfere either, nor does treatment with methyl iodide or benzoyl chloride.

**Chondrigen, Chondrin and Mucin.**

These substances are apt to be met with in glues, but may be considered as impurities in the higher grades.[13] Chondrigen occurs in hyaline cartilage, of which it forms the chief organic constituent. It is elastic, semi-transparent, insoluble in hot or cold water, and swells but slightly in water or dilute acetic acid. Upon heating for some hours with water under pressure (120°) chondrigen yields chondrin, whose solutions gelatinize upon cooling. Thus chondrigen, as its name indicates, is the parent substance of chondrin, just as collagen is the parent substance of gelatin.

[13] Mucin is a prolific source of foam in glue. It may be liberated from chondrin during manufacture of the glue.

Allen[14] gives the following procedure for preparing approximately pure chondrin: Boil costal cartilage in water for a few minutes and after scraping off the perichondrium, boil with water at ordinary pressure for 24 hours, or under pressure (at 120° C.) for 3 to 4 hours. Filter the solution to remove elastin, cellular elements, etc., and then precipitate the chondrin with a large excess of alcohol, and wash the precipitate with alcohol and ether. Re-solution in hot water followed by precipitation with alcohol will still further purify it. The chondrin thus prepared is hard, transparent, odorless, tasteless, and insoluble in cold water. Hot water dissolves it, yielding on cooling a jelly of weaker strength than that given by the same percentage of gelatin.

Some analyses of chondrin are given herewith:

| | *C* | *H* | *N* | *O* | *S* |
|---|---|---|---|---|---|
| Mulder | 49.3 | 6.6 | 14.4 | 29.3 | 0.4 |
| Fischer and Bödecker | 50.0 | 6.6 | 14.4 | 28.6 | 0.4 |
| Schützenberger and Bourgeois | 50.16 | 6.58 | 14.18 | 29.08 | 0.0 |
| Von Mehring | 47.74 | 6.76 | 13.87 | 31.04 | 0.6 |

Allen states: "It will be seen from the above figures that the elementary analysis of chondrin present considerable discrepancies, and suggest that the substance dealt with is not a definite substance but is liable to variations in composition. The results obtained by Morochowetz, and confirmed by Landwehr, Krukenberg, and Mörner, strongly support this view. Morochowetz found that on treating cartilage from various sources with lime- or baryta-water, a 0.5 per cent. solution of sodium hydroxide, or a 10 per cent. solution of common salt, mucin is dissolved out, and may be thrown down from the solution by acetic acid; while the substance left undissolved is readily convertible by boiling water into perfectly normal gelatin. According to these observations, chondrin is a mixture of gelatin and mucin, while chondrigen is a mixture of collagen with mucin or hyalogen, the latter component masking its true nature."

This led the writer to remark[15] that chondrin is probably an adsorption compound of simpler substances, i.e. gelatin and mucin.

The following table shows that chondrin behaves toward reagents like a mixture of gelatin and mucin:

[14] "Comm. Organic Analysis," 4th ed., Vol. VIII, p. 625.

[15] Allen, *loc. cit.*, p. 626.

| | *Gelatin* | *Chondrin* | *Mucin* |
|---|---|---|---|
| Solubility ..... | Insoluble in cold water, alcohol, or ether. | Same. | Same. |
| | Soluble in hot water; solutions gelatinize on cooling. | Same. | Insoluble in hot water. |
| Reaction with Acetic Acid..... | No ppt. | Ppt.; insoluble except in large excess. | Same. |
| Mineral acids..... | No ppt. | Ppt.; readily soluble in excess. | Same. |
| Tannic acid..... | Ppt. | Ppt. | No ppt. |
| Mercuric chloride.. | Ppt. | Ppt. | No ppt. |
| Lead acetate..... | No ppt. | Ppt. | Ppt. |
| Alum ..... | No ppt. | Ppt. | Ppt. |
| Boiling dilute mineral acids..... | No reducing substance formed. | Yield syntonin and reducing substances. | Same. |

For further information regarding these substances, see Chapter II, p. 23, under Glycoproteins.

By microchemical staining methods Mörner found that tracheal cartilage had a collagenous network enclosing "chondrin balls." By treatment with 0.1 to 0.2 per cent. HCl followed by treatment with 0.1 per cent. KOH he dissolved out the "chondrin balls," and then with the aid of dilute acid or superheated water converted the network largely into typical gelatin. The "chondrin balls" proved to be a mixture of free chondroitic acid, and chondromucoid. This latter is the same as the chondromucoid isolated from tendons by Gies and Buerger, and on decomposition it yields a protein fraction and chondroitic acid (chondroitin sulphuric acid or cartilage acid), which when acted upon by acids gives free sulphuric acid and chondroitin. This latter substance yields acetic acid and chondrosin, which according to Hawk reduces Fehling's solution even more strongly than dextrose.

Upon hydrolysis chondrosin is split into glucosamine and glycuronic acid.

The cartilages, which are precursors of bone, are thus related

to the mucins, and in fact free sulphuric acid has recently been found in the slime of snails. A. P. Mathews[16] points out the importance of this relation from the standpoint of evolution, for it shows a chemical relationship of mesodermal to ectodermal tissues. Thus chitin which forms the shells of arthropods (e.g. beetles) yields on hydrolysis acetic acid and glucosamine, and some sulphuric acid is also present.

[16] "Physiological Chemistry," 3d ed., 1920.

# Chapter 9.

## The Effect of Tanning Substances on Glue and Gelatin.

According to J. T. Wood [1] one of the earliest contributions to the chemistry of tanning was made by Humphry Davey on February 4, 1803, in a paper entitled "An Account of some Experiments on the Constituent Parts of certain Astringent Vegetables, and their Operation in Tanning." [2] Davey remarked: "The tanning principle in different vegetables, as will be seen hereafter, demands for its saturation different proportions of gelatin, and the quantity of the precipitate obtained by filtration is not always exactly proportional to the quantities of tannin and gelatin in solution, but is influenced by their concentration. Thus, I found that 10 grains of isinglass, dissolved in two ounces of distilled water, gave with solution of galls in excess, a precipitate weighing, when dry, 17 grains, whilst the same quantity dissolved in six ounces of water produced, all other circumstances being similar, not quite 15 grains. With more diluted solutions, the loss was still greater; and analogous effects took place when equal portions of the same solution of isinglass were acted upon by equal portions of the same infusion of galls diluted in different degrees with water, the least quantity of precipitate being always produced by the least concentrated liquor."

The amount of tannin precipitated by 100 parts of gelatin is reported by different authors as follows:

| | |
|---|---|
| H. Davey (*loc. cit.*) | — 85 |
| Lipowitz (*Jahresb. Forts. Chem.*, 1861, p. 624) | — 65 |
| S. Rideal ("Glue and Glue Testing," 1900, p. 111) | —134 |
| Mulder | —135 |
| R. Williams | — 78 |
| Böttinger | — 50 |

One cause for the discrepancies was found by Wood to be the fact that a definite excess of tannin is required to produce

[1] *J. Soc. Chem. Ind.* 27 (1908).
[2] *Roy. Soc. London, Phil. Trans.* 233 (1803).

a maximum amount of precipitate. His results are given in the following table, which shows the amount of tannin precipitated by adding 1 gram of gelatin to varying quantities of 1–100 tannin solution.

| *No. of cc. 1% Tannin Solution* | *Tannin Grams* | *Tannin pptd. Grams* |
|---|---|---|
| 100 | 1 | 0.91 |
| 200 | 2 | 1.50 |
| 300 | 3 | 1.90 |
| 400 | 4 | 2.17 |
| 500 | 5 | 2.28 |
| 600 | 6 | 2.36 |
| 700 | 7 | 2.36 |
| 800 | 8 | 2.36 |

Wood also made a quantitative record of the well-known fact that the precipitate of gelatin with excess of tannin has a different composition from the precipitate of tannin with excess of gelatin, and that a considerable amount of tannin may be removed from the latter by washing with hot water. He found that 100 parts of gelatin carried down 300 parts of tannin, of which 88 parts were given up upon washing with boiling water.[3]

In conclusion Wood observes: "An examination of the facts shows that the combination of gelatin and tannin compound is not of constant composition, nor a purely physical one, since it does not obey the solution laws, which require the concentration of the tannin in the solution and the tannin in the gelatin to maintain a constant ratio." It is therefore an error to consider "tannate of gelatin" as a definite chemical compound, for it is a typical adsorption compound, and as von Schroeder [4] has shown, the precipitation of gelatin by tannin follows the adsorption isotherm.

The precipitation of gelatin by tannin is also a typical instance of the precipitation of one colloid by another of opposite charge. Aqueous solutions of tannin are positively charged (negatively conducted), whereas gelatin is amphoteric, and as Ricevuto [5] has shown is not precipitated by tannin unless in the negative condition (positively conducted). Carefully dialyzed

[3] Apparently the excess of gelatin exercises a protective action so that part of the tannin, and some of the gelatin as well, are washed away, probably in colloidal solution.

[4] *Kolloidchem. Beihefte 1*, 1.

[5] *Kolloid Z. 3*, 114 (1908).

gelatin is not precipitated by tannin, nor is hide tanned by tannin unless it is on the acid side.

When dry the gelatin-tannin compound forms a yellowish-brown, brittle mass which melts in boiling water to a tenacious sticky mass like bird-lime. In this state it may be drawn out or spun into fibers fine as a spider's web, which have a metallic luster like silver slightly tinged with gold. When soaked in alum solution they acquire a blue tinge like polished steel. The gelatin-tannin compound is tasteless and does not yield tannin to alcohol, ether, or acetone. Prolonged boiling with water, especially in the presence of magnesia, decomposes it, probably because of the hydrolytic cleavage of the gelatin.

**Chrome.**

The chroming of gelatin does not affect its absorption of tannin, for a sheet of heavily chromed gelatin absorbs as much tannin as before chroming. This fact is not so strange as it seems if we remember that the chromium is absorbed only from basic solutions, and is apparently attracted to a different part of gelatin complex. It seems difficult, however, to reconcile with the fact that treatment with basic chromium salts renders collagen insoluble; indeed the progress of chrome tannage may be followed by immersing strips of the hide in boiling water, which causes distortion by converting any unchanged collagen into glue.[6] Chrome tannage appears to be the "mirror picture" of tannin tannage, positively charged gelatin being precipitated by negatively charged colloidal chromium hydroxide. This view is supported by the experiments of Bancroft,[7] who found that gelatin sheets took up chromic sulphate practically unchanged. If the gelatin containing chromic sulphate is washed repeatedly with boiling water, acid is slowly extracted together with some gelatin. By treating with dilute alkali, however, the acid may be removed without causing swelling or solution of the gelatin, which is combined with about 3.3 to 3.5 grams of $Cr_2O_3$ per 100 grams of gelatin.

Since Namias showed that the tanning action of chrome alum

[6] No attempt will be made here to consider the question of the tanning of hides and skins, which is even more complicated than the tanning of gelatin.

[7] "Applied Colloid Chemistry," p. 229.

was increased by adding alkali up to the point of precipitation of hydrous chromic oxide, Lumière and Seyewetz made experiments with the green basic chromic sulphate of Recoura, and found it yielded a more insoluble gelatin than did a less basic solution. Excess alkali apparently produces such a high degree of dispersion of the chromic oxide, that little or no tanning occurs.

**Organic Substances.**

Besides tannin and basic chromium solutions, many other substances are well known as tanning agents for gelatin; chief among them are alums, formaldehyde, and ferric salts. L. Meunier and A. Seyewetz [8] report having obtained the precipitation of gelatin solutions with the following organic compounds: phenol, resorcin, orcine, hydroquinone, pyrocatechin, gallotannic acid, pyrogallic acid, p-amidophenol, chlorophenol, picric acid, monochlorhydroquinone (durol), R acid (disulfo-β naphthol 2.3.6), G acid (disulfo-β-naphthol 2.6.8.), S acid (monosulfo-β-naphthol 2.6.). From their results with various substituted quinones [9] they conclude that the tanning action of a quinone increases in rapidity with decreasing power of penetration. The importance of this tanning action in the "hardening" or fixing of gelatin-coated photographic negatives or positives, must be at once manifest. "Neredol," the sulphonated phenol-formaldehyde patented product of Stiasny, is largely used to tan leather.

**Bichromates.**

A large literature exists regarding the action of light on gelatin containing bichromates.[10]

The tanning effect, which forms the basis of several photographic reproduction and engraving processes, seems to depend upon the liberation of colloidal chromic oxide, for as S. J. Levites [11] has shown $K_2CrO_4$ is reduced to $Cr_2O_3$ by most albuminoids. A. and L. Lumière and A. Seyewetz [12] have shown

[8] *Collegium*, 1908, No. 313, p. 195.
[9] *Collegium*, 1914, No. 531, p. 523.
[10] See e.g. Eder, "Reaktionen der Chromsäure und der Chromate auf organische Substanzen in ihren Beziehungen zur Photographie," 1878.
[11] *Kolloid Z.* 9, 5 (1911).
[12] *Phot. Korresp.* 6, 75, 192, 239 (1906).

that the illuminated bichromate-gelatin differs from that tanned by basic chromium salts, the chromium oxide in the former consisting of two fractions. The first, which equals 3.5 per cent. of the bichromated gelatin, represents what is held by the tanned gelatin; the second varies with the time of illumination, and arises from the reduction of the bichromate by light in the presence of organic matter. The first fraction increases disproportionately to the illumination, and decreases with increasing concentration of bichromate.

**Silicic Acid.**

Colloidal silicic acid reacts with gelatin to form a co-silicate or colli-silicate of gelatin, whose composition varies with conditions of its formation. Graham states: [13] "When a solution of gelatin was poured into silicic acid in excess, the co-silicate of gelatin formed gave, upon analysis, 100 silicic acid with 56 gelatin, or a little more than half the gelatin stated above as found in that compound prepared with the mode of mixing the solutions reversed. The gallo-tannate of gelatin is known to offer the same variability in composition."

**Alum.**

The action of alum, and other aluminium salts, in tanning gelatin, appears to be consequent upon their hydrolysis, colloidal alumina being formed and fixed by the gelatin, while the acid may be differentially washed or diffused out. The results of A. and L. Lumière and A. Seyewetz [14] are briefly:

(1) Aluminium salts and nascent alumina raise the setting point of gelatin, the effect depending on the percentage of alumina present. 0.107 grams of $Al_2(OH)_3$ per 100 grams gelatin raises the gelatinization point 1°. (2) Alum has relatively a weak effect, as is to be expected; aluminium chloride has the greatest effect. (3) Irrespective of the kind of aluminium salt used, the settling point rises with increasing alumina content, up to about 0.64 grams alumina per 100 grams gelatin. Over this the setting point remains stationary and then falls. (4) The increase in the setting point varies with the concentration of the

[13] *Phil. Trans. Roy. Soc. London 151,* 206 (1861).

[14] *Z. für wiss. Photographie 4,* 360 (1906); *Bull. Soc. chim. Paris 35,* 676 (1906).

gelatin solution. (5) Gelatin fixes a maximum of about 3.6 grams alumina per 100 grams gelatin, and gives up to the water the acid and salts with which the alumina was combined. It was concluded, therefore, that gelatin forms a definite chemical compound with alumina.

Lumière and Seyewetz also found that an excess of alkali or ammonia completely inhibited the tanning of gelatin by alumina, just as is the case with chrome salts.

In reviewing this work, H. Freundlich [15] expressed the view, in which the writer concurs, that it is more probable that a colloid complex is formed, rather than a chemical compound between hydroxide of aluminium and gelatin. The effects of selective absorption and differential diffusion are so great [16] that even potassium sulphate may be decomposed by percolating its dilute solution through a column of sand, the alkali being held by the sand, while a dilute solution of sulphuric acid issues from the bottom.

Gutbier, Sauer, and Schelling [17] report that at ordinary temperatures a higher concentration of alum is required to raise the viscosity of bone glues than of hide glues. Alum lightens both glues, and at higher temperatures, if the solution is slightly acid, forms a precipitate, which is an adsorption compound and clarifies better if it settles rapidly. On dialysing glue solutions the aluminium only is held back; the colloidal aluminium hydroxide and the H-ion concentration control the action of the alum, optimum conditions varying with kind of glue and with concentration. Hide glues seem especially sensitive to the action of an excess acidity, for with them more often than in bone glues, alum clarification causes hydrolysis resulting in foam and decreased strength. The precipitate, however, seems to adsorb impurities; it removes part of the ash-producing substances and all the added acid; and the resulting solution contains but little Al.

### Iron.

The action of iron on gelatin is well known to gelatin manufacturers, for rusty nets often produce an insoluble reddish-

[15] *Kolloid Z. 1,* 157 (1906).
[16] See J. Alexander, *J. Am. Chem. Soc. 39,* 84 (1917).
[17] *Kolloid Z. 30,* 376-95 (1922).

brown compound. According to Lüppo-Cramer [18] ferric chloride solutions precipitate gelatin. A 1 per cent. solution of gelatin mixes with the chloride without precipitation, but the color is darker than a solution of ferric chloride of the same concentration, showing that the salt undergoes, in the presence of gelatin, an hydrolysis similar to that which it suffers on boiling. Iron alum also tans gelatin, but not in the presence of an excess of alkali, which turns the gelatin dark but allows the iron to be washed out. The adsorbed ferric hydroxide can also be washed out by potassium citrate or oxalate, and by oxalic and other acids. Fifty cc. of 10 per cent. $FeCl_3$ + 50 cc. 10 per cent. gelatin solution at 50° give a thick red-brown fluid which sets and can be remelted. Here the excess of gelatin evidently acts as a protective colloid to the iron-gelatin adsorption compound. In fact, according to Stiasny the unsatisfactory tanning action of iron-salts consequent upon their rapid and complete hydrolysis, is improved by the presence of soap, blood, albumen, gelatin, and similar colloidal protectors.

**Other Salts.**

Uranic salts (e.g. uranium nitrate) act similarly to ferric salts, and auric chloride has a particularly powerful tanning action, which it likewise exerts on the skin. Copper, silver, mercury and lead salts are powerfully fixed by gelatin, and even barium chloride undergoes a partial hydrolysis in its presence. Indeed, as Van Bemmelen [19] has shown, colloids by their adsorptive action can effect a chemical decomposition of most salts. Thus if a red solution of thiocyanate of iron is added drop by drop to a 10 per cent. solution of gelatin, the ruby-red precipitate soon changes to the rust-brown color of ferric hydroxide.

Phosphomolybdic and phosphotungstic acids precipitate gelatin, and its precipitate with picric acid is used for its detection in the Stokes method.[20]

**The Halogens.**

The halogens have a powerful tanning action on gelatin. As far back as 1840, Mulder [21] described the compound formed by

[18] *Kolloid Z. 1,* 353 (1907).
[19] *Rec. Trav. chim. Pays Bas 7,* 37 (1888).
[20] *Analyst,* 1907, p. 320.
[21] *Berzelius Jahresber. 19,* 734; *J. für Chem. 44,* 489.

treating gelatin with chlorine, and Allen and Searle [22] described a similar compound with bromine, while Hopkins and Brooks [23] made like observation with respect to iodine.

According to Rideal and Stewart [24] when chlorine is bubbled through 1 per cent. gelatin solution the liquid remains clear for a time and then froths, each bubble of gas becoming encased in a white pellicle. With an excess of chlorine the liquid becomes clear again and the gelatin forms a white granular precipitate which on washing and drying yields a pale yellowish-white powder, odorless, tasteless, and insoluble in water or alcohol, but soluble in alkalis.

Cross, Bevan, and Briggs [25] found that moist gelatin spread out very thin by immersing cotton yarn in its solution, combines with 15.4 per cent. of chlorine figured on air-dried gelatin. The extremely stable substance resulting they regard as a gelatin chloramine; it is sensitive to antichlors, and when treated with sulphuric acid reverts to the original gelatin. This reaction is made the basis of a method for the detection and estimation of gelatin in tub-sized papers (*loc. cit.*, p. 263).

Lumière and Seyewetz [26] found that the best results with chlorine were obtained by adding, say, 10 grams of gelatin to 500 cc. of a saturated solution of chlorine, containing 50 grams of NaCl and held at 0° to drive back the ionization of hydrochloric acid. They found it much easier to use hypochlorites, 10 grams of gelatin in thin sheets being rendered insoluble at room temperature by a solution of 100 grams of commercial sodium hypochlorite and 2 cc. of HCl (21° Bé) in 400 cc. of water. They found that bromine acted similarly but more energetically, but were unable to render gelatin insoluble with iodine.

### Formaldehyde.

The tanning action of formaldehyde, both in solution and as a gas, has long been known and utilized. Acrylic aldehyde is said to act similarly, but acetic aldehyde acts only in the presence of water.

[22] *Analyst*, 1887, p. 258.
[23] *J. Physiol.* 22, 184.
[24] *Analyst*, 1897, p. 228.
[25] *J. Soc. Chem. Ind.* 27, 260 (1908).
[26] *Bull. Soc. Chim.* (4) 11, 344 (1912).

The maximum amount of formaldehyde fixed, when its 10 per cent. solution acts on dry gelatin, is between 4.0 and 4.8 per cent.[27] The "insoluble" formo-gelatin is decomposed by repeated washing with boiling water, as well as by heating to 110°, and by cold 15 per cent. HCl. R. Abegg and P. von Schroeder[28] half filled a test tube with a 10 per cent. solution of gelatin which had a melting point of 36°, and after the gelatin had set, covered it with a 5 per cent. formalin solution which was allowed to act for 24 hours. The upper fully tanned layer was infusible, but crumpled at 85° with the development of a brown color. Lower layers showed melting points of 48°, 42°, and 37°, whereas the bottom layer was unaffected. They also found that the time of tanning (as determined by the time needed to reach the highest melting point observed, 48°) varied inversely with the concentration of the formaldehyde.

H. Bechhold[29] prepares ultra filters by impregnating filter paper with gelatin solutions of various strengths, allowing the gelatin to set, and then immersing the treated paper in 2-4 per cent. ice-cold formaldehyde.

R. H. Bogue[30] has examined into the effect of formaldehyde on various glues. He found that the viscosity increased directly as the amount of formaldehyde added; it *decreases* with rise of temperature up to 40°, after which it rises rapidly to the setting point. It also increases with time. On the other hand the jelly strength of glues is decreased proportionately to the amount of formaldehyde added, the effect being most marked in lower concentrations and with weaker glues, some of which actually remained fluid. The higher the grade of glue and the higher its concentration, the less formaldehyde is required to produce "insolubility." Alums produce increased viscosities but have little or no effect on the jelly strength.[31]

[27] Lumière and Seyewetz, *Bull. Soc. Chim. 35,* 872 (1906).
[28] *Kolloid Z. 2,* 85 (1907).
[29] "Colloids in Biology and Medicine," p. 97.
[30] *Chem. Met. Eng. 23,* 61 et seq. (1920).
[31] Chrome alum increases the melting point however.

# Chapter 10.

## The Chemical Examination of Glue and Gelatin.

### Hydrogen Ion Concentration, or $p_H$ Value.

Since recent investigations [1] have shown the great influence of the *effective reaction* (hydrogen ion concentration or $p_H$ value) on the viscosity and jelly strength of glue and gelatin, its determination by the electrometric or the colorimetric methods may form a necessary part of factory control.[2] For colorimetric determination of $p_H$, the following indicators are used:

| *Indicator* | $p_H$ *range* | *Color change acid-alkaline* | *Solution strength* |
|---|---|---|---|
| Methyl Violet ..... | 0.1 to 3.2 | green to blue | 0.02% |
| Thymol Blue ..... (lower range) | 1.2 to 2.8 | red to yellow | 0.04% |
| Methyl Orange ..... | 3.2 to 4.4 | red to yellow | 0.02% |
| Methyl Red ..... | 4.4 to 6.0 | red to yellow | 0.02% in 60% alcohol |
| Phenol Red ..... | 6.8 to 8.4 | yellow to red | 0.02% |
| Thymol Blue ..... (upper range) | 8.0 to 9.6 | yellow to blue | 0.04% |
| Phenolphthalien ..... | 8.3 to 10.0 | colorless to red | 0.05% in 50% alcohol |

The limitations of these indicators for gelatin are still to be determined, and in all cases the percentage of gelatin present is a factor of importance. That is, a one per cent. solution may show a different $p_H$ than a 5 per cent. solution. Patten and Johnson [2a] state that gelatin does not interfere with the determination of $p_H$ colorimetrically.

Bogue even recommended the determination of H-ion concentration as part of the regular laboratory routine test of both glue and gelatin. He states [3]: "If the $p_H$ value is 4.7, the viscosity, swelling, etc., are low, and the product nearly insoluble. On either side of this point [4] these properties increase very consid-

[1] J. Loeb, R. H. Bogue and others.

[2] For details see W. M. Clark, "The Determination of Hydrogen Ions," Baltimore, 1920.

[2a] *J. Biol. Chem. 38,* 179 (1919).

[3] *J. Ind. Eng. Chem. 14,* 439 (1922).

[4] This is the isoelectric point of gelatin.

erably, attaining their maximum on the acid side at $p_H$ 3.5, and on the alkaline side at $p_H$ 9.0. At greater acidity than $p_H$ 3.5 or at greater alkalinity than $p_H$ 9.0 these properties again decrease. The $p_H$ value indicates, therefore, not only the reaction of the material, and the degree of acidity or alkalinity, but also the proximity of the substance to the points of maximum or minimum properties. . . . One per cent. solutions are best in either case, and the results expressed in terms of $p_H$ to the nearest tenth."

The experiments of Bogue and of Loeb were made with rather dilute solutions of glue gelatin. In actual practice the relatively small variations in the H-ion concentration of glues produce such slight changes in the viscosity of working solutions that they are barely perceptible with the viscosimeters in common use.

Outside of moisture and ash which have already been considered, chemical tests on glue are seldom made, unless they be tests to simulate working conditions where alum, formaldehyde or other substances are added to the glue. The reason is that most of those whose knowledge of glue transcends laboratory experience, are agreed that the chemical tests proposed for glue do not enable one to form any trustworthy idea as to its practical value, and they are besides much more difficult, expensive, and time-consuming than the very satisfactory physical tests. In the case of food gelatin it is essential to make an exact estimation by chemical methods of arsenic, zinc, copper, lead, sulphur dioxide and ash. Chemical tests are required for gelatin intended for special uses, e.g. photography (see Chapter 13, p. 208).

Acidity or alkalinity may be determined by titration. To estimate free acid, Kissling[5] soaks 30 grams of glue in 80 cc. of water for several hours and then drives over the volatile acid by a current of steam. When the distillate amounts to 200 cc., it is titrated with standard alkali, and titrate back the unused portion.

**Total Acidity.**

The total acidity of a glue may, of course, be determined directly by titration with 0.1 *N* NaOH solution, using phenol-

[5] *Chem. Z. 11,* 691.

phthalein or roseolic acid as an indicator. $H_2SO_3$ may then be determined by a separate titration with 0.1 *N* I solution. For more accurate work phenol red (effective range $p_H \simeq 6.8$ to 8.4) should be used as indicator. If the glue contains formaldehyde or other substances which react with iodine, $H_2SO_3$ must be determined by acidifying with $H_3PO_4$, distilling off the $SO_2$ in a current of steam or $CO_2$, and weighing it as $BaSO_4$. In the case of bone glues a direct titration of the acids other than $H_2SO_3$ may be made with 0.1 *N* NaOH, using as an indicator alizarin which, in the presence of at least 1 per cent. of glue, possesses the curious property of reacting only with strong acids and not with $H_2SO_3$.[6] Owing to legal restrictions the exact estimation of $SO_2$ and sulphates in gelatin is of great importance and will be referred to later.

**Determinations Involving Nitrogen.**

Clayton[7] considered the estimation of non-gelatinous substances the best single chemical test for glue. To determine non-gelatinous substances, C. Stelling[8] dissolved 15 grams of glue in 60 cc. of water made up to 250 cc. with 96 per cent. alcohol, and after thorough shaking, evaporated to dryness 25–50 cc. of the fluid filtered off after standing six hours. Trotman and Hackford[9] separated the hydrolyzed from the non-hydrolyzed products by precipitating the former by saturation with zinc sulphate and estimating them by the Kjeldahl method. H. J. Watson[10] does not regard the test as having any value.

R. H. Bogue[11] found that the ash and total nitrogen bore no consistent relation to the jelly strength of glues, but that the strongest glues showed the highest moisture content. Bogue made the following determinations on a series of hide and bone glues and a few other glue products:[12]

[6] Gutbier, Sauer and Brintzinger, *Kolloid Z. 29*, 130 (1921).
[7] *J. Soc. Chem. Ind. 21*, 670 (1902).
[8] *Chem. Z. 20*, 461; *Analyst 21*, 239 (1896).
[9] *J. Soc. Chem. Ind. 24*, 1072 (1904).
[10] *J. Soc. Chem. Ind. 23*, 1189 (1904).
[11] *Chem. Met. Eng. 23*, 61 et seq. (1920).
[12] For details regarding these methods see S. B. Schryver, "Allen's Comm. Organic Analysis," 4th ed., Vol. 8, pp. 467 et seq. Schryver recommends the addition of 2 cc. of diluted sulphuric acid (1 part concentrated acid to 4 parts water to each 100 cc. of mixed protein and sulphate solutions) for the protein precipitation, for which he used zinc sulphate. Bogue, using magnesium sulphate, found maximum precipitation to occur with ½ cc. of dilute sulphuric acid.

(1) *Total nitrogen,* by Kjeldahl's method.
(2) *Protein nitrogen,* from the precipitate formed on adding 50 cc. saturated magnesium sulphate solution to 50 cc. of water containing one gram of glue.
(3) *Protein-proteose nitrogen,* from the precipitate formed by saturating a similar glue solution with magnesium sulphate.
(4) *Proteose nitrogen,* difference between (3) and (2).
(5) *Amino nitrogen,* from the filtrate of (3), using Sørensen's formaldehyde titration method.

Bogue found that the temperature exercises a marked influence on protein precipitation, 3 to 8 per cent. more coming down at 17° than at 25°, but his determinations were made at 25° as this was more convenient.[13]

Bogue's results are given in the following table:

RELATION BETWEEN NITROGENOUS CONSTITUENTS AND JELL STRENGTH

| | *Grade* | *Protein N* | *Proteose N* | *Peptone N* | *Amino Acid N* |
|---|---|---|---|---|---|
| | $H_1$ | 92.2 | 6.3 | 1.1 | 0.4 |
| | $H_2$ | 90.4 | 7.0 | 2.0 | 0.6 |
| | $H_3$ | 86.2 | 12.0 | 1.4 | 0.4 |
| Hide and fleshing | $H_4$ | 84.6 | 12.4 | 2.6 | 0.4 |
| glues ..... | $H_5$ | 78.7 | 16.0 | 4.5 | 0.8 |
| | $H_6$ | 77.6 | 17.0 | 4.7 | 0.7 |
| | $H_9$ | 52.0 | 38.6 | 8.4 | 0.9 |
| | $B_1$ | 79.1 | 14.9 | 4.8 | 1.2 |
| | $B_2$ | 73.5 | 16.4 | 8.1 | 2.0 |
| | $B_3$ | 64.6 | 28.3 | 5.6 | 1.5 |
| | $B_4$ | 59.8 | 32.4 | 6.4 | 1.4 |
| Bone glues ..... | $B_5$ | 53.6 | 36.6 | 8.4 | 1.4 |
| | $B_6$ | 52.5 | 37.9 | 7.8 | 1.8 |
| | $B_7$ | 48.2 | 40.1 | 10.1 | 1.6 |
| | $B_8$ | 36.8 | 47.1 | 12.5 | 2.3 |
| | $B_9$ | 31.5 | 50.6 | 14.8 | 3.0 |
| *Special Glues* | | | | | |
| Russian isinglass.... | $H_2$ | 91.0 | 4.4 | 4.5 | 0.1 |
| Edible gelatin ..... | $H_3$ | 87.8 | 11.3 | 0.7 | 0.2 |
| Fish glue ..... | $B_9$ | 33.4 | 42.3 | 21.9 | 2.4 |
| Pressure tankage ... | $B_9$ | 34.3 | 46.4 | 16.3 | 3.0 |
| Peptone ..... | $B_9$ | 0.0 | 33.2 | 48.5 | 18.3 |

(Here 1 represents the highest grade and 9 the lowest.)

[13] Samples secured by empirical precipitation methods of this character, obviously represent mixtures of substances of variable composition.

These figures indicate that the jelly strength varies approximately as the protein nitrogen determined by Bogue's procedure. No consistent relation could be shown, however, between viscosity and nitrogenous constituents. The amino nitrogen is greater in bone than in hide glues, and tends to increase with decrease in jelly strength.

Bogue also treated a number of glues having uniform jelly strength (grade), but different viscosities, with various concentrations of magnesium sulphate from 50 per cent. down to 24 per cent. of saturation. Below that the precipitate was so finely subdivided and slimy that filtration was practically impossible. The results, given in the following table, show that there is no definite relation between viscosity and precipitate, except that

SHOWING PER CENT. OF NITROGEN THROWN DOWN BY VARYING PERCENTAGE SATURATIONS OF MAGNESIUM SULPHATE

| | *No.* | *Grade* | *Jell* | *Visc.* | *50 Per Cent.* | *35 Per Cent.* | *30 Per Cent.* | *28 Per Cent.* | *25 Per Cent.* | *24 Per Cent.* |
|---|---|---|---|---|---|---|---|---|---|---|
| Series 3..... | 1 | $H_4$ | 65 | 45.6 | 84.4 | 69.2 | 41.3 | — | — | — |
| | 2 | $H_4$ | 65 | 47.2 | 87.3 | 68.8 | 46.0 | — | — | — |
| | 3 | $H_4$ | 66 | 48.0 | 87.0 | 71.8 | 49.7 | — | — | — |
| | 4 | $H_4$ | 66 | 49.0 | 85.2 | 71.6 | 50.3 | — | — | — |
| | 5 | $H_4$ | 66 | 50.2 | 85.2 | 68.0 | 51.7 | — | 34.6 | 28.6 |
| | 6 | $H_4$ | 64 | 51.0 | 84.9 | 68.0 | 52.2 | — | 35.3 | 29.0 |
| | 7 | $H_4$ | 64 | 54.0 | 81.8 | 64.8 | 50.9 | — | 34.8 | 30.0 |
| | 8 | $B_2$ | 68 | 43.2 | 75.6 | — | 22.3 | — | — | — |
| | 9 | $B_2$ | 70 | 43.8 | 77.0 | — | 36.3 | — | — | — |
| | 10 | $B_2$ | 70 | 47.0 | 77.5 | — | 38.9 | — | — | — |
| | 11 | $B_2$ | 70 | 48.0 | 76.2 | — | 39.8 | — | — | — |

| | *No.* | *Grade* | *Jell* | *Visc.* | *50 Per Cent.* | *35 Per Cent.* | *30 Per Cent.* | *28 Per Cent.* | *25 Per Cent.* | *24 Per Cent.* |
|---|---|---|---|---|---|---|---|---|---|---|
| Series 4 ..... | 1 | $H_5$ | 64 | 45.4 | 81.8 | 58.2 | 44.2 | 39.2 | — | — |
| | 2 | $H_5$ | 64 | 47.4 | 87.3 | 68.8 | 46.0 | 42.1 | — | — |
| | 3 | $H_5$ | 64 | 50.6 | 74.0 | 60.6 | 49.2 | 45.8 | — | — |
| | 4 | $H_5$ | 65 | 46.2 | 85.4 | 62.9 | 47.3 | 39.5 | — | — |
| | 5 | $H_4$ | 65 | 47.8 | 87.2 | 65.1 | 46.6 | 40.9 | — | — |
| | 6 | $H_4$ | 65 | 48.0 | 75.6 | 57.6 | 44.7 | 44.7 | — | — |
| | 7 | $H_4$ | 65 | 49.4 | 76.5 | 59.0 | 47.2 | 45.3 | — | — |
| | 8 | $H_4$ | 65½ | 49.0 | 72.2 | 59.0 | 44.5 | 42.0 | — | — |
| | 9 | $H_4$ | 65½ | 50.0 | 86.0 | 66.9 | 52.8 | 44.0 | — | — |
| | 10 | $H_4$ | 65½ | 50.2 | 85.2 | 66.4 | 52.1 | 48.7 | — | — |
| | 11 | $H_4+$ | 66 | 47.4 | 83.0 | 63.1 | 47.7 | 42.5 | — | — |
| | 12 | $H_4+$ | 66 | 48.6 | 87.0 | 71.8 | 49.7 | 46.1 | — | — |
| | 13 | $H_4+$ | 66 | 48.8 | 85.2 | 71.6 | 50.3 | 46.1 | — | — |
| | 14 | $H_4+$ | 66 | 49.2 | 86.2 | 64.3 | 52.2 | 46.8 | — | — |
| | 15 | $H_4+$ | 66 | 49.6 | 85.2 | 68.0 | 51.7 | 49.0 | — | — |
| | 16 | $H_4+$ | 66 | 49.8 | 83.2 | 65.5 | 52.7 | 49.4 | — | — |

with about 30 per cent. saturation the precipitate varies as the viscosity. Bogue interprets these figures to mean that "if the jell strength be constant the viscosity will vary as the size of the protein molecule." By the "molecule" he means "a group which may not be subdivided except by chemical processes, as of hydrolysis, whereas the colloidal complex is established probably by electrical phenomena and the processes chemical condensation or hydrolysis are not involved."

Bogue also treated several glues having about the same viscosity, but varying jelly strengths, with 50 per cent. and 30 per cent. saturated magnesium sulphate. The precipitates showed the following percentage of nitrogen (Kjeldahl):

| *No.* | *Jell.* | *Viscosity* | *50 Per Cent.* | *30 Per Cent.* |
|---|---|---|---|---|
| 1 | 63 | 46.2 | 83.5 | 45.0 |
| 2 | 64 | 45.8 | 84.2 | 46.2 |
| 3 | 66 | 46.0 | 84.9 | 46.7 |
| 4 | 68 | 45.8 | 85.3 | 55.1 |
| 5 | 70 | 46.2 | 85.5 | 57.4 |

"It will be seen that at both 50.0 per cent. and 30.0 per cent. magnesium sulphate saturations, the nitrogen thrown down in the several precipitates varies directly as the jell strength, the viscosities being practically constant. This means then that at constant viscosity the jell strength will vary as the size of the protein molecule, as well as with the total amount of protein."

In the precipitation of purely empirical groups such as "gelatin," "gelatoses," and "gelatones" the larger molecular groups are for the most part thrown down more readily: but no quantitative relations are deducible from the amount of the precipitate, for the various fractions exercise a varying protective influence on each other, which accounts for the observations of Haslam [14] that part of any fraction remains in solution, while the precipitate may carry down part of a subsequent fraction.[15] With precipitates of this character we are obviously dealing with molecular groups rather than with simple molecules.

Upon investigating the crazing of glues, Bogue reports that this crackling up of the glue pieces is "due to an exceptionally great hydrolysis of the protein molecule and the consequent

[14] *J. Physiol. 32,* 267 (1905); *ibid., 36,* 164 (1907).

[15] The work of E. Zunz shows the great variation in the protective action of various albumose fractions.

inability of the resulting mixture to retain water above that minimum content below which crazing occurs." His analytical results hardly justify this conclusion, for 7 crazed glues showed an average of 11.99 per cent. moisture, while with 7 firm glues of equal grade the average moisture was 11.91 per cent. Although in the firm glues the average protein nitrogen was higher and the average proteose and peptone nitrogen lower than in the crazed glue, still one crazed glue had the highest protein and the lowest proteose and peptone nitrogen. It is true that only very low-grade glues craze, but some other factors must be reckoned with, probably differences inherent in the original raw material, for glue is no more a definite chemical entity than is gelatin. Presence of an excess of a fraction having excessive syneresis, or diminution of a fraction having protective action against a syneresis, might possibly account for crazing.

**Diffusible Nitrogen Test.**

The British Adhesives Committee [15a] evolved this test, which, they say supplies an indication of the stability of glues towards water, and furnishes a rough measure of their tensile strengths, the stronger glues generally being low in diffusible nitrogen.

The glue under test is made up into a jelly containing 2.1 grams of nitrogen in 75 cc. of water; this requires 15 grams of glue, approximately. The exact amount of glue is soaked over night in 75 cc. of water, heated to 37° C. for 2 hours, then to about 90° C. for 30 minutes (This procedure must produce marked hydrolysis. J. A.), and finally poured into a Petri dish 14 cm. in diameter, where it is allowed to set. One hundred cc. of water are now layered over the jelly and the dish is placed in a thermostat at 20° C. for 20 hours. The number of milligrams of nitrogen per 100 cc. of the supernatant fluid, as determined by Kjeldahl's method, constitutes the diffusible nitrogen number.

Since some of the constituents of the glue act as protectors to others and thus tend to peptize them (the Report even mentions this on p. 29), and since the value of the various hydrolysis products in this respect is not known, the investigation, to use the Committee's own words, is "admittedly incomplete, and the

[15a] First Report, p. 20 et seq., London, 1922.

subject requires further study. Again, the test may lend itself to the study of the size of the hydrated gelatin aggregate under various conditions. The addition to a glue solution of salts that lower the surface tension of water will tend to reduce the size of the gelatin aggregate, and presumably, consequently, to increase the amount of diffusable nitrogen." The test should also indicate the degree of hydrolysis.

The Committee tested the effect on joint or tensile strength and jelly strength of a series of sodium salts of organic acids, and also the effect of some sugars. Variations in viscosity complicate the joint tests, but sodium formate and salicylate and the sugars markedly increase the joint strength.

**Reactions of Gelatin.**[16]

Gelatin is totally insoluble in absolute alcohol, ether, chloroform, benzene, carbon disulphide, and fixed and volatile oils. It is practically insoluble in ice-cold 10 per cent. alcohol, and precipitates as a white coherent, elastic mass if an excess of alcohol is added to its aqueous solution. The precipitate swells in cold water and may be redissolved as before.

Fairbrother and Swan[16a] tested the "solubility of gelatin in cold water, and report the following results given in grams per 100 cc.: 0.02 at 0°, 0.07 at 18.3°, and 0.10 at 22°. The solubility in solutions of hydrochloric, sulphuric, nitric and acetic acids and in solutions of potassium and of sodium hydrates (concentrations varying from 0.2 to 5000 millimols per liter) were also determined. With acids the solubility passes through a minimum, rising then to about 0.2, after which solution gradually occurred. With alkalis similar results were obtained, but there was no minimum. Neutral salts decreased the solubility, their effect being approximately in the order of the Hofmeister series.

These experiments were done with Coignet's Gelatin Extra analyzing 2.24 per cent. ash and having a $p_H$ value in 1 per cent. solution of 5.6 at 20°. They should be repeated with ash-free gelatin properly freed from products of hydrolysis, for it is probable that only the hydrolysis products dissolve in cold water.

[16] See also Chapter 9. Most of the reactions apply to the more or less impure gelatin known as glue.

[16a] F. Fairbrother and E. Swan, *J. Chem. Soc.* *121*, 1273-44 (1922).

According to Zlobicki[16b] 0.5-0.8 grams of gelatin to 100 cc. of water causes a marked lowering of the surface tension of water, although further addition does not increase the effect.

As has been pointed out by Victor Lehner[17] selenium oxychloride readily dissolves glue and gelatin in the cold. This remarkable solvent likewise dissolves resins (natural and synthetic), rubber, shellacs, and asphalt.

Solutions of gelatin in strong acetic acid do not gelatinize on cooling and are used as liquid glues. Warming with dilute nitric acid yields a liquid product, but strong nitric acid destroys gelatin, giving oxalic acid and other substances. Gelatin may also be held in fluid condition by urea, zinc, magnesium, and calcium chlorides, sodium iodide and sodium and calcium nitrates, naphthalene sulphonate, etc.

Gelatin is completely precipitated by saturating its aqueous solution with ammonium, zinc, or magnesium sulphates. Phosphomolybdic and phosphotungstic acids also precipitate it, as do tannin and sufficient quantities of mercuric chloride and of picric acid. The reaction with tannin is used to detect gelatin (or vice versa), a 0.02 per cent. solution of gelatin yielding a white or buff-colored precipitate which is insoluble in presence of an excess of tannin. Without such excess the precipitate tends to dissolve in pure water, especially if hot, apparently going into colloidal solution because an excess of gelatin acts as a protector or dispersing agent toward the tannin precipitate.

Ruffin[18] proposed to determine gelatin by precipitating it with tannin, and titrating the excess of tannin with iodine.

The evidence shows, however, that the so-called "tannate of gelatin" is not a substance of definite composition. Thus H. Trunkel[19] found that 1 gram of freshly dissolved gelatin is precipitated by 0.7 grams tannin, but after standing 24 hours 0.4 tannin will precipitate it. On rewarming the original condition returns. Any excess of tannin up to 3 parts per unit of gelatin is carried down, but upon washing the precipitate with alcohol, 97 per cent. of the tannin may be removed. Trunkel's conclusion is that the gelatin tannin complex is an adsorption compound.

[16b] *Bull. Acad. Sci. Cracovie,* 1906, p. 497.
[17] *J. Am. Chem. Soc. 43,* 29 (1921).
[18] *Chem. Z. 24,* 567 (1900).
[19] *Biochem. Z. 26,* 458 (1910).

J. T. Wood [20] quotes much of the prior work on compounds of tannin and gelatin, going back to experiments of Humphry Davy,[21] who found that variable quantities of tannin were fixed by isinglass. Wood, using Coignet's Gold Label Gelatin, found that the greatest amount of tannin which could be precipitated by 1 gram of air-dry gelatin was about 2.4 grams from a solution containing about 6 grams of tannin, the volume of the solution after the addition of gelatin being 150 cc. Chromed gelatin absorbs just as much tannin as unchromed gelatin. Chromed hide powder is used for the assay of tanning materials. See Chapter 9 for the action of tanning substances on gelatin, and Chapter 8 for the effect of $CS_2$.

The halogens, chlorine, bromine and iodine react with gelatin, yielding insoluble compounds which form the basis of analytical methods. The chlorine compound described by Allen [22] is a pale, yellowish-white powder, which is odorless, tasteless and imputrescible, and insoluble in water or alcohol, but soluble in alkalis. Yet Allen found that the bromine precipitation method could not be applied to commercial gelatin and glue, which "yielded results which at present are incapable of interpretation. The completeness of the precipitation of gelatin by bromine-
ater is affected by conditions not at present understood. In
me cases the precipitation was very complete, while in other
periments, in which the conditions were but very slightly
ried, much nitrogen remained unprecipitated."

Since glues and gelatins may vary considerably in composition
d in their protective action toward the bromine compound, and
nce their "previous history" may affect their ability to form
lsorption compounds, it is not surprising that Allen reported
nfusing results.

Platinic chloride and sulphate give precipitates with gelatin,
d Crismer recommends an acid solution of chromic acid as a
ecipitant.

Northrup [23] followed the hydrolysis of gelatin by pepsin,
ypsin, acid, and alkali, and found that the early action of the
zymes and alkali were similar but different from the action of

[20] *J. Soc. Chem. Ind. 25,* 384 (1908).
[21] *Phil. Trans.* 1803, p. 233.
[22] "Comm. Organic Analysis," 4th ed., Vol. 8, p. 591.
[23] J. H. Northrup, *J. Gen. Physiol. 4,* 7 (1921).

acid. Comparing the relative velocities of hydrolysis of different peptide linkages, he found that trypsin would attack all those linkages that pepsin attacked, and some others besides. Linkages rapidly attacked by pepsin yielded only slowly to trypsin, while those most rapidly attacked by the enzymes yielded readily to alkali but slowly to acid.

For a discussion of the cleavage products of gelatin, including those resulting from bacterial decomposition, see "Allen's Commercial Organic Analysis," 4th ed., Vol. 8, pp. 594 et seq.

According to Seemann [24] oxidising agents, like permanganates, yield with gelatin such products as oxalan, $NH_2.CO.NH.C_2O_2NH_2$; ammonium oxaminate, $C_2O_3.NH_2.NH_4$; ammonium oxalate; and oxalic, succinic, benzoic, butyric, acetic, and formic acids. Sometimes benzaldehyde, propionic and valerianic acids are produced.

*Detection of Glue and Gelatin.* According to Allen [25] the property of gelatinizing on cooling is the only test from which the presence of gelatin in a complex animal liquid can be safely inferred. To detect gelatin in ice-cream or in cream, the U. S. Department of Agriculture use Stokes' picric acid method,[26] which is as follows: Dissolve 5 grams of mercury in 10 grams of nitric acid (sp. gr. 1.42), and dilute to 25 times its bulk. To 10 cc. of this solution add 10 cc. of cream and 20 cc. of water, in order to precipitate all proteins except gelatin. If gelatin be present, the filtrate will give an immediate yellow precipitate with an equal part of a saturated aqueous solution of picric acid. The nitrate solution should give no turbidity with the picric acid solution. This test will detect one part of gelatin in 10,000 parts of water.

To detect gelatin in preserves, A. Desmoulière [27] takes 20 grams of the sample and precipitates the gelatin by gradually adding 100 cc. of 90 per cent. alcohol.[27a] After standing 2–3 hours, the supernatant fluid is decanted, the residue dissolved in hot water, and tested with picric acid and tannin which give pre-

[24] *Zentr. Physiol. 18,* 285 (1904).

[25] "Comm. Organic Analysis," 4th ed., Vol. 8, p. 592.

[26] *Analyst,* 1907, p. 320.

[27] *Ann. Chim. anal. appl. 7,* 201 (1902).

[27a] Gelatin is somewhat soluble in 90 per cent. alcohol, and the preserve usually contains water; therefore stronger alcohol should be used. Since gelatin precipitates most readily at the isoelectric point, the solution should have a $p_H$ value of about 4.7.

cipitates in the presence of gelatin. A confirmatory test is to add quick-lime, which evolves ammonia.

Henzold [28] proposed the following method for detecting gelatin in foods: The specimen is boiled with water and the filtrate boiled with an excess of 10 per cent. potassium dichromate. If gelatin be present, a few drops of concentrated sulphuric acid produces a white flocculent precipitate, which gradually agglomerates at the bottom of the vessel.

E. Schmidt [29] makes a reagent said to be sensitive for glue in the presence of ammonia, by acidifying Nessler's reagent slightly with sulphuric acid. This gives a red precipitate which is filtered off, leaving a clear yellow solution which constitutes the reagent.

Dürbeck [30] uses a solution of thionin (a thiazin dye) to detect gelatin or agar in sausage. Agar gives a violet color, gelatin a deep blue.

**Gold Number.**

Glue and gelatin possess such a powerful action as colloidal protectors, that a determination of the "gold number" after Zsigmondy's method [31] may demonstrate their presence, at least differentially. The "gold number" is the number of milligrams of a colloidal substance which just fails to prevent the color change (from bright red to violet) of 10 cc. of a colloidal gold solution upon the addition of 1 cc. of 10 per cent. NaCl. The colloidal gold solution is prepared as follows:

One hundred and twenty cc. of distilled water, condensed in a silver worm, are placed in a 300–500 cc. Jena glass beaker. While heating there are added 2.5 cc. of a 0.6 per cent. solution of gold hydrogen chloride and 3–3.5 cc. of 0.18 *N* potassium carbonate solution, both of the highest purity. After boiling add promptly 3.5 cc. of dilute formaldehyde (0.3 cc. of commercial 40 per cent. formal to 100 cc. water). A bright red color should develop slowly, usually beginning as a brown or orange tint. Jena or equivalent glass should be used throughout, both in preparing the solutions and in making tests with them.

[28] *Z. offentl. Chem.* *6*, 292 (1900).
[29] *Farber Z.* *24*, 97.
[30] *Z. Nahr. und Genüssm.* *27*, 801.
[31] "Colloids and the Ultramicroscope," New York, 1909; *Z. anal. Chem.* *40*, 697 (1901).

The following table gives the gold numbers of some proteins according to Zsigmondy and Schryver:

| *Substance* | *Gold Number* |
|---|---|
| Gelatin | 0.005–0.01 |
| Russian glue | 0.005–0.01 |
| Isinglass | 0.01 –0.02 |
| Casein (in ammonia) | 0.01 |
| Egg-globulin | 0.02 –0.05 |
| Ovomucoid | 0.04 –0.08 |
| Glycoprotein | 0.05 –0.1 |
| Amorphous egg-albumen | 0.03 –0.06 |
| Crystallized egg-albumen | 2.0 –8.0 |
| Fresh egg white | 0.08 –0.15 |
| Gum arabic | {0.15 –0.25<br>{0.5 –4.0 |
| Gum tragacanth | 2.0± |
| Dextrin | 6.0 –20.0 |
| Wheat starch | 5.0 ± |
| Potato " | 25.0 ± |
| Sodium oleate | 0.4 –1.0 |
| Sodium stearate at 100° | 0.01 |
| " " at 60° | 10.0 |
| Deutero-albumose | ∞ |
| Cane sugar | ∞ |
| Urea | ∞ |
| Stannic acid sol (old) | ∞ |

W. Menz [31a] found that dilution increased the protective action of gelatin sols, which he believed to be due to the gelatin amicrons, a view confirmed by the ultramicroscopic studies of Elliott and Sheppard.[31b]

C. A. Smith [32] suggests a polariscopic method for detecting the presence of gelatin. He says: "A progressive increase in levoration (or mutarotation) obtained from a solution cooled quickly 35° C., accompanied by the production of a jelly after a change of approximately 4.7° V., is very positive proof of the presence of gelatin in any solution concentrated enough to jelly."

A. D. Little [33] has found the following qualitative tests satisfactory:

To a water solution of the material to be tested add a slight excess of acetic acid, cool thoroughly and then add a concentrated solution of tannic acid containing about 10 per cent. of common salt. The tannic acid-salt solution should be freshly filtered before use. In the presence of glue this gives a grayish yellow flocculent precipitate. It is to be remembered, of course, that

[31a] *Z. physik. Chem. 68,* 129 (1909).
[31b] *J. Ind. Eng. Chem. 13,* 609 (1920).
[32] *J. Ind. Chem. Eng. 12,* 878 (1920).
[33] Private communication.

casein and certain other soluble protein substances may give a similar reaction.

*Glue in Paper.* Boil several grams of the paper with water until the volume of the solution is only a few cc. Filter and cool thoroughly; add an equal volume of a cold 10 per cent. salt solution nearly saturated with tannic acid and freshly filtered. A light grayish yellow flocculent precipitate indicates glue (or casein). In case the paper contains starch and gives a precipitate by the above test, the test should be repeated after first hydrolyzing the starch in the water extract of the paper by means of hydrochloric acid. This is accomplished by adding sufficient HCl to give a 2 per cent. solution and adjusting on the steam bath until all the starch is converted to dextrose, or in other words, until a drop of the solution, when added to 5 cc. of very dilute iodine solution, gives no blue color.

It is important, in applying these qualitative tests, that the solutions be cold.

There are here given in condensed form essentially the methods of the Association of Official Agricultural Chemists for determining metals, etc., in gelatin.[34]

*Ash.*—Ash at low redness preferably in a muffle until the ash is white or grayish white. (Gelatin intumesces violently, and due care must be used.)

*Total Phosphorus.*—Treat the ash obtained as above with 2–3 cc. of nitric acid (sp. gr. 1.42) and evaporate on the steam bath. Repeat the nitric acid treatment and take up in hot water containing a few drops of nitric acid. Precipitate and weigh the phosphoric acid as *magnesium pyrophosphate,* according to usual analytical methods.

*Nitrogen.*—Determine by the Kjeldahl method, or by the modifications of this method by Gunning and Arnold.

*Arsenic.*—Heat 20 grams of gelatin with 75 cc. of arsenic-free hydrochloric acid, 1–3, in a covered vessel until all insoluble matter has flocculated and the gelatin dissolved. Add an excess of bromine water (about 20 cc.), neutralize with ammonium hydroxide; add either about ½ cc. of 85 per cent. phosphoric acid or 2 grams of sodium phosphate ($Na_2HPO_4.12H_2O$), or

[34] For complete details see "Assoc. Official Agricult. Chem. Methods," 1920. I must thank C. R. Smith of the U. S. Dept. of Agriculture, Bureau of Chemistry, for information supplied.

crystallized sodium ammonium phosphate and allow to cool. Precipitate the arsenic along with the phosphoric acid by an excess of magnesia mixture. The phosphoric acid or compound used should require about 20–25 cc. of the usual magnesia mixture for precipitation. After standing about an hour, wash the precipitate several times with dilute ammonium hydroxide, drain well and dissolve in dilute hydrochloric acid, 1 to 3, to 50 cc. volume, in a graduated flask. Take a 25 cc. aliquot and determine the arsenic as directed below. Run a blank determination with the sample. Arsenic impurities, if present, are usually found in the phosphate added.

The apparatus for determining arsenic consists of a 2-ounce wide-mouth bottle, closed with a thoroughly cleaned perforated rubber stopper in which is inserted a glass tube 1 cm. in diameter and 6 cm. long. A second tube like the first is connected with it by a clean rubber stopper, and on the top of this second tube is mounted in like manner a third narrower tube 3 mm. in diameter and 12 cm. long. Into the several tubes introduce the following:

*First (lowest) tube.*—A rolled piece of heavy filter paper, size about 4.5 x 16 cm., which has been soaked in 20 per cent. lead acetate solution, and dried.

*Second tube.*—Pack loosely with absorbent cotton, soaked in 5 per cent. lead acetate solution and squeezed to remove any excess. Since the test involves comparison of stains, all tubes used should have cotton of uniform moisture content.

*Third (top or narrow) tube.*—A strip exactly 2.5 mm. wide and about 12 cm. long of heavy drafting paper (similar to Whatman's cold pressed) which has been soaked for one hour in a 5 per cent. solution of mercuric bromide in 95 per cent. alcohol, squeezed free from excess solution of mercuric bromide and dried on a glass rod. Both ends of the strip should be trimmed off before using.

Place 25 cc. of the gelatin solution prepared as above described in the 2-ounce bottle and add 20 cc. of dilute arsenic-free hydrochloric (1–3) acid. Warm to 90° C., add 3 drops of stannous chloride solution (40 grams stannous chloride made up to 100 cc. with concentrated hydrochloric acid). Heat for 10 minutes and then cool the bottle and its contents in a pan containing water and ice, to a temperature of about 10° C.

When cold, add about 15 grams of arsenic-free zinc in sticks about 1 cm. long, and *immediately* connect up the chain of tubes to the bottle.

Keep the bottle in the iced bath for 15 minutes to retard the evolution of gas, and then remove it and allow the action to proceed for one hour more. Remove the sensitized mercuric bromide paper, and compare the stain with similar ones produced under identically the same conditions with known amounts of arsenic.

A standard arsenic solution is made by dissolving one gram of arsenious oxide in 25 cc. of 20 per cent. sodium hydroxide solution, neutralizing with dilute sulphuric acid, adding 10 cc. of concentrated sulphuric acid, and making up to one liter with recently boiled distilled water. One cc. of this solution contains 1 mg. of arsenious oxide ($As_2O_3$). It may be diluted as desired; e.g., if 20 cc. be made up to 1 liter, and 50 cc. of this dilute solution be further diluted to 1 liter, there results a solution containing 0.001 mg. of arsenic oxide ($As_2O_3$) per cc. Such dilute solutions must be freshly prepared just before using.

*Copper.*—Hydrolize 50 grams of gelatin with 150 cc. of dilute hydrochloric acid, 1 to 3, as directed under arsenic, heating about 2 hours on the steam bath. To facilitate filtration and separation from zinc and iron later, use the phosphoric acid or compound and magnesia mixture as before. Precipitate with hydrogen sulphide in a slightly ammoniacal solution. Allow the precipitate to settle, filter and wash with 5 per cent. ammonium chloride solution saturated with hydrogen sulphide. Dissolve off the zinc and iron sulphides, magnesium phosphate, etc., in 75 cc. of dilute hydrochloric acid (4 per cent. HCl) saturated with hydrogen sulphide. Digest both filter and copper sulphide with 4 cc. of concentrated sulphuric acid and sufficient nitric acid until the residue is perfectly colorless and fuming freely. Take up with water, add 5 cc. bromine water, boil off the bromine, and determine copper by titrating with 0.01 *N* sodium thiosulphate.

*Lead.*—If lead is present, it is shown as the sulfate mixed with some silica when the sulphuric acid residue is diluted with water in the above determination. Add an equal volume of alcohol and allow to stand several hours. Filter and wash with dilute alcohol. Evaporate the filtrate to remove alcohol and determine copper as directed above.

Dissolve the lead sulphate from the filter with 10 cc. of hot 50 per cent. ammonium acetate solution alternated with hot water until the filtrate measures about 75 cc. Add potassium dichromate solution to precipitate the lead as chromate, filter on a dry Gooch at 125–150° C. and weigh. Calculate to metallic lead using the factor 0.641.

*Zinc.*—Determine the zinc in the filtrate from the copper determination as follows:

Boil the filtrate containing the zinc, to expel hydrogen sulphide, add a drop of methyl orange indicator and 5 grams of ammonium chloride and make alkaline with ammonium hydroxide. Add dilute hydrochloric acid drop by drop, until the reaction is faintly acid, then add 10–15 cc. of 50 per cent. sodium or ammonium acetate solution and pass in hydrogen sulphide for a few minutes until the precipitation is complete. Allow the precipitate to settle, filter until clear, and wash the precipitate twice with hydrogen sulphide water. Dissolve the precipitate on the filter with a little hydrochloric acid (1–3), wash the filter with water, boil the filtrate and washings to expel hydrogen sulphide, cool and add a distinct excess of bromine water. Then add 5 grams of ammonium chloride and ammonium hydroxide until the color, caused by free bromine, disappears. Add hydrochloric acid (1–3), drop by drop, until the bromine color reappears, then add 10–15 cc. of sodium or ammonium acetate solution (50 per cent. by weight) and 0.5 cc. of ferric chloride solution (10 per cent.) or enough to precipitate all the phosphates. Boil until all the iron is precipitated.

Filter while hot and wash the precipitate with water containing a little sodium acetate. Pass hydrogen sulphide into the combined filtrate and washings until all the zinc sulphide, which should be pure white, is precipitated; filter upon a tared Gooch crucible, and wash with hydrogen sulphide water containing a little ammonium nitrate. Dry the crucible and its contents in an oven, ignite at a bright red heat, cool and weigh as zinc oxide. Calculate the weight of metallic zinc using the factor 0.8034.

The zinc may also be determined direct from the filtrate from the copper determination, by proceeding as directed in the second paragraph of the alternate method for copper and zinc, given below, beginning "Boil the filtrate," etc.

*Alternate Method for Copper and Zinc.*—Hydrolize 20–50 grams of gelatin with 100 cc. of dilute hydrochloric acid, 1 to 3, for two hours on the steam bath. Add 5 mg. of iron from 5 cc. of a standard solution of ferrous sulphate (4.9 grams of ferrous sulphate to a liter containing 10 cc. of sulphuric acid). Make solution faintly ammoniacal and saturate with hydrogen sulphide. Filter the sulphides and wash 2 or 3 times with a very dilute solution of colorless ammonium sulphide (saturate a solution of 1 cc. of concentrated ammonium hydroxide in 200 cc. of water). Dissolve the sulphides in 20 cc. of hot, dilute nitric acid, 1 to 1, and wash the filter and insoluble matter with water. Add 10 cc. of dilute sulphuric acid, 1 to 3, and evaporate all the nitric acid. Cool and add 40 cc. of water. When the soluble salts are in solution filter off silica, washing filter thoroughly with water. Saturate the filtrate with hydrogen sulphide. Heat the solution 5 minutes on the steam bath. Filter the copper sulphide on a carefully prepared Gooch crucible and wash with hydrogen sulphide water. Dry and ignite to copper oxide.

Boil the filtrate to expel all hydrogen sulphide. Make the solution strongly ammoniacal and then acidify with 15 cc. of 50 per cent. formic acid. Filter off any insoluble matter such as alumina, etc., while hot, then pass in a rapid stream of hydrogen sulphide for 10 minutes. Warm solution 15 minutes on the steam bath, remove and allow to stand for 30 minutes before filtration. Filter the zinc sulphide on a carefully prepared Gooch crucible with a very gentle suction, washing with 2 per cent. ammonium thiocyanate. Dry and ignite at the highest temperature of a Bunsen burner. Cool and weigh the zinc oxide.

*Polariscopic Constants.*—Prepare a concentration of 3 grams per 100 c. by soaking 3 grams of the sample in 40–50 cc. of cold water for about 15 minutes, heating to complete solution at about 50° C. and making to volume at 35° C. Polarize at 35° C. in a 2 dm. tube using the Ventszke scale.

Cool a portion of the gelatin solution rapidly to 10–15° C. and pour into cold dry 1 dm. tubes before jelly has time to form. Place the tube in a constant temperature bath at 15° C., and polarize after 18 hours to obtain equilibrium rotation at 15° C. Double the reading to place it on a basis of 2 dm. tube.

In order to polarize cloudy samples, digest the original 100 cc. in a stoppered flask with roughly 10 cc. of lightly powdered mag-

nesium carbonate for at least one hour at 35–40° C. and filter until clear through a folded filter, avoiding unnecessary evaporation. (This produces considerable hydrolysis J. A.)

The increase in levorotation (*mutarotation*) between 35° and 15° is an index of the jelly strength developed.

*Sulphur Dioxide.*—Proceed as in the distillation method described below, or by diffusion method as follows: Cool, in ice water, a vessel containing 100–150 cc. of water, 5 cc. of dilute hydrochloric acid, 1 to 3, 10 grams of sodium chloride and some filtered starch solution. Add a few drops of 0.01N iodine until a blue color is produced. Pour this mixture on 5 grams of powdered gelatin sample contained in a stoppered flask, replacing in the ice water. After remaining for 2 minutes with occasional mixing, add 0.01N iodine until the blue color is restored. Replace in the ice water for one minute, remove and titrate to the reappearance of the color. Repeat these operations until the color persists for one minute.

One cc. of 0.01N iodine is equivalent to 0.32 miligram of sulphur dioxide.

*Distillation Method.*—Dissolve 20 grams of gelatin in 300 cc. of recently boiled water, add 5 cc. of a 20 per cent. glacial phosphoric acid solution, and distil in a current of carbon dioxide until 150 cc. have passed over. (The current of carbon dioxide may be replaced, without material error, by using double the quantity of phosphoric acid and immediately before connecting up the condenser, dropping in a lump of sodium bicarbonate weighing less than one gram.)

The distillate should be collected in 100 cc. of nearly saturated bromine water, the condenser end dipping below its surface. On completion of the distillation boil off the excess bromine, dilute the solution to about 250 cc., add 5 cc. of hydrochloric acid (1 to 3), heat to boiling, and precipitate the sulphuric acid with 10 per cent. barium chloride solution. Boil for a few minutes longer, allow to stand overnight in a warm place, filter on a tared Gooch crucible, wash with hot water, ignite at a dull red heat, and weigh as barium sulphate.

Irving Hochstadter [35] advises the use of 27.3 grams of gelatin. The number of milligrams of $BaSO_4$ then indicate directly the number of parts per million of $SO_2$. The $CO_2$ should be washed

[35] Private communication.

through $CuSO_4$ solution to eliminate sulphides. He distils over 200 to 250 cc. and catches it in 25 to 50 cc. $\frac{N}{20}$ iodine solution.

The former official method was to collect the distillate in standardized iodine solution, and then determine the excess of iodine by titration with standardized sodium thiosulphate. This method was severely criticized [36] and was abandoned. Parts of animals slaughtered under Government supervision as well as gelatin made therefrom and gelatin containing no added sulphur dioxide or sulphites, all showed *apparent* sulphur dioxide. C. Mentzel [37] found in pure chopped meat from 0.0014 to 0.0021 per cent. of apparent sulphur dioxide equivalent to from 0.0054 to 0.0084 per cent. of sodium sulphite. When onions were added to the chopped meat, the percentage of apparent $SO_2$ was largely increased, probably owing to the presence of allyl sulphide.

The substance responsible for apparent $SO_2$ in meat, gelatin, etc., is possibly the sulphur-containing protein cystine. It should be noted that, while meat contains about 70 per cent. of water (besides much fat), gelatin contains only about 15 per cent. of water, and in it all water-soluble substances would naturally be concentrated. Meat may absorb $SO_2$ from the atmosphere,[38] and Poetschke (*loc. cit.*) found the same condition with gelatin. Another possible source of error was pointed out by Baythein and Bohrisch,[39] who observed that the limestone or marble used to generate $CO_2$ sometimes contains sulphides, and the gas should be washed with copper sulphate.

From the results of the analysis of over 1,000 samples Poetschke reports as follows: [40]

$SO_2$ CONTENT OF SAMPLE

| *Year* | *Less than 100 parts per million* | *From 100 to 500 parts per million* | *Over 500 parts per million* |
|---|---|---|---|
| 1907 | 19.65 | 44.87 | 35.48 |
| 1908 | 60.62 | 18.61 | 20.70 |
| 1909 | 66.64 | 18.92 | 14.44 |
| 1910 | 66.47 | 23.92 | 9.60 |
| 1911 | 87.90 | 9.16 | 2.94 |
| 1912 | 48.27 | 42.65 | 9.08 |

[36] J. Alexander, *J. Am. Chem. Soc. 29*, 783 (1907); E. Gudeman, *J. Ind. Eng. Chem.*, Vol. I, No. 2 (1909); P. Poetschke, *J. Ind. Eng. Chem.*, Vol. 5, No. 12, (1913).

[37] *Zeit. für Untersuch. Nahrungs und Genuss. 11*, 320 (1906).

[38] A. Kickton, *Zeit. für Untersuch Nahrungs Genuss. 11*, 324 (1906).

[39] *Zeit. für Untersuch Nahr. und Genuss. 5*, 401 (1902).

[40] The figures given are the percentage of the total number of samples.

Poetschke also found free chlorine in the $CO_2$ which would cause error by oxidizing $SO_2$; it is also removed by washing through copper sulphate. He prefers the use of iodine instead of bromine in the gravimetric method for determining $SO_2$, and finds Gudeman's steam distillation method shows no advantage. Some samples of gelatin contained hydrogen peroxide evidently added to oxidize the $SO_2$.[41]

As a result of these analytical uncertainties, traces of sulphur dioxide are disregarded in practically all jurisdictions. The figures of Poetschke, including foreign as well as domestic gelatins, show a very creditable and successful effort on the part of gelatin manufacturers to comply with the official regulations.

[41] Irving Hochstadter (U. S. Pat. 1,412,523, dated April 11, 1922), has protected the process of bleaching foods (including gelatin) with $SO_2$, and then oxidizing any traces of this gas or of sulphites that may remain into sulphates by means of $H_2O_2$ or other peroxide.

# Chapter 11.

## Technology of Glue and Gelatin.

The technical operations in the manufacture of glue and gelatin may be grouped under the following heads:

1. Stock or raw material and its treatment prior to boiling.
2. "Boiling" or cooking, that is preparing a solution of glue or gelatin from the treated stock by the action of hot water.
3. Clarifying, bleaching, filtering, evaporating or otherwise treating the dilute glue liquor.
4. Chilling the glue liquor to a jelly.
5. Cutting, spreading and drying the jelly.
6. Packing, breaking, or grinding the dried glue.
7. Testing, grading, and selecting or blending the finished product.

The treatment of glue stock varies considerably, but the operations of glue manufacture subsequent to boiling, are along the same general lines in most factories, although there are differences in apparatus and in chemical and mechanical treatment.

Special machinery may effect the consolidation of several, or the elimination of one or more of the above operations. Thus, if the glue is dried on a heated rotating drum, the operations of chilling, cutting and spreading are eliminated, the glue liquor being fed directly to the roll or drum. G. Illert[1] describes a complete plant (which he claims can be operated by three men), in which a concentrated glue-foam is fed automatically to a drying roll, the dry glue passing directly thence to the packing apparatus.

### Glue Stock.

The most motley array of raw materials find their way to the glue factory. They include slaughter-house refuse such as heads, feet and bones from the canning department; butchers' refuse;

[1] "Die neuzeitliche Einrichtung und der Betrieb einer Lederleimfabrik," *Chem. App.* 8, 78 (1921).

dried bones (junk bone) which come largely from South America or India; bones from garbage; clean bone trimmings from button and handle manufacturers; horn pith (the cornellion or interior bony support of the horn); trimmings of hides and skins such as raw hide, calves' pates, tannery trimmings and fleshings; shreds of rabbit, hare, nutria and other skins from the hatters' fur cutting industry; sinews and pizzles.

Glue stocks (and the glues which they yield) may be divided into four groups.

1. Bone stock. Besides the kinds above mentioned, this includes: *ossein,* which is degreased granulated bone from which the soluble lime salts have been leached by acid; *"spectacles" (knochen-brillen, dentelles),* a variety of ossein showing the round holes from which buttons have been cut; *acidulated horn pith,* a variety of ossein showing the original shape of the horn. Acidulated bone (ossein and horn pith) yield very high-grade glues and gelatins, fully equal to those produced from the finest hide stock.
2. Hide stock. This includes such curious things as old Turkish raw hide moccasins, lips and ears, hide bale wrappings, and discarded loom-pickers worn out from incessant impact of the shuttle. *Dried-hide* stock may have been limed before drying; thus *guaras negras* is dried unlimed, while *guaras blancas* is dried limed hide stock from South America. *Wet* or *green hide* stock is usually salted or limed to preserve it, but wet limed stock will not stand long transportation unless the temperature is low.
3. Sinew stock. Sinews are imported in dried state, but local sinew stock is usually shipped in green salted condition. With the imported dried sinew stock are often included dried bull's pizzles.
4. Tanned stock. In recent years processes have been perfected for making glue from leather waste.

**Treatment of Glue Stock.**

Before describing in detail the steps in the treatment of glue stock prior to boiling, it should be borne in mind that with some stocks the complete cycle of operations is unnecessary, while with other stocks special treatment is required.

Hide and acid leached bone stocks are limed, but rabbit skin stock is merely soaked. Many manufacturers cut or shred their hide stock so that it may be limed and extracted more readily. Sinews and pizzles are limed and treated like hide stock.

The treatment of bones varies considerably, depending on the facilities of the plant and the products desired. The finest bone glues and gelatins are made by first converting the bone into ossein, which is accomplished by an acid leaching process (see p. 156). Many packers and slaughter houses aim to secure mainly "steamed bone," and submit the fresh bone to one or two extractions in a pressure tank whereby most of the grease and a large part of the glue are incidentally obtained. Plants operating on dried or junk bone usually granulate the bone and recover the remaining grease with volatile solvents, after which the glue is extracted. The poorest bone glues are usually made by the unskillful "boiling" of uncleaned and frequently unwashed garbage or slaughter-house bone. Glues made from bones that have not been degreased, usually contain considerable grease.

In general all salted stock is washed free from salt, limed stock, if old, is washed to get rid of old carbonated lime, and dried stock is soaked to soften and swell it. The glue manufacturer must be on the alert to discover adulterations—thus a precipitate of barium sulphate is sometimes formed on hide stock to add to the weight. Even preservatives and disinfectants legitimately used may cause trouble; for example, arsenic is often used in curing hides, and the customs regulations of some countries require disinfection against anthrax, for which purpose bichloride of mercury, formaldehyde or sulphur dioxide may be officially prescribed.

The following diagrammatic table shows the usual schemes of handling various stocks:

*Bone Stock.*

| | |
|---|---|
| Green, fresh or packer bone<br>Refuse, town, or garbage bone | a. Wash and boil.<br>b. Wash, grind, degrease and boil. |
| Dried or junk bone<br>Steamed bone | c. Wash, grind, degrease, acidulate, lime, wash, neutralize and boil. |
| Acidulated bone (ossein)<br>Acidulated horn pith | Soak, lime, wash, neutralize and boil. |

*Hides and Sinew Stock.*

| | |
|---|---|
| Green or fresh trimmings or fleshings, also the same stock partly limed<br>Salted hide or sinews | Wash, lime, wash, neutralize and boil. |
| Dried hide pieces or fleshings (whether previously limed or not) | Soak, wash, lime, wash, neutralize and boil. |

*Leather or Tanned Stock.* Soak, detan, lime, neutralize and boil.

Modern practice is to cut up hide and leather stock to render subsequent operations more rapid.

*Water Supply and Sewage Disposal in the Glue and Gelatin Factory.* Whatever salts or other non-volatile impurities exist in the water with which the glue solution is made, will be found in more concentrated state in the dried product. Where such impurities accumulate in a steam boiler, they may be largely removed by blowing down the boiler from time to time; but there is no practical way of removing them from the glue solution.

The importance of pure water in a gelatin factory, is, therefore, obvious, and for especially pure products filtration or even distillation may be advisable.

An abundant supply of water is essential, as well as adequate provision for the disposal of the large volume of wash-water which may cause difficulty owing to the fact that at times it is liable to putrefy. Putrid glue has a peculiarly nauseating odor, and the sewage from a glue factory requires even more consideration than that from a tannery.

## Methods and Apparatus for Preparing Glue Stock.

**Bone Stock.** To remove metallic and other impurities which would otherwise cause costly damage to machinery or contaminate the glue or gelatin, the bones are first sorted and passed before a powerful electro-magnet which removes bits of iron concealed by fat or dirt.

The bones then pass to the crushing or granulating machines. In Europe heavy-toothed steel rolls driven by powerful spur gears are generally used for crushing, but high-speed percussion mills

may be used providing the bone dust is sifted out. Stamp mills are also used.

Bones are often given a preliminary boiling to remove the major portion of the grease.

For the extraction of grease from the crushed bone, petroleum, benzine or naphtha is generally employed, although coal tar benzene, carbon tetrachloride, or carbon bisulphide may be used. According to H. G. Bennett, the petroleum fraction boiling at about 212° F. is now used in most British factories. All of it must be volatile at 280° F., and the last traces are blown out of the bones with steam at 80 lbs. pressure. L. Thiele [2] reports the use for bones of a mixture of 20 parts benzol, 60 parts toluol, and 20 parts xylol, and gives distillation tables of this and of three satisfactory benzines. A suitable benzine, according to Thiele, should have a sp. gr. of 0.745 and boil at about 100° C.; 99 per cent. should distil over at 130° C. and any balance between 130° and 140° C.

Carbon tetrachloride has a low boiling point, gives light-colored grease and above all is non-inflammable; but its cost is high, special tin-lined apparatus is necessary, and the loss in solvent value is high. The use of naphthalene as a solvent has been patented, but it is not used.

There are many types of apparatus suitable for extraction, and since improvements appear from time to time, the makers of such machinery should be consulted for latest details.[3] Descriptions of extraction apparatus are also to be found in most standard books on chemical engineering.

The general principle on which most extraction systems work, is to have the solvent percolate through a granulated bone contained in a closed tank, the fat-containing solvent being caught in a still set at a lower level. The solvent vapors from the still are condensed and the fat-free solvent is again sent through the bone. When extraction is complete, the condensing solvent is diverted to a storage tank, and the solvent remaining in the bone is driven out by steam, a special separator being used to part the solvent and the condensed steam. The apparatus is then

[2] "Glue and Gelatine," 2d ed., M. Jänecke, Leipzig.

[3] Nothing is to be gained here by burdening the book and the reader with a diagram of some selected form of extraction apparatus and details regarding the operation of its valves, etc. This would simply serve to perpetuate types which may soon become obsolete because something better has been discovered.

opened, the extracted bone removed, and the solvent-free fat run off.

Before boiling, the degreased bones are freed from dirt and adhering meat by being rotated in a large perforated screen or drum called a rattler or cleaner, in which they are polished by auto-attrition.

Thiele reports the following average yield from raw bones:

| | | | |
|---|---|---|---|
| Crushed bone | 50.3 | to | 59.5 per cent. |
| Bone dust | 8.6 | " | 13.0 |
| Bone fat | 7.7 | " | 10.3 |
| Hoofs | 2.7 | " | 13.1 |
| Horns | 0.01 | " | 0.04 |
| Tendons | 0.19 | " | 0.8 |
| Iron | 0.02 | " | 0.10 |

The degreased bone has from 5 to 6 per cent. of glue and about 60 per cent. of calcium phosphate.

The acidulation of the degreased bones is usually accomplished by dilute (about 8 per cent.) hydrochloric acid and takes place in large wooden vats, which may be subjected to intermittent rotation, or through which the acid solution is slowly circulated by pumps. The counter current system of circulation is used, and the time required for treatment varies with the nature and size of the bone, being from 8 to 10 days with occasional limits of 4 to 14 days, according to Bennett, and from 2 to 3 days, according to Thiele. Degreased bones are attacked more rapidly than steamed bone. According to Bogue [3a] American practice is to use from 2 to 5 per cent. hydrochloric acid, one pound of bone requiring roughly one pound of 22° Beaumé acid for complete extraction.

In acidulation the main reactions are as follows:

$$Ca_3(PO_4)_2 + 4HCl = 2CaCl_2 + CaH_4(PO_4)_2 \text{ (acid phosphate)}$$
$$Ca_3(PO_4)_2 + 6HCl = 3CaCl_2 + 3H_3PO_4$$
$$Ca_3(PO_4)_2 + 4H_3PO_4 = 3CaH_4(PO_4)_2$$

The acid phosphate is then precipitated by carefully adding milk of lime:

$$CaH_4(PO_4)_2 + CaO = Ca_2H_2(PO_4)_2 + H_2O$$
$$H_3(PO_4)_2 + 2CaO = Ca_2H_2(PO_4)_2 + H_2O.$$

(Secondary reactions occur here to some extent.)

To avoid an excess of lime which would cause a reformation

[3a] "The Chemistry and Technology of Gelatin and Glue," McGraw-Hill Book Co., 1922.

of tricalcium phosphate, filtered samples of the acid liquor are tested from time to time with molybdic acid solution. When the acid phosphate and free phosphoric acid are both completely converted into $Ca_2H_2(PO_4)_2$, a precipitate of ammonium phosphomolybdate no longer forms, and the addition of lime is stopped. Should an excess of lime be accidentally used a suitable quantity of acid liquor may be added to retrieve the error. The precipitate, $Ca_2H_2(PO_4)_2$, is then washed free from calcium chloride in a filter press. Since the phosphate readily hydrolyzes, as little wash water as possible is used. It is known as "precipitated bone phosphate" and is largely used in the manufacture of bone china and fertilizers. The "acid phosphate" is used in making phosphate baking powder.

The soft collagen, after washing and neutralization of the residual acid with lime water, may be made directly into glue or gelatin. If dried at a low temperature it yields commercial ossein.

Other acids than hydrochloric may be used to acidulate bone. Sulphurous acid is the one most employed, though phosphoric acid has been tried. Sulphuric acid is not suitable owing to the formation of insoluble calcium sulphate which blocks the process.

In the process patented by Grillo and Schroeder bones are disintegrated by moist sulphurous acid gas or by liquid sulphurous acid according to the equation:

$$Ca_3(PO_4)_2 + SO_2 + H_2O = Ca_2H_2(PO_4)_2 + CaSO_3.$$

Bones thus treated readily dissolve upon boiling or steaming, yielding a "mud" which forms a valuable fertilizer after the calcium sulphite is oxidized to sulphate.

The Bergmann process for decalcifying bones is as follows: The degreased bones are placed in closed tanks and a solution of sulphurous acid is percolated through them, its strength being maintained by continuous additions of sulphurous acid gas. This leaves a thoroughly bleached ossein which is washed free of acid. The leach liquor is heated in a lead lined tank, liberating free $SO_2$ for further use, and precipitating calcium phosphate and calcium bisulphite.

The bisulphite is decomposed by hydrochloric acid, freeing a further quantity of $SO_2$ for return to the process, so that all told only about 5 per cent. of $SO_2$ is lost.

Dentelles consists of ossein made from button makers' bone refuse. It is often called "spectacles" because the pieces of bone contain round holes which recall the appearance of eye-glasses. Prepared horn pith is a variety of ossein made from the cornellion or interior osseous core of the horn. Since it does not come in contact with flesh, and has a porous structure that renders easy its extraction, it produces high-grade gelatin. The acidulated horn pith of commerce keeps its original shape. Pieces of the skull bones are often left on, and if not properly leached, constitute "dead bone," which adds to the weight but reduces the percentage of gelatin yielded. The yield from ossein is said to vary from 65 to 85 per cent.

**Hide and Sinew Stock.**

The liming of hide, sinew and ossein stock has for its object the thorough swelling or "plumping" of the stock and the elimination of mucin. The lime pits are square wooden or cement tanks about 4 feet deep, sunk in the ground like those of a tannery. The soaked and washed stock is thrown into a saturated solution of lime contained in the pits, and the stock is occasionally stirred up or transferred from one pit to another, with the aid of a long-handled fork, the lime solution being agitated, renewed or strengthened as often as necessary. Thick hide pieces often have to remain in the lime vats several months before they are properly limed; thin fleshings or skivings lime much quicker, and in general liming proceeds more slowly in winter.

In some plants to save labor the stock is pumped in and sucked out of the vats by large centrifugal pumps similar to those used in dredging operations, or else is handled by bucket chains.

To shorten the time of liming, the hide pieces may be cut up or shredded. Furthermore caustic soda is often added to the lime liquor to "sharpen" it and produce a quicker swelling—too much must, of course, be avoided. With gelatin stock sodium peroxide is often used to produce a bleaching action at the same time.

**Washers.**

To swell up and soften dried hide or sinew stock or to free it from salt or lime, it is treated in mechanical washers.

The type most popular in America is the "cone washer." This consists of a heavy slatted, hollow cone about 5 feet long, that is rolled by a rotating arm around a shallow circular tank about 10 feet in diameter through which a current of water is passing. The cone presses and kneads the stock and the agitation results in thorough washing by the water which enters at the center and runs off through perforated grids at the outside of the tank.

In Europe smaller tanks with rotating paddles are in common use.

G. Illert [4] describes a series of washers having horizontal arms turning at 90–100 R.P.M., the hide stock being passed from one to the other by bucket elevators. A copious spray of water removes lime, etc., through the perforated sides and bottom and the washed stock is automatically fed to a press, where it is squeezed before dumping into the boiling tanks. A capacity of 10 tons per hour is claimed.

After washing limed stock, it is usual to add some hydrochloric or sulphuric acid to the last wash water and let the stock soak in it so that any remaining lime may be neutralized. This is readily determined by cutting open a piece of swollen stock and testing with litmus or phenolphthalein. Alum is also frequently added to the last wash water.

The remaining acid is then washed out and the stock is transferred to the cookers. Sulphuric acid usually clouds the glue by forming a small quantity of calcium sulphate. Hydrochloric acid forms calcium chloride which keeps the glue clear but which lowers the jelly strength if much be present, for whatever is left in the stock remains in the finished glue. In making photographic gelatin it is particularly objectionable to have any quantity of salts left; calcium chloride is, of course, hygroscopic.

### Tanned Stock.

H. R. Procter [5] suggested that chrome tanned hide may be stripped of chrome for glue manufacture by a solution of Rochelle salt or other salt of an hydroxy-acid. M. C. Lamb [6]

[4] *Chem. App.* 8, 78 (1921).
[5] *Soc. Chem. Ind. Annual Rept. on Appl. Chem.*, 1916, p. 232.
[6] *J. Soc. Chem. Ind.* 38, 572A (1919).

leaches the cleaned disintegrated chrome leather for 48 hours in a 15–40 per cent. solution of organic acids containing two or more hydroxyl groups, oxalic acid being preferred. The chrome is precipitated as hydroxide from the extract, and the regenerated hide, after washing in weak alkali, is limed as usual to make glue. The results are said to be very satisfactory, which can hardly be the case with the drastic process of A. Wolff,[7] who dissolves chrome leather waste in at least its own weight of 5 per cent. sulphuric acid. After removing the separated fat, the chrome is precipitated as hydroxide by lime, the lime removed and the filtrate dried for glue.

S. R. Trotman[8] dechromed hide for glue making by oxidizing the chrome to sodium chromate with sodium peroxide. W. Prager[9] converts the basic chrome salt into the normal soluble salt by a 2 per cent. solution of sodium bisulphite.

Lime and other bases have also been used to de-tan chrome leather,[10] but the acid methods seem to be preferable.

**Boiling Apparatus and Methods.**

In the extraction, cooking or "boiling" process, the prepared stock is subjected to the solvent action of hot water or steam, whereby the swollen collagen is changed into gelatin or glue. Hofmeister regarded the change in a definite hydrolysis proceeding in two stages according to the equations:

$$C_{102}H_{149}N_{31}O_{38} + H_2O = C_{102}H_{151}N_{31}O_{29}$$
Collagen + water = Gelatin

$$C_{102}H_{151}N_{31}O_{39} + 2H_2O = C_{55}H_{85}N_{17}O_{22} + C_{47}H_{70}N_{14}O_{19}$$
Gelatin + Water = Semiglutin + Hemicollin

Considering the fact that the final disintegration products of gelatin are amino-acids, and that progressive heating results in progressive degradation, it is obvious that the much used term "hydrolysis" simply conceals our real ignorance of what actually does occur. In fact, Emmett and Gies claim that the process is one of molecular rearrangement and no hydrolysis at all. But even this does not seem to coincide with the experimental facts.

[7] *J. Soc. Chem. Ind. 38,* 331A (1919).
[8] *J. Soc. Chem. Ind. 30,* 1462 (1911).
[9] *J. Soc. Chem. Ind. 32,* 501 (1913).
[10] See e.g. German Patent 202,510.

With colloidal substances like gelatin, chemical changes are so closely associated with physical changes, that no line of separation can be drawn between the two. Indeed, the whole boiling process appears to be a gradual breaking up of colloidal adsorption complexes (or of large "molecules" held together by residual molecular attractive forces), with accompanying changes in free surface and amount of water adsorbed.

The higher the extraction temperature, and the longer the stock and liquor are exposed to it, the more rapidly these degenerative changes proceed, and the lower in test and darker in color the glue will be. Therefore, it is desirable to extract the stock as quickly as possible, and at the same time to keep the extraction temperature low. Since these two factors oppose each other there is a wide range of possibilities. Usually with gelatin where color is important, the temperature is kept low, even though the extraction period is thereby lengthened. With glues, especially bone glues, higher temperatures are generally used.

Since the stock dissolves quicker in pure water than in glue solution, attempts to produce too concentrated a liquor unduly protract the period of extraction.

It is really a misnomer to call the extraction process "boiling," for the boiling temperature is seldom reached, except in pressure tanks, or when extracting residues. The temperatures generally used vary from about 70° to 90° C., depending upon the kind and condition of the stock. Too low a temperature, which would favor bacterial growth and decomposition, must be avoided.

Whereas slow circulation of the glue liquor (i.e. by rotary pumps) aids solution, unnecessary agitation is harmful as it tends to lower viscosity and jelly strength and cloud the liquor.

Two types of kettles, or cookers, are used: (1) open tanks; (2) pressure tanks.

**Open Kettle or Tank.**

The open tank, used mainly for hide, sinew or ossein stock, usually consists of a rectangular or round wooden tub having a closed steam coil over which is placed a perforated or slatted false bottom of wood or iron, so as to leave a circulating space

between the two. Upon the false bottom is placed a layer of excelsior or straw often topped with a thin layer of hair, thus forming a rough strainer, upon which the stock is thrown until it reaches within a foot or so of the top of the tank. Sufficient pure water is then added (almost enough to cover the stock) and steam is turned into the coil until the desired temperature is reached; whereupon the steam is cut down to minimum necessary to maintain this temperature. As a rule the first "run" or "boiling" is made at about 70° C. (158°), and subsequent runs are made at progressively increased temperatures.

During boiling the stock is occasionally "opened up" by stirring with a long pole, so as to permit a more perfect circulation of the liquor, or a circulating "chimney" is provided. From time to time a sample of the liquor is tested by a hydrometer or by chilling it in a cup, and when a sufficiently concentrated "soup" is obtained, it is run off, fresh water is added, and another "run" or boiling is made. A boiling usually takes from 2 to 6 hours, depending on the nature of the stock and the temperature used. The last run or wash water is extracted at boiling heat and is usually so weak that it is added to another kettle or to a stronger run, or else must be evaporated.

The residual tankage is used for fertilizer. It may contain considerable grease or insoluble lime salts of fatty acids. In this event it is boiled with sulphuric acid to liberate the grease, which is then squeezed out in an hydraulic press.

During boiling in open tanks, most of the grease contained in the stock rises to the surface and is skimmed off. With fleshing stock the yield of grease is heavy, often exceeding the yield of glue in the case of machine fleshings which take in relatively little of the hide or skin substance.

**Pressure Tank.**

The pressure tank is largely used for extracting untreated or degreased bone stock, and acidulated bone. The tanks are vertical steel cylinders with convex ends, having large manholes at the top for filling, and at or near the bottom for discharging the spent bone. Hide glues made in pressure tanks are usually weak, and the tank is seldom used for hide stock.

The bones may be boiled with water under a pressure of from

10 to 20 lbs.; or hot water may be allowed to trickle in from the top while steam enters from the bottom (English process); or the condensation of the steam may supply the necessary water. The successive runs of glue obtained are more concentrated than those yielded by open tanks, though they do not have so strong a jelly. They are drawn off from time to time through a perforated false bottom. The most modern practice is to work pressure tanks in gangs upon the counter-current principle. Pressure tanks with circulating mechanism for handling ground bone have also been patented.

If bones are intended for making bone black, they are either degreased by volatile solvents or else they are given one light cooking to remove most of the fat and but little of the glue. If too much of the nitrogenous matter is removed, the bone black will be of poor quality.

After extraction of the glue, the bones are dried in a rotary drum dryer, ground in a high-speed rotary mill, and sold for fertilizer. Thiele reports the following analyses of bones from which the glue has been extracted.

| | *Bones previous extracted with benzine* | *Boiled bones* | *Steamed bones* |
|---|---|---|---|
| Water | 8.54 to 9.25 | 10.81 | 10.79 to 12.18 |
| Organic matter | 17.10 " 19.53 | 25.97 | 22.48 " 24.62 |
| Ca and Mg carbonates | 7.50 " 8.74 | 6.28 | 6.33 " 6.89 |
| Alkali | 0.32 " 0.59 | 1.07 | 0.86 " 1.25 |
| Ca phosphate | 61.22 " 64.10 | 53.15 | 53.94 " 57.23 |
| Iron oxide | Trace | 0.27 | Trace |
| Insoluble | 1.38 to 1.78 | 2.45 | 1.68 to 1.74 |
| Nitrogen | 0.68 " 1.05 | 1.91 | 1.64 " 1.72 |
| Equivalent in glue | 3.79 " 7.06 | 10.06 | 9.10 " 9.56 |

He also gives a detailed description of the operation of a diffusion battery of four pressure tanks in making bone glue (*loc. cit.*, p. 50). He also describes (p. 98) a gelatin extractor with false bottom, in which the stock is treated with a spray of superheated water. He recommends glass enameled vessels for handling the gelatin liquors, which come off continuously and may be collected into one "run" or divided into various runs or fractions.

The liquor resulting from the first extraction of the stock is known as the "first run," that from the second extraction the "second run," etc. The first run, having been subjected to less

heat for a shorter time naturally yields the strongest glue. The various runs may be kept separate (known as "successive glues") or they may be mixed together and dried as one batch. The cheaper bone glue liquors are often mixed with hide glue liquors, the resulting glue being known in Germany as "misch-leim." In America such mixtures are usually made by mixing the separately granulated glues, although factories producing both hide and bone glues often mix the liquors.

## Clarification, Bleaching and Evaporation of Dilute Glue or Gelatin Liquors.

While the removal of relatively coarse particles from glue liquors is readily effected by a strainer or filter press, the high protective or deflocculative action of glue makes difficult the separation of any finely subdivided or colloidal matter, which renders the glue turbid. The methods of clarification, apart from simple straining, fall into three groups:

1. *Mechanical* (settling or centrifugation).
2. *Adsorptive* (filter-mass or bone black).
3. *Formation of precipitates* (albumen, phosphoric acid, alum, etc.).

About twenty years ago the writer was able to clarify gelatin liquor in an ordinary cream separator (DeLaval type) but the modern super-centrifuge (Sharples type) is, of course, much more efficient and is said to be much used in the United States for gelatin liquors.

The use of paper or cellulose filter-mass, along the lines of modern brewery practice, and of bone black as per the methods used in sugar refining or glucose clarification, give excellent results; and they are widely used for gelatin.[11]

An old method of clearing liquors was to add blood or a solution of blood or egg albumen and then boil. The albumen in coagulating would carry down most fine turbidity; but the heating weakened the glue or gelatin, and gave a soapy smell to the gelatin. In the Grillo and Schroeder process the precipitate of calcium sulphite carries down most suspended matter,

[11] Several varieties of filters are on the market, details regarding which may be obtained from the makers.

and the use of phosphoric acid is very common in clarifying gelatin liquors. If made from bone stock they may have sufficient lime to give the desired precipitate of calcium phosphate; otherwise milk of lime may be added as is done in the defecation of sugar liquors. The glue liquor must be warm enough to let the precipitate settle, but the temperature must be kept as low as possible to avoid loss of strength. Phosphate of soda may be used, but has the disadvantage of leaving soluble sodium salts. Oxalic acid may also be used, producing a precipitate of calcium oxalate.

Alum or aluminum sulphate is the clarifying agent most used for glues. It seems to undergo hydrolysis in the presence of the colloid, and the nascent hydrate of alumina combines with the glue or the impurities giving a precipitate which can be settled or filtered out. Lambert [12] gives the following details:

"A bucketful of liquor, which should have a temperature of about 80° C., should be drawn from each vat, the necessary quantity of alum stirred in, and the contents thoroughly mixed in the mass, the heat at the same time being raised to 100° C. by means of a steam pipe. After boiling ten minutes the steam is turned off and the liquor allowed to settle, during which the heavier mineral and organic impurities fall to the bottom, while the lighter form a coagulated scum on the surface."

Such a procedure is obviously very injurious to the strength of the glue; in fact, as a general rule highly clarified glues are relatively weak, strength being sacrificed for appearance.

Schwerin [13] has patented a process for clarifying gelatin and glue liquors by electroösmosis, but this method has not yet found general application.

H. Fleck [14] describes a process for improving glue by precipitation with ammonium sulphate or sodium bisulphite, which removes some of the products of hydrolysis. He warned against the danger of boiling glue solutions.

Of course, any grease rising to the surface of the glue liquors is skimmed off, though with cheap bone glues, when grease is

[12] "Glue, Gelatine and Their Allied Products," London, 1905.
[13] "*Kolloid Z.* 20, 64 (1917), Ger. Pat. 293,188 (1918).
[14] "The Manufacture of Chemical Products from Animal Offal," Brunswick, 1878.

low in price, as much grease as possible is kept in the glue liquor and helps to render the glue free from foam besides increasing the yield.

For the bleaching of glue, sulphurous acid or bisulphites are most commonly used because of their cheapness. The stock may be bleached before boiling, or $SO_2$ gas or its solution may be added to dilute or to concentrated liquors. Sodium hydrosulphite and analogous compounds (zinc hydrosulphite, sodium formaldehyde sulphoxalate) have certain advantages, but are more expensive.

For bleaching gelatin liquors, hydrogen peroxide may be used, but it is preferable to bleach the stock before boiling, in which case sodium peroxide or sulphurous acid are also of service. Peroxides also oxidize sulphites to sulphates.

**Evaporation.**

Many first or second run gelatin and glue liquors, as they come from the boilers, will set firmly enough to be chilled, cut, and dried; but in most cases it is necessary to evaporate or concentrate the liquors.

The old style steam worm rotating in a trough-like tank has been superseded by the modern double or triple effect evaporator. It is not proposed to burden the reader with a detailed description of the various types of vacuum evaporators, which are being continually improved; for the apparatus used to-day may be superseded by a superior form soon after publication of this book, if not before. The apparatus is well known, is described in many elementary books, and the various manufacturers are at all times ready to give the latest details.

The film evaporators (Yaryan, Kestner, Lillie, and Blair-Campbell) are in high favor, since in them the glue liquors are but a short time under the action of the heat, and this is conducive to maintenance of strength.

The degree of evaporation depends upon:

1. The jelly strength of the original glue liquor;
2. Its initial concentration;
3. The temperature of the outside air. Weaker glues and warm weather naturally demand greater evaporation.

**Antiseptics.**

Among the antiseptics commonly used in glues are zinc sulphate (which tends to precipitate odor-producing decomposition products), boracic acid, borax, sulphurous acid and bisulphites, and formaldehyde (about 1–10,000). Beta-naphthol may be used in the limes if desired. Phenol (carbolic acid) has also been used on stock and in liquors, but the odor is very objectionable.

**Chilling.**

In most factories the glue liquors, after evaporation and other treatment, are poured into small rectangular galvanized iron pans and allowed to gelatinize, preferably in a chill room. Sometimes the pans are allowed to stand in cold water. Many European factories have *casting tables,* which form the glue directly into cakes. These tables have hollow tops through which is circulated cold water or brine; the upper surface (of metal or glass) is divided into square or oblong recesses which are filled with the glue liquor and which often have a trade mark etched in, so that it appears on the glue cake.

Many devices have been patented for chilling concentrated glue liquor into a continuous sheet, which is automatically spread upon nets. None of these have found general widespread use, and several have been tried and abandoned. One of the early patents was that of Peter Cooper-Hewitt (U. S. P. No. 11,426, issued 1894). Some of the European gelatin factories are said to employ such apparatus. In the United States the apparatus of Maurice Kind (U. S. P. No. 1,046,307, issued 1912) is employed by several factories.[15] This machine has an endless belt which passes through a refrigerating tunnel, and on which is formed a continuous sheet of jelly of the desired thickness. The continuous sheet of chilled jelly coming from the belt is automatically cut into sheets of the required size, and these sheets are automatically spread upon drying nets of the usual kind. Fifteen minutes after leaving the evaporator, the first sheets are ready to enter the drying room.

A single unit of the Kind machine is said to have a capacity

[15] See Arthur Lowenstein, *Trans. Am. Inst. Chem. Eng. 10,* 105 (1917).

of 4300 lbs. of dry glue per day of 20 hours with a 16 per cent. jelly spread ¼″ thick. The unit occupies a space of about 85 feet by 10 feet, takes about 10 horse power, and requires about 10 to 12 tons of refrigeration.

The advantages of such an automatic machine are obvious. Since gelatin solution is a particularly good culture medium, the more rapidly a glue jelly can be handled and dried, the less chance there is of contamination and bacterial decomposition. This applies especially to food gelatins which, because of the absence of antiseptics, are especially susceptible to the attack of bacteria. There is furthermore a big saving in labor, and more or less freedom from the effect of weather conditions.

Glue and gelatin are frequently dried upon steam-heated rolls, which may rotate in a vacuum (Passburg system) or which may have a close-fitting hood forming a narrow channel through which a rapid blast of air carries off the evaporated moisture.

Where vacuum or forced draft drying rolls are used, the chilling process is eliminated, for the concentrated glue liquor is fed to them direct. A recent German method (Ruf system) is to beat the thick glue liquor to a foam before feeding it to a steam-heated drum.[16]

**Cutting, Spreading and Drying.**

Where glue has been chilled on casting tables, the sheets of jelly are picked off by hand and spread upon the drying frames or nets. But where the glue has been chilled in pans or boxes, the large jelly blocks are removed (usually by dipping the pans for an instant in hot water) and cut into slices which are spread upon the nets. With large pans, the jelly blocks are first cut into several smaller blocks before slicing.

Any heavy impurities or particles of dirt in the glue or gelatin liquor settle to the bottom of the jelly blocks, while grease and light particles float to the top. Therefore in most factories the blocks are sliced horizontally, and the "tops and bottoms" dried separately to make an inferior grade of goods, or else they are worked into another batch. If the jelly blocks are sliced vertically, the top and bottom impurities, if any, are distributed throughout the sheets.

[16] See G. Illert, *Chem. App.* 8, 78 (1921).

Most slicing contrivances have a grid of steel wires through which the jelly block is forced by a plunger; the distance between the wires regulates the thickness of the slices. Or the blocks on a moving belt are carried against wires, some distance apart, and fixed at different heights. With hand cutters the jelly block is sunk below the surface of a table and raised intermittently by a ratchet; between each successive elevation, a wire stretched on a bow-like handle is drawn through the block, which is thus cut into slices whose thickness depends on the number of teeth in ratchet.

For very stiff jellies, knife cutters are sometimes used, but as a rule highly concentrated jellies show rough or wavy marks which detract from the appearance of the finished glue.

The slices of jelly as spread on the nets vary from about 3 to 10″ in width, to about 6 to 12″ in length. A certain automatic chilling and spreading machine which was operated for a time in one plant, spread a strip the size of a whole net. Ribbon glue is cut in strips about 1½ to 2″ wide and about 8″ long. Noodle glue is cut in strips having a cross section about ½″ square. Bazaar glue is similar to noodle glue, but of about 1″ cross section.

The thickness of the finished glue depends upon two factors: the thickness of the jelly slice, and the concentration of the jelly. The sheets of glue suffer more or less distortion on drying, and usually show the marks of the nets on which they were dried. In Japan nets of bamboo are evidently used, as some Japanese glues show distinctly. Cast glues are usually highly concentrated, and the cakes tend to hold their shape well. The so-called Scotch glue is dark and comes in cakes about 10 x 12″ with a loop of string through one of the long ends. Thin flake glue is usually made from jellies strong enough to be dried with little or no evaporation. But thinness of flake is no criterion of quality, since concentrated jellies of weak glues are often cut very thin to simulate this supposed ear-mark of quality. Gelatins are usually quite thin cut, especially those which are to be put up in paper packages holding about 1 pound.

The drying nets upon which glue and gelatin are dried, usually consist of rectangular wooden frames about 3 x 5 feet, upon which are stretched pieces of galvanized iron wire netting with a mesh resembling chicken wire fencing. Some factories use cotton or

linen fish net, especially for gelatin, because if the zinc protection cracks off the wire net, rust forms which injures the appearance of the product and may actually form insoluble brown flakes.

The net frames may, of course, be made of metal, but each frame has a small block or leg in each corner so that when the nets are piled up into stacks there is a space, usually about 1 inch, between the frames for air circulation.

The stacks of nets are mounted upon wheeled tracks or bogies, which generally run on tracks, and pass on into the dry room.

In the old days the dry room was simply an upper floor or loft with louvred sides, and the drying was left to the vicissitudes of the wind and weather. Warm weather would melt the glue on the nets or foggy weather would pit or mold it. In all modern plants the glue is dried by passing a current of warm air over the stacks packed in narrow alleys or tunnels.

Some prefer the forced draft or blower type of fan, whereas others prefer the exhaust or ventilating type which sucks the air through the tunnels. Since the latter type fan has a tendency to churn if pulling against much resistance, the positive pressure fan is generally used.

The air entering the alleys is heated by passing it over banks or stands of steam pipes, any of the approved systems being used. Exhaust steam is utilized where possible. In order to prevent the air short circuiting and thus passing through the alley without taking up its quota of moisture from the glue, it is, of course, necessary that the stacks of nets fill the alley as completely as possible, and that the top, bottom, and sides of the alley be air-tight. Windows are usually provided, through which the temperature at various parts of the alley may be read from the outside.

The alley itself may be straight or U shaped. The glue entering the end furthest removed from the source of heat, first comes in contact with moist air that has suffered a drop in temperature because it has already evaporated water from the forward stacks. The stacks move progressively toward the pipe coils, near which they get their final "baking" before being removed from the alley.

Considerable experience and care are required to get the best results from a drying alley. Improper adjustment of the tem-

perature to the particular batch may cause the glue to melt and run through the nets or sink into the mesh, forming pendulous drops ("titted" glue), which are difficult to remove without damage to the nets. The formation of a very thin surface skin (skinning over) is a partial protection against these difficulties, but if the surface skin is too thick or formed too rapidly, it impedes further evaporation. The addition of a small quantity of formaldehyde to the concentrated glue liquor is said to facilitate the formation of a skin, besides acting as an antiseptic and giving a stronger jelly. Excessive drying must also be avoided, for besides diminishing the yield, it is apt to cause the glue to crack or check.

The time required to dry a batch of glue depends upon the concentration of the liquor, the thickness of the jelly slices, the strength or quality of the glue and the weather conditions. Good practice requires the drying to be completed as speedily as possible, usually within about 24 hours. Any plant where drying takes from 10 to 30 days, as claimed by E. Sauer,[17] must needlessly tie up an enormous amount of capital, product and apparatus.

In England and on the Continent the prevailing practice is to pick the dried sheets or cakes off the nets and pack them into bags or other packages. In America the glue is simply dumped off by inverting the net, and the sheets are still further crushed by passing them through a rough breaker whence they issue in flakes (flake glue). A large percentage of glue in America is ground to about 8 to 16 mesh in high-speed percussion mills. At one time barrels were the leading packages here, but bags are coming into wider use, both for flake and ground glues.

The different batches or boilings of glue and gelatin, which may amount to several hundred or even several thousand pounds, are as a rule kept separate for testing and grading. Where large lots of one grade are required, it is common practice to mix a number of boilings and then to test the mixture.

According to Thiele the various brands of sheet gelatin, which are usually sold in 1-pound packages for food purposes, are selected as follows:

[17] *Kolloid Z.* **17**, 130 (1915).

| *Run of Gelatin* | *Brand or label* | *Average number of sheets per lb.* |
|---|---|---|
| 1 (or 1 and 2) | Non plus ultra | 285 |
| 2 and 3 | Gold extra and Gold | 227–200 |
| 3 alone | Silver | 180 |
| 4 and 5 | Copper | 136 |
| 5 alone | Black | 90 |

The finished glue should be stored in a place of mean humidity and temperature. Excessive moisture is apt to cause the glue to soften and even be decomposed by molds or bacteria, whereas excessive heat and dryness tends to make the glue crack and check. According to E. Sauer [18] if sheets of glue that have been stored for a long time in a very dry room are transferred to a very damp room, many sheets, often with a loud report, may burst into small pieces. The unequal absorption of water, which naturally takes place at first on the surface, produces a tension which forcibly relieves itself by breaking the sheet of glue. Low grade, highly hydrolyzed glue are especially apt to check or crack.

**Blow-down Processes.**

Since the cutting, spreading and drying operations are expensive, and both time and space consuming, attempts are being made to "spray-dry" the concentrated glue and gelatin liquors along the lines now successfully used in making milk powder. If the cost can be gotten down, such processes should have a big future, especially for gelatin.

**Percentage Yield of Glue Stock.**

Since glue stock is for the most part a highly variable commodity, definite yields can hardly be predicted without examination; but the following table, prepared by a factory manager, gives the results of his experience with several varieties of stock:

| *Kind of Stock* | *Percentage Yields* *Glue* | *Grease* |
|---|---|---|
| Green bones | 10–12 | 8–10 |
| Dry bones | 14–16 | 5– 7 |
| Green calf | 18–22 | 3– 5 |
| Green salted hide | 16–18 | 3– 5 |
| Dry hide | 45–50 | — |
| Salted sinews | 18–22 | 3– 5 |
| Dry sinews | 40–50 | — |
| Dry fleshings | 30–35 | 10–12 |
| Wet fleshings | 8–12 | 12–15 |
| Horse hide (wet) | 10–15 | — |
| Sheep skin (wet) | 6–10 | 10–18 |

[18] *Kolloid Z. 17,* 133 (1915).

# Chapter 12.

## The Testing and Grading of Glue and Gelatin.

At the outset let it be emphasized that there is no single chemical or physical test which will satisfactorily gauge the value of a glue or gelatin for all purposes. Many authors have recommended individual tests and while these may have some value for special purposes, the wisest and safest way for the factory or sales manager is to run a series of connected tests which will grade the glue or gelatin against preceding lots of the same type, and thus render possible uniform deliveries to the consumer, whatever his business may be.

This view was voiced by E. G. Clayton,[1] who says: "In conclusion the observations seem to show that, whilst it would be rash to form a judgment on glue from a single test the evidence afforded by a number would be irresistible. Glue may be shown by certain tests to be suitable for one purpose, though less perfectly adapted for another. The expert's wisest system appears to be, not to rely upon single short-cut tests of general quality but to employ a number of methods, including any having especial bearing on the present or prospective uses of the glue, and then to base his conclusions on a consideration of all the results together."

Before describing a connected series of general physical test methods which are, with more or less modification, used in glue testing laboratories, let us review briefly some of the methods that have been proposed for testing and grading glue. Secrecy has been traditional in the glue trade, with the result that treasured methods may be inferior to other methods known to all. The references given are to books and papers published in the usual scientific journals. An exhaustive review attempting to fix priorities is not attempted. Some partially completed methods of the National Association of Glue and Gelatin Manufacturers are given in an Appendix.

[1] "The Technical Examination of Glue," *J. Soc. Chem. Ind.* **21**, 675 (1902).

**Jelly Strength.**

One of the first tests proposed was the consistency test of Lipowitz [2] which determines the relative capacity of the jelly for bearing a weight. The instrument used [3] consists of a saucer-shaped piece of tinned iron, 1 inch in diameter, having a thin metal rod soldered vertically to its concave side, the upper end of the rod being provided with a small metal funnel. The rod slips loosely in a perforated metal strip which supports the apparatus. The saucer is placed with its convexity next to the jelly, and shot gradually poured into the funnel until the saucer breaks through. The total weight required to rupture the jelly indicates the jelly strength. Of course, the determination must be made at a definite temperature, and with a definite concentration of glue. A thick top "skin" must be avoided.

More recently R. Kissling [4] stressed the importance of jelly strength test, which is often called Kissling's test. J. Fels [5] describes it as "correct as a comparative method," and more recently R. H. Bogue [6] has found that this test alone would correctly gauge about 75 per cent. of all glues. It is also known as the "shot test," the "jelly test," and the "finger test," the last from the fact that the relative jelly strength may be easily fixed by pressing the jellies with the finger tips. F. Davidowsky [7] describes a jelly tester used by the factory at Hamborn. It consists [8] of a hemispherical weight, bearing a vertical scale, which slides in a guide cylinder that rests on the jelly with a broad flat rim, and bears also an adjustable pointer to fix the zero point. According to K. Kieser [9] Davidowsky's method is largely used in Germany to determine jelly strength. This corresponds to our "shot test" in all practical particulars.

Many mechanical contrivances have been suggested to fix the jelly strength of glue. Some depend upon measuring the depression produced by placing a definite weight on the jelly, without

[2] "Neue Chem.-tech. Untersuchungen," Berlin, 1861, pp. 37-42.
[3] "Allen's Comm. Organic Analysis," 4th ed., Vol. 8, 607.
[4] *Chem. Z.* *17*, 726 (1893); *ibid.*, *22*, 450 (1898).
[5] *Chem. Z.* 56 and 70 (1897).
[6] *Chem. Met. Eng.*, July, 1920.
[7] "Glue and Gelatin Manufacture," 5th ed., p. 26.
[8] This instrument is practically the same as that described in Technical Note No. F-32 of the Forest Products Laboratory, Madison, Wisconsin, who will supply working drawings on request.
[9] *Kolloid Z.* *28*, 186.

breaking through. The jelly tester of E. S. Smith [10] consists of a pressure chamber whose bottom contains a thin elastic rubber diaphragm; attached is a rubber air bulb to produce pressure and a manometer to measure the pressure produced, that required to produce a certain depression being taken as jelly strength.

E. T. Oakes and C. E. Davis [10a] described the jelly tester of A. Schweitzer, which consists of a balance, one arm of which carries a plunger below and a beaker above, so that on adding water to the beaker a definite compression of the jelly beneath the plunger may be produced.

W. H. Low [11] has proposed a modification of Smith's instrument. The Forest Products Laboratory [12] describes the construction and operation of a tester with a constant weight plunger, whose depression in the jelly is a measure of the jelly strength.

All these methods involve testing the jelly strength through the skin of uncertain strength and thickness which forms on the upper surface of the jelly. J. Alexander [13] devised a jelly tester which avoids this disturbing factor by casting the jelly into truncated conical blocks in brass cups of definite size, and then removing the blocks and determining their resiliency. The troublesome skin is placed at the bottom, and a gradually increasing weight is applied to the top of the jelly block, until a definite compression of the jelly is produced. The jellies are cast in round brass caps 6 cm. high, 5.5 cm. in diameter at the open top and 5 cm. in diameter at the bottom, which is closed with a tight-fitting external friction cap. The truncated cones thus formed should be exactly 4.5 cm. high, the cups being filled only to that level. If the jellies do not push out readily on removing the cap, the closed cup may be dipped for an instant in hot water. After removal the jellies are placed in a thermostat until they reach the desired temperature.

S. E. Sheppard [14] states that all modifications of the "shot test"

[10] U. S. Pat. 911,277; see *J. Soc. Chem. Ind. 28,* 252 (1909).

[10a] *J. Ind. & Eng. Chem. 14,* 706 (1922).

[11] *J. Ind. Eng. Chem. 12,* 255 (1920).

[12] United States Dep't of Agriculture, Madison, Wisconsin, in Technical Note F-32.

[13] U. S. Pat. 882,731; see *J. Soc. Chem. Ind. 27,* 459 (1908).

[14] Sheppard, Sweet, and Scott, *J. Ind. Eng. Chem. 12,* 1007 (1920).

err because the stress applied affects both elasticity of the bulk and elasticity of the figure. For more exact results he devised a torsion dynamometer which measures the force required to twist to the breaking point jellies which had been chilled for 3 hours at 0° C. Above 10° the jelly strength begins to diminish rapidly, though no material change occurs till then. Using this instrument, Sheppard found that no simple relation holds between the concentration of gelatin and the jelly strength; and that jelly strength values determined for a single arbitrary concentration give a very arbitrary comparison of the jelly strengths, because the curves relating these values for different concentrations of commercial gelatins are not of a common family and often cut each other. He concludes that there is no definite relation between the jelly strength at a given concentration, and the glue-joint or tensile strength of a dry glue joint. While the H-ion concentration affects jelly strength, there is no simple relation between the two.[15]

C. R. Smith[16] describes a simple jelly-testing device which may be rigged up in any laboratory. An 80 mm. short-stemmed funnel accurately formed to a 60° angle, is closed at one end, and 120 grams of mercury are poured in, forming an upper surface 3 cm. in diameter. Over the mercury is layered 50 cc. of gelatin solution, which is allowed to set in a horizontal position (fixed by a spirit level) in a constant temperature bath at 10° C. The mercury is then run out, the funnel is connected with a water manometer, and a reduction pressure (6 dm. of water) is produced. The depression of the upper surface of the jelly produced by the suction is measured with a micrometer and indicates the jelly strength. Smith's figures show, however, that the jelly strengths thus determined do not exactly parallel those estimated by his polariscopic method. This consists in finding the increment in the specific rotation of a 3 grams per 100 cc. solution between 35° C. and 10° C.

Most commonly the jelly strength is determined by the finger test against standard samples, for this method is speedy and of sufficient accuracy for commercial purposes. Besides it fits in readily with other tests and does not require any special ap-

[15] Sheppard and Sweet, *J. Am. Chem. Soc.* *43*, 539 (1921).
[16] *J. Ind. Eng. Chem.* *12*, 878 (1920).

paratus. But it demands the possession of standard glues for comparison. These, however, may be obtained by careful selection, aided by any of the mechanical devices.

**Viscosity or Running Test.**

A viscosity determination in the form adopted by J. Fels [17] is largely used in Germany.[18] Fels originally proposed taking the viscosity of a 15 per cent. solution of glue at 30° C. with the Engler viscosimeter; but finding that some very high test glues would not flow at this temperature, later he [19] raised the testing temperature to 35° C.

Fels' test, as it is sometimes known, is therefore the viscosity of a 15 per cent. glue solution at 35°, as fixed by the Engler viscosimeter; and it is a single test of great importance. It is interesting to note that the temperature finally fixed by Fels is that recently shown by C. R. Smith [20] to be the one at which incipient gel formation begins, as is evidenced by the polariscope. R. H. Bogue [21] has also recently recommended what he terms a "melting point" determination, which he says gives "a truer evaluation of product than by the use of the old and time-honored methods." Curiously enough Bogue's method, though evolved independently after numerous experiments, is practically the same as that of Fels. Bogue advises the use of the MacMichael viscosimeter [22] to determine the viscosity ("melting point") of a 30 to 100 solution of glue at 83° F. (32° C.).

It has been the general practice in glue testing laboratories in the United States to take viscosities with a simple pipette at temperature more closely approaching those at which the glue is ordinarily used, and with concentrations varying from 10 to 25 parts per 100 of water, depending on the strength of the glue. J. Alexander [23] adopted as standard a pipette of the following dimensions:

[17] *Chem. Z. 21,* 56 and 70 (1897).
[18] See J. Rudeloff, *Mitt. K. Materialprüfungsamt 36,* 2 (1918).
[19] *Chem. Z. 25,* 23 (1901).
[20] *J. Am. Chem. Soc. 41,* 135 (1919); *J. Ind. & Eng. Chem. 14,* 435 (1922).
[21] *Chem. Met. Eng.* 23, July, 1920.
[22] See Winslow Herschel, *J. Ind. Eng. Chem. 12,* 282 (1920).
[23] *J. Soc. Chem. Ind. 25,* 158 (1906); "Allen's Comm. Organic Analysis," 4th ed., Vol. 8, p. 605.

| | |
|---|---|
| Capacity | 45 cc. of water at 80° C. |
| Internal diameter of effluent tube | 6 mm. |
| External diameter of effluent tube | 9 mm. |
| Over-all length of effluent tube | 7 cm. |
| Smallest diameter of outlet (about) | 1.5 mm. |
| Outside diameter of bulb | 3 cm. |
| Length of bulb | 9.5 cm. |
| Length of upper tube | 22 cm. |

This pipette should permit the efflux of 45 cc. of hot water at 80° C. from the bath in which the glue testing glasses are immersed, in exactly 15 seconds. The viscosities of glues vary greatly as will be seen from the table showing the viscosities and jelly strengths of the glues chosen as standards.

Considerable care is necessary to make pipettes that will give concordant results. The size and form of the outlet hole, and the length and diameter of the effluent tube are the main factors controlling the time of delivery. The efflux hole is made by cutting the effluent tube square across, and holding it pendant in a Bunsen flame with constant rotation. As the glass softens the hole gradually draws together, and after a few trials can be brought to the desired size. It is necessary to have the lower graduation point just about where the effluent tube joins the bulb, for with very viscous glues there might otherwise be much uncertainty due to dribbling of the last few drops.

The time of efflux is taken with a stop-watch, and care must be used to see that no particles of paper, wood, dirt, undissolved glue, or glue slime clog the outlet even for an instant during a determination, at the conclusion of which the pipette is washed out with hot water from the bath.

A refinement is to keep the pipette in a simple water jacket thermostat while running; in this case a glass stop-cock or a rubber tube and pinch-cock is used to control the pipette. They impede rapidity of work without corresponding increase in accuracy. More complicated viscosimeters like the Rideal-Slotte [24] though more accurate than a simple pipette are not practical for routine work where speed is essential.

The results of R. H. Bogue (*loc. cit.*) indicate that the viscosity test alone, would correctly classify about 75 per cent. of all glues—that is viscosity determined at about 60° C. As E.

[24] *J. Soc. Chem. Ind. 10,* 615 (1891). Bogue ("The Chemistry and Technology of Gelatin and Glue," p. 384) describes many of the various viscosimeters that have been suggested.

Sauer [25] points out, glue is not a pure substance, but may contain impurities having a viscosity of their own, or substances which materially affect the viscosity of the glue. He concludes that "viscosity measurement is no absolute method for determining the quality of a glue, especially when dealing with products of different origin, which according to their raw materials and methods of making may contain more or less foreign material and which therefore cannot be compared with each other. For practical purposes, however, it is important, especially if certain other characteristics be taken into consideration." Sauer here speaks of Fels' test at 35° C.; he says that viscosity is good for factory control purposes if foreign additions, etc., remain unchanged.

H. J. Watson,[26] while placing most reliance upon jelly strength, found it necessary in many cases to take the viscosity as well, by Fels' method. Trotman and Hackford [27] also regard jelly strength as the more reliable physical test, although they mistakenly place more reliance upon chemical tests.

**Water Absorption Test** [28] (Schattermann's Immersion Test).

A known weight of glue or gelatin is soaked 24 hours in water at room temperature. The excess water is drained off, and the amount absorbed estimated by weight. High test glues absorb from 10 to 15 times their weight of water, weaker glues take up only 3 to 5 parts, and very weak glues may actually go into solution, forming a slime. Considering the many factors influencing water absorption (see p. 87), it is obvious that this test can give only a crude approximation as to value. It is, of course, impossible to apply it to finely broken or ground glues or gelatins.

**Hygrometric Test** (Cadet's Test).

This practically obsolete test is based upon the amount of moisture absorbed by a glue exposed to damp air, and is even less reliable than the preceding.

Where a glue or a glued article is to be exposed to a damp

[25] *Kolloid Z. 17,* 130 (1915).
[26] *J. Soc. Chem. Ind. 23,* 1189 (1902).
[27] *J. Soc. Chem. Ind. 23,* 1072 (1902).
[28] *Dingler's J. 96,* 119 (1845).

climate, some form of hygrometric test, simulating conditions of service, is desirable. An unpublished report by E. Bateman and G. G. Town of the Forest Products Laboratory (U. S. Dep't of Agriculture), Madison, Wisconsin, indicates that at about 30 per cent. moisture content glue becomes weaker than wood, and that above 33 per cent. moisture results in molding, against which no harmless preservative has been found. Hygroscopic salts greatly increase the water absorption, whereas tanning agents seem to decrease it. In Technical Note F-10 of the Forest Products Laboratory it is indicated that the moisture resistance of animal glues is proportional to the viscosity, jelly strength, and grade. High-grade glues absorb water more slowly.

**Melting Point.**

Glue and gelatin jellies soften gradually when warmed, and show no sharp line between solid and liquid. The "melting point" will therefore depend upon definition, and is an uncertain factor. As a general rule it varies as the jelly strength.

N. Chercheffsky [29] described a simple apparatus for determining the melting point. A 250 cc. beaker is filled with refined paraffin oil into which is hung a wire with a horizontal end on which are threaded several small blocks of jelly. When these lose their rectangular form on warming, the melting point is read on a thermometer which hangs as close to them as possible. Increased accuracy is had by placing the oil beaker in a water jacket which is gradually warmed.

Cambon's *fusiometer* [30] consists of a small brass cup which is held to a brass rod by a jelly made by dissolving 10 grams of the glue or gelatin under test in 40 cc. of water. The brass cup is hung at the surface of a beaker of water which is slowly warmed, and the melting point is taken as the temperature of the water when the brass cup drops off. A cane ferrule weighing about 7 grams will serve as the cup.

A. W. Clark and L. Du Bois [31] propose to determine the percentage of glue or gelatin which just maintains a solid jelly at

[29] *Chem. Z. 25,* 413 (1901).

[30] Cambon and Bergmann, *Monit. Scient.,* June, 1907; *J. Soc. Chem. Ind. 26,* 703 (1907).

[31] *J. Ind. and Eng. Chem. 10,* 707 (1918).

10° C., but this is a troublesome method and the work of Sheppard indicates that it is not dependable. C. F. Sammet [32] determines roughly the comparative melting point of glues by placing their jellies on an inclined brass plate, which is warmed by dipping one end in hot water. The weaker glues melt and slide down the plate more rapidly than the stronger ones. Bogue's suggestion (*loc. cit.*) is to continue the low temperature viscosity curve so as to determine, by extrapolation, the point where the solution would cease to flow, i.e. the viscosity would reach infinity.

H. Bechhold and J. Ziegler [33] propose to determine the melting point of jellies by observing the temperature at which 5 grams of mercury breaks through. To prevent skin-formation from interfering with this test, it would seem wise to protect the upper surface of the jelly with a layer of oil or wax, which may be removed later on. J. Herold [34] observed the temperature at which a thin tube containing jelly dropped from a thermometer about which the jelly had set.

Sheppard and Sweet [35] describe an apparatus which serves to measure both melting point and setting point. An intermittent stream of air bells (bubbles), under constant pressure, is passed through the test solution whose temperature is lowered or raised by a surrounding bath. The setting point is fixed as the temperature at which the bubbles cease to pass, and the melting point as the temperature at which they begin to pass. They also describe a simpler melting point apparatus consisting of an annular brass weight which slips over a thermometer and rests on the jelly by three equidistant wedge-shaped feet. The thermometer is centrally imbedded in a wide tube of jelly, the bulb being just below the surface, and the melting point taken as the temperature at which the weight sinks just above the feet.

The results of Sheppard and Sweet show that melting point and setting point are not identical, and also that, as they had previously found with jelly strength,[36] the concentration of the gelatin solution is a factor which may of itself change the order of grading. Furthermore the order obtained by melting or

[32] *J. Ind. and Eng. Chem. 10,* 595 (1918).
[33] *Z. physik. Chem. 46,* 110 (1906).
[34] *Chem. Z. 35,* 93 (1910).
[35] *J. Ind. Eng. Chem. 13,* 423 (1921).
[36] *J. Ind. Eng. Chem. 12,* 1007 (1920).

setting point determinations may not coincide with the order of grading according to jelly strength.

**Setting Point.**

The determination of the setting point or temperature of gelatinization, as proposed by K. Winkelblech [37] is just as difficult and uncertain as that of melting point. The apparatus of Sheppard and Sweet has been referred to above. C. R. Smith likewise fixed the setting point by the air bubble method, using his polariscope tube.

**Strength Test,** also called **Shear Test, Joint Test, etc.**

Since glue is used very largely for gluing wood, and since its binding strength on wood is a good indication of what it will do on other service, it is only natural that a large number of strength tests have been described and recommended. The trouble with them all is the great difficulty of obtaining concordant results because of the many variable factors involved. Some authors naïvely discard from their averages results that are from 100 to 300 per cent. too low, which shows that one single test may be very misleading. While impractical as a routine laboratory test in a glue factory, the strength test is valuable, and has been largely used to check other tests on glues for airplane propellers, etc.

R. H. Bogue (*loc. cit.*) points out one highly important source of error in this test, namely the joining pressure. He found that the strength of a glued joint varies directly as the joining pressure applied, up to about 1,000 lbs. per sq. in. Below 200 lbs. per sq. in. the variation is large, but beyond that it is small. S. Rideal [38] tried to avoid the uncertainty due to the variability of wood, by using porcelain blocks. Further variable factors are the conditions of drying, the amount of glue spread, temperature and moisture content of the wood.

The old method of the Königliche Artillerie-Werkstatt at Spandau was radically defective in that the glue solution to be tested was first boiled down to $\frac{5}{9}$ of its original weight, which naturally hydrolyzed the glue but indicated what it would do if

[37] *Z. angew. Chem. 19,* 1260 (1906).
[38] "Glue and Glue Testing."

mishandled by workmen. Among the various other methods may be mentioned those of Rudeloff,[39] who uses red beech blocks, and of A. H. Gill,[40] who tests maple briquet-shaped blocks in a cement tester. Gill also tried unsuccessfully paper impregnated with glue in the Mullins paper tester which recalls the similar test of Setterberg,[41] and briquets of sawdust, sand, fullers' earth, etc., which recalls the work of Weidenbusch,[42] who used impregnated rods of plaster of Paris.

P. A. Houseman [43] used straight grained walnut wood. G. Hopp [44] dissolved and redried the glue, cutting it then into strips of definite size which were tested for strength and stretch in a tensile machine. One hide glue showed an average tensile strength of 13,240 lbs. per square inch, while another glue showed 8,523 lbs.

The method of the Forest Products Laboratory [45] used in testing airplane glues is as follows:

"Two blocks of hard maple, about 1 x 2 x 12 inches in size, are glued together lengthways along their flat grain, and after standing about a week to dry out the glue, are each cut into four shear specimens having a glued area of 4 square inches. The shearing pressure to separate the blocks is then noted by a testing machine, and the percentage of wood torn out by the glue is estimated.

If the failure occurs entirely in the glue, a measure of the strength of the glued joint is obtained, but if the failure is entirely or partly in the wood, as frequently happens, the full strength of the glue is not developed, and the test may have to be repeated, using stronger blocks.

The same method has been used in securing data on the strength of wood in shear. Consequently when the strength of glue has been determined it can be compared with that of any wood whose average shearing strength is known.

[39] *Mitt. K. Materialprüf. 36,* 2 (1918); *J. Soc. Chem. Ind. 37,* 743A (1919).
[40] *J. Ind. Eng. Chem. 7,* 102 (1915).
[41] *Schewed. teknisk Tideskrift 28,* 52 (1898); *Chem. Z.,* 1898, p. 283.
[42] *Dingler's J. 152,* 204.
[43] *J. Ind. Eng. Chem. 9,* 359 (1917).
[44] *J. Ind. Eng. Chem. 12,* 356 (1920).
[45] See their Bulletin No. 66, Washington, 1920; also *Mech. Eng. 41,* 382 (1919). A detailed description of this test and of several other similar official tests, is given by Clyde H. Teesdale in his book with C. Mortimer Bezeau, entitled "Modern Glues and Glue Handling," The Periodical Publishing Co., Grand Rapids, Mich., 1922.

Four specimens are usually broken and an average taken of their individual values. The variation in the values can be kept at a minimum if the specimens are selected, prepared, and tested under as nearly the same conditions as possible. A very important factor is the selection of the wood. The species should be the one upon which it is proposed to use the glue, or one at least equally strong. Hard maple is the standard wood in use at the Forest Products Laboratory. Other woods of equal or greater strength which might be used are sweet birch, black locust, flowering dogwood, canyon live oak, persimmon, big shell-bark hickory, and western yew.

It is a good plan to test hide glue at three or four different dilutions. Four different sets of specimens should therefore be prepared, using 2, 2¼, 2½ and 2¾ parts water, respectively, to 1 part of glue. An exceedingly high-grade glue may work best at three to one, and there are low grades which will give best results with less than two parts of water to one of glue. Other types of glue should also be tested under conditions which will permit them to develop their full strength.

On account of the variable nature of wood and the impossibility of doing perfect gluing, the test is far from perfect as an absolute measure of the strength of a glue, but no other strength test has been found to be nearly so good. It merely gives an idea of the ability of the glue to hold wood together. If only one or two specimens are tested, the results are apt to vary widely and be misleading, so it is desirable to base conclusions upon data from a considerable number of tests.

As a means of judging whether the glue is being used to the best advantage, the shear block test is very valuable. The specimens can be prepared from almost any piece of glued work, provided the laminations are not thinner than about one-fourth of an inch and the grain in adjacent laminations runs parallel. It is preferable that the specimens be cut to the size (4 sq. in.) but it is not absolutely necessary; smaller sizes can be used if conditions require.

The highest grades of animal glue are the strongest glues used in wood working. Their strength is greater than that of the woods they are used upon, and when they are properly applied they are exceedingly reliable, so long as they are not exposed to moisture. The certified glue used in propeller manufacture

was sufficiently strong for the highest type of woodworking, but still higher grades of glues are obtainable. The certified glues were required to have an average shearing strength of 2,400 pounds per square inch, with a minimum of not less than 2,200 pounds per square inch. Most of them, however, actually show an average shearing strength of between 2,500 and 3,000 pounds per square inch. The shearing strength of the lower grades of animal glue, such as 1¼ and less (See p. 191), is somewhat lower, but by careful application fairly high values can be obtained from them.

The water resistance of animal glue is low; but the high grades, which have high jelly strength, will stand dampness for longer periods than the low grades, which have low jelly strength."

From the results of a large number of shear tests made by the above method, using a joining pressure of 200 lbs. per sq. in.[46] R. H. Bogue's [46a] experiments show that the joining strength of a glue is a function both of its viscosity (at 60° C.) and its jelly strength, but it is directly proportional to its viscosity at 32° C., which he mistakenly calls its melting point.[46b] Bogue's results indicate that the viscosity at about 30°–35° C. is the best single measure of joining strength. It is, so to say, a combination viscosity—jelly strength figure; for then, as C. R. Smith has shown, the glue is just beginning to gelatinize. Bogue also showed that on heating a certain hide glue for twelve hours, its joining strength dropped from 2,940 lbs. per sq. in. to 1,965 lbs. per sq. in. In his strength tests he used the procedure of the Forest Products Laboratory.

The technique of the British Aeronautical Inspection Department is as follows: [47]

Carefully selected pieces of hard, dry, straight-grained, American walnut, two inches wide, nine inches long, and three-eighths

[46] Very high pressures are unsafe as the glue may be mostly squeezed out, leaving a "starved" joint. The joining pressure in the above test has not been accurately defined, which is a defect common to most descriptions of such tests.

[46a] *Loc. cit.;* also *J. Ind. Eng. Chem. 14,* 435 (1922).

[46b] Bogue originally started out to determine the melting point with a series of viscosities at successively lower temperatures, plotting the resultant curve, and taking the point of infinite viscosity as the setting point. This being very laborious, he found that the viscosity taken at 32° C. gave him a figure that was relative to the setting point so determined. This figure was therefore taken in subsequent investigations in lieu of the true setting point.

[47] See First Report of the Adhesives Research Committee, London, 1922, p. 18.

of an inch thick, are planed true on the flat face and then toothed with a toothing plane having 25 teeth per inch.

The glue (concentration not stated) is soaked 24 hours at room temperature,[48] heated a short time between 60° and 80° C., and allowed to cool to 60° before application.

Two pieces of warm wood, which have remained several hours in a constant temperature oven at 35° C., are glued on the prepared surfaces by the warm glue solution applied by the finger, carefully avoiding the formation of air bubbles. The two glued surfaces are then placed together so as to form a one-inch overlap, giving two square inches of glued area, and a pressure of 400 pounds per square inch is applied for 12 to 18 hours by tested box springs.

After removal from pressure the joint stands three days and its breaking stress is then determined in an adapted form of the Avery or Buckton cement-testing machine, the mean of four tests being taken.

The Committee comments that this test cannot in itself be regarded as an absolute criterion of the value of a glue, since a number of disturbing factors arise, such as variations in porosity of the wood, in the heating of the glue, in the temperature of the wood, the thickness of glue films, in atmospheric humidity and temperature, and in application of the test load. If these factors are carefully held in mind, and any obviously erroneous test rejected, the experimental error will generally not exceed 10 per cent.

The British Engineering Standards Association have fixed the following standards for 4½-inch test pieces:

| *Class* | *Breaking Stress* | *Use* |
|---|---|---|
| Propeller glues ..... | 1,100 lbs. per sq. in. | —Airscrew manufacture. |
| Class I ..... | 1,000 " " " " | —Important stress-bearing work. |
| Class II ..... | 900 " " " " and under | —No stress-bearing work. |

**Laboratory Test Series.**

Many people mistakenly attempt to judge glue by its color, odor, clearness, fracture, shape, or thinness of flake, etc. Since glue is used for a multitude of different purposes, the use for

[48] Obviously thick cake glue was mostly used, for ground glue soaks up within an hour or less.

which a glue is intended should always be borne in mind when submitting it to test or technical examination. Frequently special tests must be devised which simulate special conditions under which the glue is to be used, so that it is obviously impossible to include all tests in any ordinary laboratory series.

There is here given, however, a connected series of tests which may be conveniently and quickly run. They cover practically all that is needed to gauge the value of a glue, especially when one has had practical experience with other lots of the same glue.

Thin blown glasses about 3½ in. high and 2½ in. in diameter are convenient for making these tests. Twenty-five grams of each glue to be tested is broken into small pieces and soaked in 100 cc. of cold water until thoroughly softened. Thick sheet or flake glue should soak overnight in a cool place.

With the glues under examination, there are at the same time soaked up a number of glues of known strength (*standards*) for tests of glue are preferentially *comparative* to avoid the great loss of time involved in fixing *absolute* conditions. It is desirable and convenient to use the standards described later (p. 190), since they cover the range of glues and gelatins ordinarily met with, and are familiar to most American manufacturers and dealers, and besides to many others.

In cold weather or in testing high-grade glues, 20, 15 or even 10 grams of glue to 100 cc. of water may be used, providing unknowns and standards are treated alike in all respects. In warm weather low-grade glues must sometime be tested 30 to 100, unless ice is available to chill the jellies. Gelatins are usually tested from 3 to 10 grams to 100 cc. of water.

When the glues are thoroughly softened, the glasses are placed in a simple rectangular water bath having a double bottom to prevent the glasses from being too close to the flame, and the temperature of the glues raised to 80° C., the contents being meanwhile thoroughly stirred from time to time to insure complete solution. Insufficient soaking or stirring is apt to leave some undissolved glue, which vitiates the test. Upon complete solution, the following tests are made in the order given.

1. *Reaction.* This is determined by a strip of litmus paper which is then allowed to adhere to the right-hand edge of the test sheet. If the exact degree of acidity or alkalinity is desired a separate titration must be made. The degrees of acidity, alka-

linity, foam, grease, and odor are conveniently noted on an arbitrary scale of 1 to 5; thus under acidity, 1 would mean *practically neutral,* 2 would mean *slightly acid,* 3 would mean *fairly acid,* 4 would mean *strongly acid,* 5 would mean *very strongly acid.*

2. *Odor.* While the glues are being dissolved, or at any other convenient time during the test, the odor is noted. This gives some indication as to the stock from which the glue was made; in fact, the odor was once seriously proposed as a test of quality. Decomposition, though often masked by antiseptics or essential oils, is readily detected, for decomposed glue or gelatin has a peculiarly nauseating odor. Glues are rated as "sweet" (1) or "off" (2 to 5). The ordinary stock odor of a glue is not an objection, but with food gelatins freedom from all odor is desirable.

3. *Viscosity.* The viscosity is then taken on each sample and each standard, by running the hot glue solution from a pipette (previously warmed each time by the hot water of the glue bath which serves also to wash it out between determinations), noting the time of efflux with a stop-watch. Any convenient pipette may be used, but Alexander's standard pipette described on page 177 is of convenient size and shape. The average time for a viscosity determination with it is about 40 seconds. Special caution must be used to see that nothing interferes, even momentarily, with the efflux of the glue solution. If anything clogs the pipette it must be cleaned and the viscosity run anew.

4. *Grease.* A flat camel's hair brush is dipped in the glue solution, worked into a little aniline or pulp color on the corner of a piece of hard sized paper. The colored glue is then painted out upon the sheet, where whitish spots or "eyes" appear whose number is roughly proportionate to the amount of grease present. For an exact determination of grease a separate determination must be made.

5. *Foam.* Beat the glues rapidly with the glass stirring rod, using, say, 30 double strokes (across the diameter of the glass and back), and then note comparatively how the foam fades away or persists. With some glues the foam dies away speedily or even instantaneously, and such are rated 1 (foam free).

This is especially the case with greasy glues. Other glues are rated from 2 to 5 depending upon the persistence of the foam.

For more accurate estimation of foam, the glues may be reheated after the tests are concluded, and agitated in a small bowl by an egg-beater, the foam being measured in mm. after the glues have been poured back into their glasses, or into graduated cylinders.

While very undesirable in a veneer glue, foam is advantageous in a gelatin used for making marshmallow confectionery. Trotman and Hackford [49] give a method for determining foam, which is similar to that described above. They found that peptones, overboiling, and alkali (which causes hydrolysis) produce foam. H. J. Watson [50] found that foam was favored by free alkalis or alkaline earths, free acid, zinc compounds, overheating, and mucin (Rideal).[51]

6. *Comparative Set.* The glasses are now taken from the water bath and set aside to cool. Note is made of the order in which the jellies set, which is usually in the order of their jelly strengths. In warm weather, especially with glues of low strength or in weak concentrations, the glues are placed in cold or even iced water.

7. *Jelly Strength or "Finger Test."* After the glue solutions have set or gelatinized, the glasses are arranged in order of the strength or firmness of their jellies. This is done by pressing the jellies with the middle finger or with two fingers, and noting their comparative resiliency. The unknown glues group themselves as equals to or as stronger or weaker than the various standards used on the test. The standards should be so chosen that they cover the range of the glues on test, which may then, if necessary, be graded in between the standards. The difference between each standard "grade" is divided into ten "points," and the differences fixed in increments of two points.

The concentration and temperature of the glue jellies must be such as to permit the ready detection of small differences of jelly strength, which is difficult if the jellies are too stiff.

With the finger test the personal equation is naturally a factor;

[49] *J. Soc. Chem. Ind. 25,* 104 (1906).
[50] *J. Soc. Chem. Ind. 25,* 209 (1906).
[51] Technical Note No. F-9 of the Forest Products Laboratory deals with foamy glues.

but given proper standards, it is speedy and sufficiently accurate for commercial purposes. Mechanical devices are apt at times to give erroneous results, particularly in attempting to take the jelly strength under "absolute" conditions without the steadying effect of standards. Slight differences in temperature, time of set, and amount of evaporation or surface skin will make any instrument, no matter how perfect mechanically, give variable results. But with standards which are treated the same as the unknown glues, there is little chance of *serious* error. Where glues are nearly alike in strength, their jellies may be broken up with the fingers to see the difference between them, if any.

8. *Keeping Properties.* The glasses are now allowed to stand at room temperature to see how the glues resist bacterial attack and mold. If it is necessary to know the keeping properties under certain conditions (i.e. at greater dilutions, at higher temperature, if mixed with color or other ingredients) these conditions must be simulated in a special test.

9. *Appearance of Jelly.* Practically all glues are turbid and a rough description *clear, cloudy,* or *opaque* will answer. Note should be made of any flocculent precipitate or sediment. "Opaque" usually means that some whitener has been added, i.e. oxide of zinc.

With gelatins the clarity of the jelly is usually a very important matter, especially when they are intended for photographic or table use. The clarity is best measured against other samples of gelatins. S. E. Sheppard [52] has described a turbidimeter which may be used to determine the degree of clarity of gelatin and other substances.

**Standard Glues.**

As these form the fixed scale by means of which unknown glues are to be measured, their careful selection and preservation in moisture tight packages is a matter of great importance. In the past, results of glue tests by various investigators have not been comparable because they used different glues, different methods, and had no standards of comparison.

Although no official unanimity of standards exists even now, for nearly a century American manufacturers have been using

[52] *J. Ind. Eng. Chem. 12,* 167 (1920).

a series of loosely fixed standards based upon those established by Peter Cooper, the well-known philanthropist, who was an American manufacturer of glue. And about twenty years ago J. Alexander attempted to fix these standards [53] so that uniformity might prevail and all use standards of the same strength. The table below gives sixteen arbitrarily established, nearly equidistant *grades* which cover the range of jelly strength usually met with. The jelly strengths were determined by Alexander's jelly tester, and the viscosities by Alexander's viscosity pipette, both previously described. The viscosity figures are based upon many years of laboratory experience with glues tested 25 grams of glue in 100 cc. of water; but since glues of the same jelly strength may vary greatly in viscosity, there is indicated in the table reasonable limits for such variation. Opposite each standard is the corresponding Cooper grade, and also the grade recently suggested by Bogue, based upon the viscosity in centerpoises of an 18 per cent. (dry basis) solution of glue, at 35° C.[54]

| *Standards* | *Viscosities (in seconds) at 80 Solution 25–100* | *Permissible Variation in Viscosities (in seconds)* | *Jelly Strength (in oz.) at 10* | *Jelly Strength (in grams.) at 10* | *Equivalent Cooper Grade* | *Equivalent Bogue Grade* |
|---|---|---|---|---|---|---|
| 160..... | 40 | 12 | — | — | — | — |
| 150..... | 34 | 8 | — | — | — | — |
| 140..... | 28 | 5 | — | — | — | — |
| 130..... | 26 | 3 | 258 | 7.314 | A Extra | 12 |
| 120..... | 25 | 1 | 236 | 6,691 | No. 1 Extra | 11 |
| 110..... | 24 | ¾ | 214 | 6,067 | 1 | 10 |
| 100..... | 23 | ¾ | 192 | 5,443 | 1x * | 9 |
| 90..... | 22 | ¾ | 170 | 4.820 | 1¼ | 8 |
| 80..... | 21 | ½ | 148 | 4,196 | 1⅜ | 7 |
| 70..... | 20 | ½ | 126 | 3,572 | 1½ | 6 |
| 60..... | 19 | ½ | 104 | 2,948 | 1⅝ | 5 |
| 50..... | 18 | ½ | 82 | 2,324 | 1¾ | 4 |
| 40..... | 17 | ¼ | 60 | 1,701 | 1⅞ | 3 |
| 30..... | 16½ | ¼ | — | — | 2 | 2 |
| 20..... | 16 | ¼ | — | — | — | 1 |
| 10..... | 15½ | ¼ | — | — | — | — |
| Water.... | 15 | | | | | |

* Called "one cross."

[53] *J. Soc. Chem. Ind.* **25**, 158 (1906).

[54] These were kindly furnished by Dr. Bogue in a private communication. Their parallelism to the Cooper grades can be regarded only as approximate because of the variability between the jelly strength and viscosities of the standard samples. Hide glue grades are known as $H_{12}$, $H_{11}$, etc., bone glues as $B_{12}$, $B_{11}$, etc.

In the old days, manufacturers or dealers would occasionally check their standards by mutual exchange or by purchasing some of a known grade in open market. Nothing has been published regarding the origin of the nomenclature of the Cooper grades, but from information received it is probable that they represent the distance that a certain weighted foot-rule would compress a certain bowl or vessel of glue jelly of known concentration and temperature. A weak glue allowed it to sink 2 inches, a strong glue only 1 inch; and the intermediate grades were measured in eighths of an inch. The instrument must have resembled the jelly tester described by Davidowsky (see p. 174).

**C. R. Smith's Polariscopic Method.**[55]

Smith's own description follows:

"In grading gelatins or glues polarize 3 g. per 100 cc. at 35° to 36° C. in a 2-dm. tube; cool a portion of the solution rapidly to 15° (or 10°) and transfer, before the sample has jellied, to a cold 1-dm. tube. This procedure avoids contractions in the jelly which may produce poor readings. If the samples need clarification, digest the solution with 5 cc. of light powdered magnesium carbonate at 30° to 40° C. for one hour or longer, and filter until clear, avoiding appreciable evaporation. Occasionally it has been found advantageous to add 0.10 g. of ammonium citrate to the filtrate to avoid the formation of insoluble calcium compounds, but this does not appear to be necessary if the magnesium carbonate has been used in sufficient quantity and the digestion has not been too short. The procedure for clarification outlined has not been found to change the polariscopic results when applied to clear samples.

"In place of a constant temperature bath the tubes can be placed in a large vessel of water in a portion of the ice-chest where the temperature ranges between 13° and 16° and left overnight. The next day the temperature can be controlled for 4 to 7 hours at $15 \pm 0.4°$. If a constant temperature bath is used the tubes may be read at once in the morning.

"Considering a sample which polarizes $-20.5°$ at 35° C. and $-40.0°$ at 15° C. in a concentration of 3 g. per 100 cc.,

[55] *J. Ind. Eng. Chem. 12,* 878 (1920). The original must be consulted for tables of results on a large number of glues and gelatins, and for any further details desired.

it is suggested that the strength be expressed as 19.5 points at 15° C., the increment in rotation in Ventzke degrees. Referring to Table II (omitted here) we see that a 25-point gelatin at 15° represents the maximum strength obtained. In factory control the jelly strength determinations can be made by the polariscope in the progress of the extractions, evaporation, or drying. The solutions are diluted to approximately 3 g. per 100 cc., controlled by rotations at 35° C. The jelly strength at 15° is determined as usual and calculations made by simple proportion to reduce rotations to the average basis of —20.5° V. at 35° C. An actual test in factory control gave the strength of a first extraction as 17 points at 15° C.; after evaporation it was 10 points. The evaporated extract was mixed with some unevaporated material, bringing the strength to 11.5 points; after drying it tested 11.6 points. These figures obviously represent poor extraction, and considerable loss of strength in the evaporator, but show no loss from bacterial action in drying.

"Jelly strength tests made on samples direct and after incubation for 24 hours at 37° C. show little or no loss in strength of nearly sterile gelatins, while those in active state of decomposition show considerable loss with the development of bad odors. The following results illustrate this:

| *No.* | *35° C. 3 g. per 100 Cc.* | *Rot. at 15° C. Before Incubation* | *Rot. at 15° C. After Evaporation* | *Odor After Evaporation* |
|---|---|---|---|---|
| 1..... | —20.3 | —33.4 | —33.8 | Sweet |
| 2..... | —20.5 | —39.7 | —36.8 | Bad |
| 3..... | —20.3 | —35.6 | —31.6 | Bad |

"The solutions were filtered through magnesium carbonate to clarify. The increase in rotation in No. 1 was probably due to evaporation and experimental error. The loss of jelly strength in Nos. 2 and 3 was quite pronounced, with corresponding production of disagreeable odors."

In a lot of bone glues the mutarotation at 15° V. varied from 10.4 to 24.9, and in a lot of hide and sinew glues, from 3.9 to 24.1; the ratio $\frac{\text{rotation 15° V.}}{\text{rotation 35° V.}}$ varied with the bone glues from 1.55 to 2.14, with the hide and sinew glues from 1.20 to 2.15. The polariscope results checked with the jelly strengths, those showing the greatest mutarotation having the highest jelly

strengths and requiring the smallest amounts to produce a jelly of certain standard strength.

In addition to the foregoing laboratory series of tests, it is sometimes desirable to determine moisture and ash.

**Moisture.**

From two to three grams of glue or gelatin are roughly granulated and dried at 110° until constant in weight. If the product is commercially dry, the estimation of water is of no practical value, for it varies rapidly with atmospheric conditions, and any unusual percentage would at once register itself in reduced viscosity and jelly strength.

R. H. Bogue [56] found that the water content of air dry glues varies directly as the jelly strength, being 13.66 per cent. in a fairly strong hide glue and 10.68 per cent. in a weak bone glue. D. Jordan Lloyd [57] reports that a specimen of Coignet "Gold Label" gelatin had 20 per cent. of moisture removable by drying 6–8 hours in a hot air oven at 110°.

**Ash.**

The ash of *gelatin* is of importance for in it may be sought certain forbidden impurities. Besides, in some jurisdictions, the ash of gelatin, if above certain allowed percentages, is regarded as an adulteration. No manufacturer would intentionally raise the ash, for this would lower the test; but excessive ash may indicate careless manufacturing.

The ash of *glue* runs usually between 3 and 4 per cent. Some bone glues contain considerable calcium phosphate, while hide glues are apt to have calcium sulphate or chloride resulting from neutralization of the lime used in preparing the stock. In the ash also appear various whitening agents such as zinc oxide, lead sulphate, or carbonate, chalk, clay, etc.

To estimate ash, place 2–3 grm. glue in a large platinum crucible and heat slowly, as the glue at first intumesces violently. Ash at a low redness, preferably in a muffle, using a few drops of nitric acid to insure the oxidation of all the carbon. According to Kissling [58] the ash of bone glue fuses in the bunsen burner,

[56] *Chem. Met. Eng. 23,* 105 (1920).
[57] *Biochem. J. 14,* 148 (1920).
[58] *Chem. Z. 11,* 691 and 719.

*Laboratory Test Sheet*

20 GRMS. GLUE TO 100 CC. WATER SOAKED 14 HOURS

| *Glass Number* | *Description of Specimen* | *Acid* | *Alk.* | *Sweetness* | *Foam* | *Grease* | *Viscosity* | *Comparative Set* | *Jelly Strength* | *Jelly* | *Remarks* | *Litmus Strips* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Low-grade hide glue..... | 1 | 2½ | 2 | 1 | 2 | 17¾ | 13 | 58 | Separates | | |
| 2 | Medium-grade hide glue.... | 1 | 1 | 1 | 1 | 3 | 20¼ | 6 | 102 | Opaque | | |
| 3 | High-grade hide glue..... | 1 | 1 | 1 | 1 | 1 | 27 | 1 | 160 | | | |
| 4 | Low-grade bone glue..... | 2 | 1 | 1 | 3 | 1 | 16½ | 19 | 34 | | | |
| 5 | Medium-grade bone glue.... | 1½ | 1 | 1 | 1 | 1 | 17 | 11 | 76 | | | |
| 6 | High-grade bone glue..... | 2 | 1 | 1 | 1 | 1 | 18½ | 5 | 110 | Clear | | |
| 7 | Second run ossein stock.... | 1 | 1 | 1 | 1 | 1 | 21 | 3 | 150 | | | |
| 8 | French square sheet (bone). | 2 | 1 | 1 | 1 | 2 | 16 | 16 | 50 | | | |
| 9 | English oblong sheet (bone) | 2 | 1 | 1 | 2 | 1 | 16½ | 14 | 60 | | | |
| 10 | German oblong sheet (bone) | 1 | 1 | 1 | 2½ | 1 | 16 | 17 | 48 | | | |
| 11 | English "skin glue"..... | 1 | 2 | 1 | 1 | 1 | 18½ | 8 | 90 | | | |
| 12 | German "lederleim" ..... | 1 | 1 | 1 | 1 | 1 | 18¾ | 10 | 80 | | | |
| 13–40 | Standard ..... | — | — | — | — | — | — | 18 | 40 | | | |
| 14–50 | " ..... | — | — | — | — | — | — | 15 | 50 | | | |
| 15–60 | " ..... | — | — | — | — | — | — | 12 | 60 | | | |
| 16–80 | " ..... | — | — | — | — | — | — | 9 | 80 | | | |
| 17–100 | " ..... | — | — | — | — | — | — | 7 | 100 | | | |
| 18–130 | " ..... | — | — | — | — | — | — | 4 | 130 | | | |
| 19–150 | " ..... | — | — | — | — | — | — | 2 | 150 | | | |

is neutral and contains phosphoric acid and chlorides, while hide glue ash is infusible, alkaline, and generally free from phosphates and chlorides.

Since hide and bone glues are frequently mixed both in the liquor and dry form, it is obviously unsafe to draw any conclusion from the ash as to the raw material used. The composition of the ash usually depends upon the process used, and its estimation, in glue, unless for some special purpose, is not commercially necessary.

**Recording Tests.**

There is given on page 195 a specimen of a laboratory test sheet used in recording the connected series of tests just described.

In 1898 Friman Kahr [59] proposed a series of four tests designed especially to grade glues for joining use. These were:

1. *Adhesion* = the weight of dry glue necessary to make up 100 lbs. of liquid having the proper viscosity or body at 60° for use in making joints. This figure varied from 60 lbs. in the case of the lowest grade glues to 29 lbs. with the highest grades. It is in effect a viscosity determination.

2. *Economic value* = adhesion figure x cost per pound. Thus Kahr's figures showed $29 \times 18 = \$5.22$ per 100 lbs. of liquid made with the highest grade of glue (cost 18¢ per pound) and $60 \times 5\frac{1}{2} = \$3.30$ per 100 lbs. of liquid made with the lowest grade (cost $5\frac{1}{2}$¢ per pound).

3. *Cohesion or strength* = crushing strength of the glue jelly at the concentration indicated under 1, and determined at 65° F. (about 18° C.). This was subsequently fortified by a joint strength test.

4. *Congealing test* = temperature at which the glue made up as indicated under 1, would gelatinize. This figure ran between 91° and 75° F., and gave some indication as to speed of set.

One radical defect in this system is the latitude left open in fixing the "proper viscosity" under 1. But this is the method most consumers of glue follow—they find by rough trial about how much glue is needed to make up a ready-to-use solution and then figure its cost. Since many tests are needed to establish the *minimum* amount of glue needed, they usually stop when

[59] International Fisheries Congress, Bergen, Norway, 1898; also a periodical, *Glue*, published by him at East Haddam, Conn.

an approximate figure is reached. In any event Kahr's tests are practically intended for joint work, and cannot serve for a general laboratory method intended to indicate the value of glues for most ordinary uses.

R. H. Bogue,[60] after reviewing the various testing methods, proposes a system of evaluation based on measurement of the viscosity of an 18 per cent. dry basis (equal on the average to 20 per cent. of commercial glue) solution of glue in the Mac-Michael viscosimeter at a temperature of 32° to 35°, the latter being preferred since there is less change of viscosity with time. This is the one *primary* test; and Bogue's figures show that it correctly classifies glues on the basis of their joining strength. Speaking of the jelly strength and viscosity at 60° which the primary test is to supersede, Bogue says: "They may, however, be of great value in *secondary* evaluation, i.e. in determining the adaptability of a given glue to a given service. For example, the jelly consistency would be of value in selecting glue for the printer's rollers, and the rapidity of setting of the jelly as well as the viscosity at working temperatures would be desirable data for the wood-working industry."

While Bogue's primary test is the best single test of the value of a glue or gelatin for joint work, the fact that the similar test of Fels, proposed in 1897, has not met with general acceptation indicates that neither is self-sufficient. Bogue's statement above shows that the secondary tests are *essential.* A glue test may be considered from the standpoint of 1, the seller or manufacturer; 2, the buyer or consumer; 3, the chemist or tester. Let us review these.

**Tests from the Standpoint of Seller, Buyer and Chemist.**

The manufacturer or dealer receives different lots of boilings of glue from the factory, and before offering them to his customers, must test them to find *all their principal characteristics* so that the lots may be graded and selected to be sent out to the trades for which they are most suitable, and especially so that *deliveries to each consumer are uniform* in working properties and free from objectionable defects. Nothing is more essential to the buyer than *uniform deliveries,* and this is therefore equally

[60] *J. Ind. Eng. Chem. 14,* 435 (1922).

important to the seller. Even in the absence of absolute standards, the seller keeps reserve samples of his deliveries, against which he can test subsequent shipments. He must give his customer glue *the same as last.* Some wood-workers use the same glue for both joint and veneer work. It would be a serious mistake to give such a consumer a glue that would foam in his veneering machine, although a slightly foamy glue would serve for a shop where only hand gluing was done.

As will be seen in the next chapter, entitled "The Uses of Glue and Gelatin," each industry and, in fact, often each consumer has peculiar requirements. The seller must have all the test figures he can reasonably get out of his laboratory test; therefore it is better to have a jelly strength and a working viscosity figure than it is to have the lower temperature viscosity figure alone. Besides *all* the other figures are of *primary* importance, for one objectionable characteristic is enough to divert a lot of glue from one industry to another, i.e. greasy glues are suitable for veneering (if foam free), but not for paper sizing. In matching competitors' samples it is also wise to consider *all* of its characteristics, for most buyers do not give specifications and some even fail to give the seller essential information or samples.

The buyer should test his glue to be sure that he is getting what he ordered, and that the delivery has no objectionable features. Different lots of glue are seldom identical, and any accidental or intentional variation in delivery may cause damage and loss—even too good a glue sent in error may do this. Since by the exercise of ordinary prudence the buyer may test his glue, he cannot hold the seller legally liable for any loss he may suffer from its use; but can claim only the difference in value between what was delivered and what was paid for. The buyer should furthermore be in a position to test small samples of glues offered by competing sellers, and by eliminating those of least value, minimize the number to be selected for practical factory trial. The majority of buyers trust the sellers, using the new lot just the same way as its predecessor, and their confidence is never intentionally violated by reputable sellers.

Most generally complaints made by consumers may be traced to causes other than the glue, i.e. grease spots on paper to ma-

chine oil, poor joints to a broken window pane which in winter allowed the glue to chill.

Since the majority of manufacturers and sellers do their own testing, the consulting chemist is usually employed by some user of glue, or else as an arbitrator or an expert in disputed cases. Should his practice in this line be insufficient for him to have absolute standards, he can readily obtain from his clients samples with which to make comparative tests.

# Chapter 13.

## The Uses of Glue and Gelatin.

There are certain simple principles which govern the practical handling of glue and gelatin, no matter for what purposes the various grades or qualities may be used.

Glue and gelatin should be stored in a place that is neither too hot nor too damp. Storage in a hot dry place causes considerable loss of moisture, and unless due allowance is made for this, too much actual glue will be weighed off. (For all strictly accurate or scientific work, calculation must be made of the actual amount of glue in solution, by drying at 110°C.)

Dampness is apt to cause the glue to take up so much moisture that it will mold and undergo bacterial attack, which injure its strength and of course, ruin food and bacteriological gelatin. Ground glue and ground gelatin in barrels are less apt to suffer from these changes, but to be safe in tropical climates, glue and gelatin should be stored in air-tight containers. Even with these, trouble may be caused by "sweating."

The first essential is to select the grade of glue or gelatin best suited for the work in hand. This involves consideration of the quality and cost of the product to be made. Some suggestions as to selection will be given later, but in many cases the safest procedure is trial under actual working conditions which must be simulated in laboratory tests.

The second essential is to test carefully each delivery or lot to be sure of its uniformity. Any difficulty may then be traced to its real cause instead of simply blaming it on the glue.

The third essential is to establish correct methods and definite formulas for preparing and using the product. While formulas must differ with each class of work and even with weather conditions, the following principles should be observed in each case.

1. Use *definite weights* of glue and gelatin. Do not depend upon measures, for owing to variations in thickness of cut, glues are apt to vary greatly in specific gravity, even in the ground

product. Definite quantities of water and other ingredients must also be used.

2. Soak the glue or gelatin in clean cold water until it is thoroughly softened. Ground glues soak up very quickly and are preferable for this reason, besides occupying less storage room. They should be stirred into the water to prevent the formation of lumps containing dry glue, which will not readily dissolve. Thick sheets are usually soaked overnight, and when bent after soaking should show no whitish "bone" at the bending line, but be thoroughly softened.

3. Melt preferably in a water or steam-jacketed bath and keep at as low a temperature as the work will permit. *Use a thermometer,* and preferably do not heat above about 65° (150° F.). Glue loses strength continuously under the action of heat, and it is more advantageous to heat up successive small lots rather than to have a large lot "cooking" all the time. In many cases, e.g. with leather belting cement, flexible padding or book-binding glue, etc., large lots are made up and cast into pans, the jelly blocks being kept and heated up a little at a time.

4. Make good evaporation from open glue pots. Some users who neglect this, fail to notice loss in strength due to prolonged heating, because the concentration of the solution due to evaporation may in a large measure compensate for the deterioration of quality.

5. Use clean utensils and keep them clean, and add antiseptics if necessary. Glue and gelatin are particularly subject to bacterial attack, which speedily destroys their strength, usually with the development of a nauseating odor. The preservatives added to commercial glue are sufficient to preserve it only when in fairly strong solutions. Dilute solutions require additional quantities of preservatives, among which may be mentioned carbolic, cresylic, sulphurous, and boracic acids, borax, formaldehyde, sodium bisulphite, etc. Low temperatures as well as chloroform and toluol are commonly used to preserve gelatin solutions used in scientific work.

Glue and gelatin are used for such a multitude of purposes that it will be possible to consider in detail only a few of them. The principal uses of glue and gelatin classified as to general nature, are as follows:

**Adhesive.**

Wood Joints—including furniture, pianos, musical instruments (violins), wooden-ware, house-trim, barrel heads, dowels, blocks, coffins, toys, etc.

Veneers—for furniture, house-trim, seats, shipping packages, etc.

Paper Boxes—"setting up" and covering paper boxes, sealing cartons, etc.

Packaging—making and wrapping paper packages on automatic machines.

Leather Goods—bags, belts, pocket-books, dress suit cases.

Leather Belting—most machinery belts are made with glue and many are made "endless" with glue to avoid lacing. This is especially desirable on high-speed machinery.

Bookbinding—for case making, rounding and backing, tabbing, etc.

Padding—making pads, "blocks," calendar pads, etc.

Gummed Cloth and Paper Tape—"passepartout" (for making picture frames), "tape" to replace string in sealing cartons, packages, etc.

Fireworks—as an adhesive and to cause the flaring of mixtures otherwise explosive.

Abrasives—in making sand, emery and garnet papers, as well as wheels and belts, glue holds the abrasive to the base.

Glue, gelatin and isinglass are also important components of many adhesive mixtures and cements.

**Sizing or Stiffening.**

Paper—including wall paper, writing paper, stocks, bonds and government currency.

Hats—most straw hats, some felt and cotton hats are stiffened with glue or gelatin.

Textiles—including silk, ribbons, burlap (for wall coating linoleum, etc.), shade-cloth.

Barrels—for oil, alcohol, turpentine, etc.

Wood—cheap furniture, reed furniture, including baby carriages, broom and other handles, are sized to prevent absorption of too much varnish.

Walls—before papering or painting.

Calsomine—for walls; both hot and cold water "paints."
Scenery—after sizing, is painted with distemper colors sized with glue.
Leather—dressing leather, making polishes, etc.

**Compositions.**

Matches—forms and binds the heads and reduces the explosion to a flare.
Dolls' Heads—including flexible "rubber" toy heads.
Printers' Rollers—used in practically all printing presses.
Gas Tubing—the flexible glue is hidden by a cotton tube.
Spangles—used in decorating dresses.
Gelatin Foils—used for wrapping, "windows" in boxes, toys, decoration.
Hectographs—the gelatin-glycerin duplicators are sold under a variety of names.
Microscopy—for glycero-gelatin mounts.
Bottle Capping—made flexible with glycerin.
Moldings—for picture frames, room molding, etc.
Plaster Casts—glue is used for making flexible molds in which are made large numbers of plaster of Paris casts.

**Colloidal Protector or Colloidizer.**

Electrolytic Refining of Metals and Electroplating, i.e. lead, copper, etc.
Electroplating—to make a fine-grained coherent deposit.
Making Colloidal Precipitates—i.e. colors, insecticides, pharmaceuticals, etc.
Inhibiting Crystallization—i.e. diminishing the sensitiveness of the explosive, lead azide.
Making Emulsions—i.e. of fats, oils, etc., for insecticidal uses, etc.
Stabilizing Foam—i.e. in Fire Foam extinguisher.
Photography—gelatin has practically superceded collodion, and all other colloids in making plates and films, and is largely used in printing out papers.
Plaster of Paris—glue is used as a retarder.

**Miscellaneous.**

Gelatin Printing—bichromated gelatin is made into a negative usable on printing presses.

Glass Chipping—high-grade glue, in drying, tears the surface from glass, making a rough "frosting." Dissolved salts influence the nature of the surface.

Rubber—glue is incorporated with rubber and is said to improve greatly the wear of solid tires and of casings.

Artificial Silk—one variety is made from gelatin. Recently Dr. A. D. Little made some gelatin from pigs' ears, converted this into "silk," from which a purse was knitted. He thus accomplished the supposedly impossible task of "making a silk purse out of a sow's ear."

Millinery—artificial fruits, and flowers are made with the aid of gelatin, which is also used to glue together silk threads to form a brilliant "straw."

Directing Chemical Change—certain reactions are influenced by the presence of gelatin.

Pharmacy—gelatin is used for coating pills, making capsules for powders (e.g. quinine) and oils (e.g. castor oil); also to make emulsions [1] and colloidal remedies (e.g. collargol, etc.).

Bacteriology—gelatin is an important constituent of culture media and serves to make ultra filters.

Medicine—gelatin is used for certain poultices and as an invalid food. It is also used intravenously, although gum arabic has superseded it in cases of shock. Formogelatin is a wound dressing.

Food—gelatin is used in confectionery (marshmallows), ice cream, jelly powders, and in the household to make various desserts.

Some of the uses of glue and gelatin will now be considered in more detail.

## Wood Joints. [1a]

No adhesive makes as strong a joint as the higher grades of animal glue. When properly made with seasoned wood, they

[1] H. N. Holmes and W. C. Child, *J. Am. Chem. Soc.* *42*, 2049 (1920), have made a careful study of gelatin as an emulsifying agent.

[1a] A description of modern glue-room practice is given by C. M. Bezeau in "Modern Glues and Glue Handling," by Teesdale and Bezeau.

are thoroughly reliable if not exposed to undue heat or moisture, and they are stronger than the hardest wood. Specifications for glues to be used in making airplane propellers called for an average shearing strength of 2,400 lbs. per sq. in. with a minimum of 2,200 lbs., but most of the glues showed actually between 2,500 and 3,000 lbs. per sq. in.[2] The glue mostly used tested about 130, but some factories got satisfactory results by careful manipulation of somewhat lower grades.[3]

While the water resistance of animal glues is low, the higher grades stand dampness longer than the lower grades. Outside of this feature there is no object in using a glue that is much stronger than the wood, unless warranted by cost or some other factor.

The wood surfaces should be true with well joined surfaces, which are often scratched or slightly roughened to increase the total area available.[4] The wood should be dry, seasoned and warmer than the room temperature if possible, certainly not colder. The glue should be liberally applied and quickly put under pressure of the clamps before it gelatinizes, or the joint will be weak. Cold wood, a cold room, or a chilly draft (e.g. from an open or a broken window) may chill the glue to a jelly which has practically no adhesive strength. Too high a pressure may squeeze out too much glue and give a weak "starved" joint. The pressure must be uniformly distributed.[5]

R. H. Bogue found that the shear strength of joints increases rapidly with increase in joining pressure up to about 200 lbs. per sq. in., after which it increases more slowly to 1,000 lbs. per sq. in. The glued joint should remain under pressure about 24 hours and must stand about a week to dry out thoroughly, before it develops into its maximum strength. Wood when glued should usually show a moisture content of from 8 to 12 per cent.

The best proportions of glue and water will vary with the grade of glue. U. S. Navy Specification No. 11-B for hide glue

[2] S. W. Allen and T. R. Truax; report No. 66, Forest Products Laboratory, 920.

[3] See C. R. McKee, "Interpretation of Glue Analysis," Bulletin of Chicago Section of A. C. S. 8, 66 (1921); *Chem. Abs. 15*, 1419.

[4] The Forest Products Laboratory, Technical Note No. F-5, found practically no difference between smooth and scratched joints (made with a tooth plane) in hard maple.

[5] See Technical Note No. 92, Forest Products Laboratory.

certified for use in airplane construction, says that the sample shall be mixed with water in four different proportions, namely 2 to 1, 2¼ to 1, 2½ to 1, and 2¾ to 1, by weight, and the proportion at which the greatest strength is indicated will be used in judging the strength of the glue.[6]

The consensus of opinion among experienced woodworkers is that hide glues running in jelly strength from about 70 to 130 are most satisfactory for joints. Some bone glues give good results, Bogue's results showing some that gave a joint strength of over 2,100 lbs. per sq. in. Much depends upon the quality of the bone glue, the feeling against it being partly due to the fact that most of the bone glue sold is low grade.

Where great strength is not essential, bone and hide mixtures, and even straight bone glues may be used for joints.

**Veneers.**

Since veneers undergo little strain, have large surfaces, and are usually protected by paint or varnish, a lower grade of glue may be used for them than for joint work. Hide, bone or mixed glues testing from about 50 to 70 are usually employed. Higher test glues are apt to give trouble by setting too quickly, though this is not a factor where hot cauls are employed. Besides higher grade glue is more resistant to moisture.[7]

Since most veneers are glued on a machine with revolving rolls, freedom from foam is essential; for foam means blistered or loose veneer.

Overheating the glue, or running the spreader idle or too rapidly may cause foam. Among the foam preventatives used are milk and soluble oils and fat emulsions.

**Paper Boxes.**

For "setting up," quick setting glues testing from 70 to 90 are best. For "covering" or "stripping," low grades testing from 30 to 60 are used; automatic machines work best with the higher

[6] See also "Selection and Testing of Animal Glue for High Grade Joint Work," by G. M. Hunt and W. L. Jones, Forest Products Laboratory, 1920.

[7] Technical Note No. F-10 of the Forest Products Laboratory gives the results of moisture tests on veneers. A high-grade glue stood 98 per cent. humidity at 80° for 198 hours as against 24 hours for low grades.

grades. The ratio between cost and water-taking capacity usually influences the selection. When the boxes are used for silver-ware freedom from tarnish-producing sulphur compounds is essential.

**Leather Belting.**

Here strength, flexibility and resistance to moisture are essential. The belt cements usually employed are mixtures of high-grade glue or gelatin, with glycerin and some antiseptic. The highest test glue (130 to 160) is preferable as less of it remains in the finished joint, and it is more resistant to moisture.

**Sizing and Stiffening.**

The "crackle" of new banknotes is produced by sizing them with a solution of high-grade (test about 150), light-colored glue to which alum has been added. For tub-sizing writing paper, to give a smooth surface which will hold the ink, a light-colored glue is essential; the stronger it is the more dilute it can be used. About 2–4 per cent. of alum is used with the glue, and the sizing increases the strength of the paper materially. Glue can also be used to size paper in the beater. E. Heuser [8] reports that precipitation methods were unsatisfactory, but upon adding sufficient talc, the glue was adsorbed and held by the paper. In making wall paper, the glue solution serves to "free out" or deflocculate the clay and color with which it is mixed, as well as to bind it to the paper. Any sweet free-flowing foam-free glue may be used, grades testing about 60 being most employed. In sizing barrels, it is essential that the glue should not melt even in hot weather, while its jelly is being dried out inside the barrel. In the turpentine belt, a concentrated solution of cheap glue is used, but where care and attention prevail, the best and most economical results are had with high-grade glues, the melting point of the jelly being sometimes raised by the addition of chemicals. Gelatin is largely used to stiffen straw hats, but many factories get good results with light-colored glues of medium strength.

[8] *Papier Z. 41,* 1365 (1916).

**Compositions.**

Many plastic compositions are made with the use of glue as a binder. High grades (120–160) are most often employed, and they are often made insoluble with formaldehyde, etc., and rendered flexible by glycerin. For printer's rollers the highest grades of glue (140–160) are used, and a similar flexible gelatin-glycerin composition is used for hectographs and similar duplicators. Flexible glues used for book-binding, padding and the like consist mainly of glue, glycerin and preservatives, as also does flexible gas tubing composition.

Gelatin foils and spangles are made by drying gelatin solutions on level sheets of plate glass. The solutions are often colored or treated to give beautiful surface effects and usually contain some glycerin.

In making plaster of Paris casts, only the highest grade of glue or gelatin should be employed. If too weak the glue mold may soften from the heat of the setting plaster, or break, especially when being pulled off from "undercut" work.

**Photography.**[9]

Chemical analysis and physico-chemical tests are subordinate to actual trial of the gelatin in emulsions, which is depended upon to show its ability to develop the purely photographic qualities of the sensitive materials. But the chemical and physical tests are not only helpful but indispensable in judging how the gelatino-silver halide emulsions will behave during development, washing, fixing, after-treatment (hardening), drying; also in judging its transparency, freedom from color, from objectionable impurities, from excessive acidity or alkalinity.

The tests suggested are the following:

*PHYSICAL TESTS.*

*Jelly Strength.* For most purposes, a high jelly strength (130–160) equal to or greater than the highest Cooper grades, is necessary, although for some purposes gelatins of lower jelly strength may be used. The jelly strength test is generally more

[9] This is based on information kindly supplied by Dr. S. E. Sheppard, of the Eastman Kodak Company.

useful than the melting point, which has been used to classify gelatins as "hard" and "soft."

*Viscosity.* A 6 to 10 per cent. solution is prepared under carefully standardized conditions, and the viscosity determined (preferably in centipoises) at 100° F. and 150° F.

*Melting Point and Setting Point.* These vary materially according to the method used. Using Sheppard's apparatus (see p. 175) and 10 per cent. solutions, "soft" gelatins set at from 19° to 23° C., "hard" gelatins at from 23° to 27° C. The melting points of these solutions are 2° to 3° higher.

All these tests should be checked by comparison with the ash analysis, and a determination of the hydrogen ion concentration ($p_H$ value), which fixes the effective, as opposed to the total acidity. Photographic gelatins usually have a $p_H$ of between 5 and 6, the generally allowable limits being 4 and 7. The $p_H$ value greatly affects the physical behavior, as also does the presence of aluminium salts.

*Clarity, color, odor, and appearance* of the gelatin are, of course, always noted.

*CHEMICAL TESTS.*

*Moisture.* The usual limits are 8–15 per cent. More than 15 per cent. means bad keeping properties and liability to bacterial infection, whereas an over dried gelatin is likely to cause trouble.

*Ash.* Though gelatins with 3 per cent. are sometimes used, 2 per cent. is a safer limit. Within these limits, CaO, $Na_2O$, $K_2O$, $SO_3$, Cl and $P_2O_5$ are harmless. Iron and copper should not exceed 50 to 60 parts per million; lead not over 50 parts per million. $Al_2O_3$ should not exceed 0.2 per cent. of the weight of the dry gelatin. (C. R. Smith [9a] found that a bromide emulsion made with ash-free gelatin was transparent and could be "cooked" indefinitely without ripening. He concludes that ripening is controlled by the ash constituents. They evidently oppose the protective action of the gelatin.—J. A.)

Sulphur dioxide (determined by distillation into iodine) should not exceed 0.1 per cent.

[9a] *J. Am. Leather Chemists' Assoc.*, Oct., 1922.

*Ammonia.* Not more than a trace should be present.

*Acidity or Alkalinity.* Determined by titration of a 2 per cent. gelatin solution with $\frac{n}{10}$ KOH or $\frac{n}{10}$ HCl, using phenolphthalein as an indicator.

*Reducing Substances.* Generally, photographic gelatins should not reduce cold ammonical silver nitrate solution in the dark, within 12 hours. To be of much utility this test must be worked in conjunction with emulsion trials.

*Grease.* The usual test (see p. 188) for "eyes" or "comets" is made and the finely powdered gelatin may be extracted with benzene. Only a negligible amount should be present.

*Mucin, etc.* A 2 to 5 per cent. solution should give no precipitate when acidified with acetic acid (to about 3 per cent.). One to 2 per cent. of alum or chrome alum should cause no precipitate.

**Inhibiting Crystallization.** In the manufacture of lead azide ($PbN_3$) which is used as a "starter" for explosives, some glue, gelatin or dextrine, is added to the reacting mixture of sodium nitride and lead nitrate to prevent the formation of normal crystals of the lead azide which tend to explode spontaneously, probably by fracture.[9b]

**Directing Chemical Change.** The presence of glue in a reaction mixture may change entirely the nature of the reaction and of the end products. Thus Kohlschütter[10] says: "In aqueous solution the action of chlorine on ammonia leads speedily to complete decomposition with the evolution of nitrogen; on the other hand an addition of glue brings about the initial formation of chloramide, which because of the increased viscosity of the solution has a chance to form hydrazine with a second molecule of ammonia, so that the process follows one or the other of the following reaction equations:

*With addition of glue*

$$NH_3 + Cl_2 \longrightarrow NH_2Cl + HCl$$
$$NH_2Cl + NH_3 \longrightarrow NH_2.NH_2 + HCl$$

*In aqueous solution*

$$2NH_3 + 3Cl_2 = N_2 + 6HCl$$

[9b] See A. G. Lowndes, *Kolloid Z.* 28, 238 (1921).

[10] V. Kohlschütter, "Die Erscheinungsformen der Materie," Berlin, 1917, p. 300.

This explains the results of H. Raschig, who, applying some principles of Pope and Barlow, added glue to the solution of hypochlorite and ammonia, and increased the yield of hydrazine from a few per cent. to a commercially possible yield of 40–60 per cent.

**Bacteriology.** Gelatin is largely used in bacteriology as the chief ingredient in many culture media, for the exact preparation of which the reader is referred to books on this subject. Certain classes or kinds of bacteria may be distinguished by the way they act on the jelly; some fluidify it. Only the purest gelatin should be used for this work, because metallic impurities may exert an effect of their own on bacterial growth. This effect need not necessarily be an inhibitive one, for minute percentages of antiseptics may act as stimulants. Still the bacteriologist should be as free from disturbing factors as possible.

Leffmann and La Wall [11] found two samples of imported bacteriological gelatin with 265 and 835 parts per million of $SO_2$, and they believe that such gelatin is unsuited for bacteriological work. P. Poetschke,[12] however, found that on preparing nutrient media with a gelatin containing 0.1108 per cent. $SO_2$, the nutritive solution after heating under pressure, contained only 0.0009 per cent. $SO_2$ instead of the 0.0133 per cent. required by calculation. That is, 93 per cent. of the $SO_2$ was lost in the process of preparation, which may account for the fact that bacteriologists have had no trouble on this score.

**Formogelatin.**

Very small amounts of formaldehyde (less than 1 in 10,000) do not exert an appreciable effect on gelatin solutions apart from a preservative action. With increasing amounts of formaldehyde, especially with concentrated gelatin solutions or those containing free alkali, the gelatin is converted upon drying into an insoluble substance known as formogelatin, which seems to be an adsorption compound.[12a] Acrylic aldehyde is said to produce a similar product, but acetic aldehyde reacts only in the absence of water.

[11] *Analyst 36*, 271.
[12] *J. Ind. Eng. Chem. 5*, 980 (1913).
[12a] See Allen's "Comm. Organic Analysis," 4th ed., Vol. 8, p. 600.

The rubbery but rather brittle jelly of formaldehyde-treated gelatin is insoluble in cold or in boiling water, but in contradistinction to the corresponding casein compound, it dissolves upon treatment with dilute (1.34 sp. gr.) sulphuric acid for 12 hours.

When dried and powdered, formogelatin is used in surgery as an antiseptic dusting powder for wounds. In preparing it, any free acidity in the gelatin is neutralized (e.g., by agitation with calcium carbonate), and any trioxymethylene formed is dissolved out with boiling water which does not affect the formogelatin. If unaffected gelatin be present, the filtrate will gelatinize, especially when chilled.

**Gelatin as a Food.**

The value of gelatin in food products is threefold. In the first place its peculiar physical properties enable it to give a desirable body, stiffness, or texture to many foods. Gelatin jellies are used in almost every home, and those who know its value, use gelatin in preparing ice-cream, charlotte russe, Bavarian creams, and many other desserts. Gelatin colored red is also used to garnish meats, and gelatin acts the part of a binder in aspics, head-cheese, and cold meat or fish served "en gelée."

A second function of gelatin in many foods is to render them more digestible by virtue of its action as a protective colloid. This applies especially to milk and milk products, such as ice-cream. About ½ per cent. of gelatin had long been used both by cooks, housewives and by practical ice-cream manufacturers who knew that it gave the ice-cream a smooth, velvety texture much desired by consumers. Following the passage of the U. S. Food and Drugs Act of 1906 (the Pure Food Law), there was promulgated by the U. S. Department of Agriculture a series of so-called standards for various foods. The standards for ice-cream excluded gelatin, and even eggs which are essential in the manufacture of the "French" ice-cream.

Upon investigating the facts J. Alexander [13] found that gelatin not only improves the product by inhibiting the formation in ice-cream of sharp crystals which make the product gritty or sandy to the taste, but it also actually renders the fat and casein

[13] *Kolloid Z.* 4, 86 (1909); 5, 101 (1909).

present more digestible by preventing the formation of large fatty or greasy curds which are particularly hard to digest.[14]

An investigation of the medical evidence showed that even prior to 1888 Jacobi [15] had recommended the addition of gelatin and similar protective colloids to cows' milk and infants' diet. Experiments *in vitro* showed that gelatin inhibited or delayed the coagulation of cows' milk by acid and by rennin, making it resemble mothers' milk in this respect; and ultramicroscope observations checked the results. Since fatty or greasy curds are particularly difficult to digest, the value of a protective colloid in ice-cream was evident. A case was brought to trial in Washington in which the so-called government standard was overthrown, and the official view regarding official legislation as to how foods should be prepared, has undergone considerable modification. Any reasonable mixture is allowed which does not mean fraud on the consumer or danger to the public health.

Harper F. Zoller and Owen E. Williams [16] report that with ice-creams containing very high percentages of evaporated skim milk, even the presence of gelatin may not prevent the "sandiness" due to the separation of crystals of the relatively slightly soluble lactose. Zoller [17] has also shown that gelatin facilitates freezing, for gelatin solutions develop crystals more quickly than does pure water. Gelatin thus reduces the tendency toward super-cooling.

As R. H. A. Plimmer [18] remarks, the proteins must be regarded, biologically, as mixtures of the various amino-acids, which are re-shuffled in digestion and absorption. During digestion the proteins are hydrolyzed into their 18 or 20 constituent amino-acids; but the animal body cannot synthesize these acids or convert one into another, an exception being the simple glycine which may be formed under certain conditions. Consequently although animals may be maintained upon a diet whose protein content is replaced by the proper selection of essential amino-acids, the animal will fail if fed on real proteins which lack any

[14] See *J. Soc. Chem. Ind. 28,* 280 (1909); *Kolloid Z. 6,* 197 (1910); *J. Am. Chem. Soc. 32,* 680 (1910); Alexander and Bullowa, *Arch. Pediatrics 27,* 18 (1910); *J. Am. Med. Assn. 45,* 1196 (1910).

[15] A. Jacobi, "The Intestinal Diseases of Infancy and Early Childhood," 1889.

[16] *J. Agri. Research 21,* 791 (1921).

[17] *Ice-Cream Trade J.,* 1921, and private communication.

[18] *J. Soc. Chem. Ind. 40,* 227R (1921).

one of these essential amino-acids. To secure growth of the animal as well as maintenance, *variety* as well as *quantity* of protein is necessary. This shows another danger of establishing a diet merely upon a fat, protein, carbohydrate, calorie basis. Colloidal protection, vitamines, soluble salts and protein variety are also essential factors. Therefore though gelatin, zein (from corn) and gliadin (from wheat) show marked deficiencies, they are nevertheless rich in many essential amino-acids. The disease pellagra appears to be caused by unbalanced protein diet (corn) and it would be interesting to see to what extent gelatin would help to supplement the diet in such cases. Gelatin, however, lacks the following amino-acids which are essential to nutrition—cystine, tryptophane, tyrosine. It is not, therefore, a complete food, nor even a complete protein food; but neither are most other foods. Nevertheless it is pure and easily digested, and the use of calves' foot jelly and consommé for invalids is a long-established custom based on favorable experience.

In his book on "Infantilism" [19] Herter describes a condition of arrested development, consequent upon the non-absorption of food and its subsequent putrefaction in the lower intestine. The patients excreted practically all the calcium ingested, which accounts for the failure of a skeletal growth; and the feces contained neutral fat, fatty acids, and soaps in marked excess, which indicates impaired fat absorption. Herter found that adding gelatin to the milk fed, caused increased absorption and recommends its use (*loc. cit.*, pp. 101, 105). The gelatin evidently exercises its well-known protective and emulsostatic action, facilitating the digestion of fats, and thereby combating intestinal putrefaction; for the cream layer or fat of milk contains from 100 to 500 times as many bacteria as the whole milk.[20] Herter observes that "in sparing protein small quantities of gelatin appear to have about as much effect as larger amounts." This accords with the view that gelatin functions as a protective colloid, for only small quantities are essential to make protection effective.

[19] "On Infantilism from Chronic Intestinal Infection," by C. A. Herter, The Macmillan Co., 1908.

[20] U. S. Dept. Agri., Bull. *56*, 737.

**Food vs. Technical Gelatins.**

How shall gelatin be distinguished from glue? There is, in fact, no sharp distinction; for any clear, light-colored glue of high strength may with justice be termed a gelatin. A sharp line, however, must be drawn between *technical gelatins* intended for manufacturing purposes, and *food gelatins* intended for human consumption.

In the first place food gelatin must be free, or practically free, from injurious substances of all kinds. Sulphur dioxide, arsenic, copper and zinc are the impurities most often tested for. Owing to refinement of analysis and the practical impossibility of eliminating all traces of metals, the following permissable limits have been tentatively fixed by the U. S. Department of Agriculture (Bureaus of Chemistry and Animal Industry):

| | | |
|---|---|---|
| Arsenic ..... | 1.4 | parts per million |
| Copper ..... | 30.0 | " " " |
| Zinc ..... | 100.0 | " " " |

No definite figure for sulphur dioxide has been announced, although excessive amounts are considered obnoxious. The quantity is not supposed to exceed 350 parts per million, this figure being to cover the errors in analysis. The State of Pennsylvania prohibits *all* sulphur dioxide, but since bone and hide from animals slaughtered under Government supervision showed apparent $SO_2$ on the official test, it is evident that due allowance must be made for the imperfections of analytical methods.

Besides satisfying the chemical tests, food gelatin must be free from objectionable color, odor, and bacteria. It should be made from clean stock under clean conditions, and be kept clean subsequently.

# Chapter 14.

## Fish Glue and Fish Isinglass.

Fish glue, which is usually marketed in liquid form, is made from fish heads, bones, and skins, that form an offal in the fishing industry. In the United States and Canada the chief sources of supply are the cod, haddock, cusk, hake, and pollock, the refuse from the salting factories yielding a large part of the supply. Many fish residues are now unutilized, although some of them, i.e. that of the mullet found in our southern waters, yield excellent glue. Generally whenever it is too troublesome or expensive to separate the glue or glue-forming stock from admixed "gurry," salt, oil, and foreign proteins, it is more profitably converted into "chum," which is sold as poultry food, or into fertilizer, which always finds a ready sale.

Sturgeon refuse, and the skins and scales of menhaden and herring have also been used as a source of glue. According to Green and Tower [1] one ton of menhaden yields 20 pounds of dry scale yielding 10½ pounds of dry gelatin (moisture content 16 per cent.). The "stick," obtained by concentrating the waste liquors of the menhaden industry, owes its adhesiveness to the glue present in it; but it is sold for fertilizer. According to a German patent (131,315) glue is made from whale blubber, after removing by volatile solvents the fat left after cold pressing. Unsuccessful attempts have been made to produce glue from the gray fish (Squalus acanthias); the dark skin pigment darkens the glue and the fish contains a large amount of oil and water. Excellent fish glue is produced on the Pacific coast of the United States.

Generally speaking, to render the extraction of fish glue profitable, in addition to simplicity of handling, the stock must be available in abundant and steady supply.

The fish glues of commerce are classed as (1) head glues, (2)

[1] United States Fish Com. Bull., 1901, pp. 97-102.

bone glues, (3) skin glues, according to the stock from which they are made; and they are valued generally in the order given, skin glues being the strongest and most valuable. With the bones are usually included the trimmings from the salted fish.

The manufacture of fish glue is extremely simple. The stock is first washed thoroughly with fresh water to remove dirt and blood from the fresh fish fragments, and salt from the salt fish offal. The old method was to boil the washed stock in open kettles for 10 hours with live steam, according to Lambert; or from 6 to 10 hours, according to Tressler,[2] who says that two runs are made. Newer methods make use of autoclaves in which the stock is extracted by heating under pressure for several hours, a steam-jacketed kettle being used. Or the stock is placed within the inner, perforated section of a double boiler, from which the glue liquor filters into the outer shell, where it can be drawn off continuously. Or steam and cold water may be used on the stock alternately until exhaustion is complete. The residue left after squeezing out the boiler residue contains 45 to 55 per cent. protein matter and is useful for poultry food or fertilizer.

The dilute glue liquors are strained or filter pressed, bleached if desired with sulphur dioxide gas, and then evaporated in open pans heated by closed steam coils, or in vacuum evaporators. After evaporation to the desired constituency, usually about 50 per cent. water, the glue is drawn off into storage tanks or barrels, preservatives and essential oils having first been added and mixed in. Among the preservatives used are boric acid, phenol, and cresol, while sassafras and wintergreen are the most popular perfumes added to mask the odor of fish.

In the production of highly clarified skin glue for photo-engraving work, the dilute liquors may be treated by a high-speed centrifuge of the Sharples type, or else forced through a special pulp filter.

Sometimes animal glues of low jelly strength are added to fish glue to stretch the yield, and acetic acid or salts may be added to depress the jellying temperature.

A small quantity of fish glue is produced dry in the form of cakes or broken sheets which are very hygroscopic and readily

[2] Private communication.

soluble in cold water. The drying is a matter of difficulty, but the concentrated glue may be "skinned" over in pans and then transferred to nets to complete the drying. Oiled or waxed surfaces may also prove useful.

*Properties of Fish Glue.* The liquid fish glues of commerce are viscous liquids which gradually thicken as the temperature is reduced and finally gelatinize at about 5° to 10° C. (40°–50° F.). If the glue thickens by evaporation beyond the average of 50 per cent. of water it usually contains, it will chill more readily and should therefore be warmed and reduced with water or acetic acid (vinegar will serve) well stirred in.

The color of the glue varies with the stock, the method, and the care used. Skin glues usually are more clear and the photo-engraving grade is transparent. Head and bone glues are usually turbid and may be brown or, if bleached, a light yellow. As with ordinary animal glues, zinc oxide may be added to produce a light tone. Taste and odor, which are usually very pronounced, depend on the same factors as color, and in addition upon the preservatives and essential oils added.

Tressler [3] says that dry skin and fish waste glues contain about 1 per cent. of ash; head glues may contain from 1–5 per cent. He gives the following representative analysis of the ash of a fish skin glue:

| | *Per Cent.* |
|---|---|
| Ash (in water-free glue) | 0.96 |
| Silica ($SiO_2$) | 12.7 |
| Calcium Oxide (CaO) | 10.5 |
| Magnesia (MgO) | Trace |
| Potash and Soda ($K_2O$ and $Na_2O$) | 13.9 |
| Sulphur Trioxide ($SO_3$) | 34.0 |
| Phosphorous Pentoxide ($P_2O_5$) | 24.9 |
| Chlorine (Cl) | 3.2 |
| Ferric Oxide ($Fe_2O_3$) | Trace |

Due to the fact that it is tenacious and dries slowly, fish glue will spin out long thin "spider webs," which are popularly thought to be an indication of great adhesive power, and indeed for many purposes it serves admirably. Contrary to the prevailing opinion, however, fish glue does not begin to equal good animal glues for making joints. The joint strength of a common commercial fish glue was only 260 lbs. per square inch, but

[3] Private communication.

according to the Forest Products Laboratory (Technical Note F-2), high-grade skin glue should average 1,700 to 1,800 lbs.

**Isinglass.**

*Isinglass* is probably a corruption of the German *hausenblase* (Dutch *huisenblas*), literally "sturgeon's bladder," which has for centuries been the main source of the celebrated Russian isinglass, a product that found its way from the great fair at Nijni Novgorod to London, and the other markets of the world. Several varieties of the sturgeon, the beluga (*Acipenser huso*), the osseter (*A. guldenstadtii*), the sterlet (*A. ruthenus*), the common sturgeon (*A. sturio*), and the starred sturgeon or seuruga (*A. stellatus*), as well as the catfish (*Silurus glanis*), and carp (*Cyprinus carpio*), which are found in the Volga and other great rivers, in the Caspian and Black Seas, and in the Arctic Ocean, yield "Russian isinglass." The sounds of many other varieties of fish also appear on the market as isinglass, supplies coming from the East and the West Indies, Penang, Brazil, Bombay, Manila, Venezuela, Canada and the United States. Brazilian isinglass, also called "Cayenne isinglass," is obtained from *Silurus Parkerii*, and rat's tail isinglass is made from the cod (*Morrhua vulgaris*), the hake (*Phycis Americanus*) and other fishes. The tongue sounds exported from Penang and Bombay are also called purse sounds, for they are purse-shaped with fringed edges. Tongue sounds, lump and pipe isinglass, are also exported from Venezuela and Brazil; they are inferior to the Russian isinglass.

The fish sound or swim bladder (air bladder) is a hollow compressible sac, containing a gas (oxygen, nitrogen or carbon dioxide) and is situated in the abdominal cavity. Its main use appears to be a mechanical one, for by compressing or expanding it the fish can regulate its specific gravity so as to rise, to sink, or to remain at a certain level.

The sound seems to be a homolog of the lung, and in some fishes may assume the functions of that organ. Its size varies greatly, but in the sturgeon, hake, catfish and carp it is very large. It is made up of several layers, the inner one being thin, often of a silvery luster, containing crystalline substances, and sometimes covered with epithelium. The next layer is thick

and fibrous, and contains the collagen which yields commercial isinglass.

Leaf isinglass (also known as Astrakan leaf, Saliansky leaf, and Samovy or Taganrog leaf) is prepared by soaking the sounds in hot water and removing the dirt and mucous membrane. The sounds are then split, and dried with exposure of the inner membrane; the outer membrane is removed by rubbing or beating. The unopened sound is called "pipe," "purse" or "lump" isinglass, depending upon its appearance. When folded and dried they form "book" isinglass, and when rolled they form "ribbon" isinglass. Trimmings are often compressed into "cake" isinglass, or they may be dissolved and the strained solution dried out. "Long staple" isinglass and "book" isinglass, which include the largest pieces, are most valued; a 2 per cent. solution jellies on cooling and yields only 0.05 per cent. of insoluble matter.

The chief source of North American isinglass is the hake, but some is obtained from the cod and the sequeteague. Hake caught in the deep water off the Newfoundland coast have large sounds, one ton of fish yielding 300 to 500 sounds, weighing from 40 to 50 pounds. Hake caught in shallow water are smaller, one ton yielding about 600 sounds, weighing about 30 pounds. The average hake sound yields about 85 per cent. of gelatin; they are easily separated from the backbone of the fish and are usually salted on the fishing vessels. Cod sounds are smaller, much harder to separate from the backbone (part of which often clings to them) and yield only about 50 per cent. of gelatin. The sequeteague yields a good quality of isinglass, but the production from this fish, once 30 tons annually, has now sunk to practically nothing. Experiments by White [4] show that the tilefish (*Lopholatilus chameleonticeps*) also yields good isinglass.

White also gives an account of isinglass manufacture in the United States, where the industry was initiated at Rockport, Mass., in 1821. Ribbon isinglass is the principal product. The sounds, after being washed and soaked until soft, are run into a cutting machine having a roller and a set of knives which reduce the sounds to small pieces. After mixing and macerating between a set of iron rollers, the material passes to the sheeting rollers, which are hollow, water-cooled, and provided with a

[4] U. S. Bureau of Fisheries Document 854 (1917).

scraper. The isinglass issues from the sheeting rollers in sheets of variable length, 6–8 inches wide, and one eighth to one quarter of an inch thick. It then passes to the ribbon rollers which without widening it, stretch it to long ribbons about one sixty-fourth of an inch thick. These ribbons are suspended in a warm, dry room, and when dry are wound up on wooden spools. The yield is about 80 per cent. of the weight of sounds originally used.

A product called transparent or refined isinglass is manufactured by dissolving New England isinglass in hot water and spreading the solution on oiled cloth to dry. The very thin, transparent sheets thus formed serve as a good glue, but have a fishy odor.

*Properties of Isinglass.* Pure isinglass is odorless, practically tasteless, tough, fibrous, and should be white with a yellowish tinge, opaline or translucent. The toughness, transparency, and flexibility of isinglass, coupled perhaps with the fact that it came from Russia, which was the original source of clear mica (muscovite or Muscovy glass), has led to much popular confusion of these terms, the mineral mica being erroneously termed isinglass. It contains from 15 to 20 per cent. of water, and according to Mulder its percentage composition is as follows: Carbon, 50.76; hydrogen, 6.64; nitrogen, 18.32; oxygen and sulphur, 24.69.

Isinglass is a nearly pure collagen. When soaked in cold water it swells greatly without losing its organized, fibrous, thread-like structure. Boiling converts it into gelatin which, probably because of the ease of its formation, yields a very strong jelly. When treated with hot water, Russian isinglass dissolves completely, swelling uniformly to produce a whitish opaline jelly. This distinguishes it from gelatin, which swells irregularly in hot water, giving in most cases a more transparent solution. Adulterated or inferior isinglass may give considerable residue and usually has a bad odor.

Isinglass is insoluble in alcohol, but dissolves in most dilute acids or alkalis. If bleached by sulphur dioxide it may give a precipitate with barium chloride due to traces of sulphates. Russian isinglass leaves on ignition from 0.4 to 0.9 per cent. of reddish ash, which contains a little calcium carbonate. Gelatin, on the other hand, yields at least 1.5 per cent. of ash, consisting

mainly of calcium carbonate and phosphate, with traces of chlorides and sulphates.

The following table prepared by F. Prollius [5] gives results on a number of specimens of Russian isinglass, and some inferior grades; the viscosities were determined on filtered solutions of 1 part of isinglass dissolved in 90 parts of water:

| *Kind of Isinglass* | *Ash Per Cent.* | *Water Per Cent.* | *Residue Insoluble in Hot Water (Per Cent.)* | *Viscosity in Seconds* |
|---|---|---|---|---|
| Astrakhan isinglass ..... | 0.20 | 16.0 | 2.8 | 507 |
| " " ..... | 0.37 | 18.0 | 0.7 | 485 |
| " " ..... | 1.20 | 17.0 | 1.0 | 500 |
| " " ..... | 0.80 | 19.0 | 3.0 | 491 |
| " " ..... | 0.50 | 19.0 | 0.4 | 480 |
| " " ..... | 0.40 | 17.0 | 1.3 | 477 |
| Hamburg " ..... | 1.30 | 19.0 | 2.3 | 470 |
| " " ..... | 0.13 | 19.0 | 5.2 | — |
| Rolled northern fish bladder... | 3.20 | — | 10.8 | 467 |
| Iceland fish bladder..... | 0.60 | 17.0 | 21.6 | 463 |
| Indian isinglass ..... | 0.78 | 18.0 | 8.6 | 437 |
| Yellow (unknown origin)..... | 2.30 | 17.0 | 15.6 | 360 |

White agrees with Prollius in advising microscopic examination for examining isinglass for adulteration with gelatin, which is often rolled in alternate layers with isinglass. The gelatin becomes more transparent on swelling and is structureless, whereas the isinglass shows its characteristic fibrous structure.

The British Adhesives Research Committee [6] found that the Hausmann numbers of isinglass do not differ appreciably from those of gelatins obtained from mammalian tissues. In contradistinction to glues and gelatins, isinglasses yield, upon heating their solutions to 100°, a precipitate of coagulated albumin. Russian isinglass gave 4.57 per cent. coagulable protein, whilst Brazilian isinglass gave 13.05 per cent.

The Hausmann numbers of the coagulable protein approximates those of egg albumen, and are much higher than those of ordinary glue and gelatin. The results are:

| *Albumin from* | *N percentage (dry wt. basis)* | *Amide N* | *Humin N* | *Diamino N* | *Monoamino N* |
|---|---|---|---|---|---|
| Brazilian Isinglass ..... | 14.45 | 6.92 | 2.90 | 20.62 | 69.56 |
| Russian " ..... | 14.67 | 6.20 | 3.06 | 19.43 | 71.31 |
| Egg ..... | 14.06 | 7.82 | 2.91 | 21.27 | 68.00 |

Removal of the albumin materially altered the adhesive

[5] *Abst. J. Chem. Soc. 45*, 647 (1884).
[6] First Report, London, 1922, p. 27.

strength of the isinglass, that of Brazilian isinglass dropping from 946 to 550.[7]

*Uses of Isinglass.* Good edible gelatin (formerly called "patent isinglass") is now produced in large quantity and at so low a price that it has replaced isinglass practically entirely in making jellies, confectionery, etc. Fish sounds especially mixed with tongues, are used as food, and fried cod sounds are said to taste like fried oysters.

The advent of prohibition in the United States has curtailed one of the main uses of isinglass, for it has long been used as a fining or clarifying agent for cider, wines, beers, etc. It acts both mechanically and as a colloidal adsorbent, removing tannins and turbidity-producing particles.

For white wine the isinglass is swollen in water and then beaten with wine and a little tartaric or sulphurous acid and strained through linen before adding to the wine. One ounce of isinglass usually serves to clarify 200 to 500 gallons of wine in 8 to 10 days. For a barrel (36 gallons) of beer about ⅛ ounce of isinglass is cut or softened in cold water containing a little acetic or sulphurous acid, and without dissolving is poured in and allowed to settle slowly through the barrel, carrying with it the "cloud." One pound of isinglass will clarify 100 to 500 barrels of beer. Gelatin is now used also, to replace isinglass as a fining material.

The formulæ and recipes found in old books show that isinglass was once largely used as an adhesive, even for postage stamps, envelopes and gummed paper. For these purposes it is now obsolete, being replaced by dextrins, gums, glues, etc. It is still used in leather belting cements, jewelers' cements, and in special adhesives such as those used in covering iron rolls in textile mills.

White gives the following recipe for making court plaster with isinglass as the adhesive: 10 grams of isinglass are dissolved in 120 grams of water, and one half of this solution is painted out on about 38 square centimeters of taffeta silk stretched on a frame. When this layer is dry, a second one is applied, consisting of the other half of the isinglass solution, to which has been added 1 gram of glycerin and 40 grams of alcohol. Finally the other face of the taffeta is painted out with tincture of benzoin.

[7] This is an interesting instance of cumulative protection. See J. Alexander, *J. Ind. Eng. Chem.* 1923.

## APPENDIX.

The National Association of Glue and Gelatin Manufacturers (of the U. S. A.) have for some time been considering the establishment of official methods for testing glues and gelatin, and while nothing has been officially published, the Technical Section has agreed upon the following details:

*Preparation of sample.* Samples of unknown origin should be ground to 4 mesh before weighing.

[Since a lot of commercial glue usually consists of a mixture of several boilings which may be of different grades, it is of course essential that the sample be drawn with this in mind. With this product segregation sometimes occurs in the barrel, because of jarring due to transportation and differences in size and specific gravity of the glue fragments. Therefore to secure a representative sample, the glue should be well mixed and a large sample ground in a suitable mill.]

*Concentration of test solutions.* Present practice shows from 11.11 to 15 per cent., and 12½ per cent. was tentatively fixed as the standard concentration for viscosity and jelly strength tests. This means 1 part of glue to 7 parts of water, by weight.

*Temperature of soaking.* At 10° C. for at least 12 hours, or overnight.

*Temperature of glue solutions* should not exceed 63° C. Viscosities should be taken at 60° C. The glue should not be subjected to the heat of the water bath for more than 30 minutes.

*Cooling procedure.* Allow to cool at room temperature. Then hold the sample at 10° C. for not less than 18 hours, nor more than 22 hours.

As stated before (Chapter 12) the prevailing practice in this country is to grade glues on jelly strength and on viscosity taken at working temperature. While the instruments have not yet been officially adopted, through the kindness of W. D. Richardson there is given below a description of the construction and operation of the Bloom Gelometer, which represents the type of instrument that will probably be adopted by the Committee for determining jelly strength.

An instrument of the same general type, but working on a different mechanical principle, is being developed by F. S. Williams, who is also working on a system of grading based on an *imaginary* perfect glue, whose jelly strength and viscosity, each assumed to be 100 per cent., are not equalled by any commercial glue or gelatin. To bring the measurements within the con-

sumner's range, Williams proposes as a solution of standard viscosity, one containing by weight 2 parts of water to 1 part of absolutely dry "60 per cent. glue, a grade approximately equalling Cooper's 1¼, Alexander's 90, or Bogue's 8.

Regarding viscosity, W. D. Richardson informs me that the Committee favors a pipette or capillary flow type of viscosimeter for glue testing, since it can be standardized to give viscosity in absolute units (centipoises). By calibrating such pipettes with fluids of known viscosity (e.g. glycerin, sugar solutions, castor oil) the variations of different instruments, with respect to the time interval of efflux, may be reconciled. J. R. Powell, of the Committee, has just (Jan., 1923) succeeded in calibrating pipettes in this manner. Starting with a pipette as near standard size as possible, he found that only one high and one low reading were necessary to calibrate the instrument satisfactorily.

F. D. Williams is working on a pipette made of carefully ground and fitted glass sections, and having an air jacket to serve as a thermostat. The effluent tube is without end constriction or tip, the length of the efflux tube controlling the time of outflow.

One distinct improvement suggested by the Committee, is the use of 8-ounce wide-mouth bottles (salt mouths) having tall, tight-fitting soft rubber stoppers, instead of glasses as test vessels. The stoppers prevent evaporation and skinning, but of course care must be taken to see that no water of condensation on the upper portion of the bottle interferes with the determination of jelly strength. The stopper is removed when making the several tests.

### DESCRIPTION AND WORKING DIRECTIONS OF BLOOM GELOMETER.

*Name:*

The name of the instrument is the Bloom Gelometer (called by the National Association of Glue and Gelatin Manufacturers, "Association Style A Gelometer").

*Purpose:*

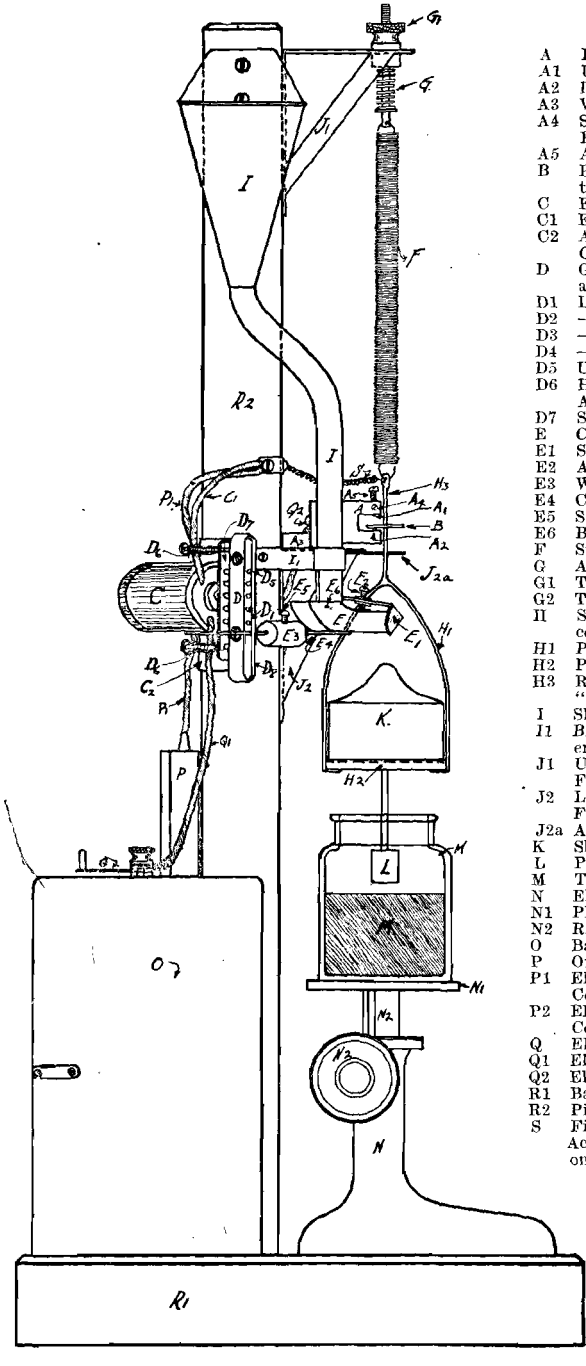
The purpose of this instrument is to afford a device for determining the jelly strength of glues and gelatins, which will be automatic in its action, which will be reproducible and which will give readings in terms of weight required to produce a definite depression of a plunger of definite diameter in the glue jelly.

*Standard Units Adopted for the Machine:*

The diameter of the plunger is exactly 12.7 mm. (½ inch) and is constructed of aluminium, the sharp lower edge being

# BLOOM—GELOMETER

## Description and Working Directions.



A Brass Contact Point Bracket.
A1 Upper Contact Point.
A2 Lower Contact Point.
A3 Wood Fibre Support for "A".
A4 Set Screw to Hold Adjustment Screw in Position.
A5 Adjustment Screw for "A".
B Pure Silver Disk (⅝ in. dia. & 1/16 in. thick).
C Electro-magnet.
C1 Electrical Connection from "C" to "S".
C2 Adjustment Screws for Adjusting Pitch of Clamshell Cut off "E".
D Guide Bar of Automatic Shot Control Mechanism.
D1 Lower Dog.
D2 ——— Dog 2.
D3 ——— Dog 3.
D4 ——— Dog 4.
D5 Upper Dog.
D6 Hair Spring Coil to Keep "D7" in Position, Acts against Electro-magnet.
D7 Soft Iron Bar Supporting Dogs. "D1-D5".
E Clamshell Spout.
E1 Stationary Clamshell Jaw.
E2 Adjusting Screw to Regulate Closure.
E3 Weight.
E4 Clamshell Arm.
E5 Set Screw to Clamp Weight to Clamshell Arm.
E6 Bearing on Which Cut-off Mechanism Turns.
F Spiral Spring (No. 8 Steel Music Wire).
G *Adjustable Support for Spring "F".*
G1 Thumbscrew Nut.
G2 Tension Spring.
H Suspended Pan and Pan Arm for Shot Receiver, Disk "B" and Plunger "L".
H1 *Pan Arms.*
H2 Pan.
H3 Rod Attached to Pan Arms Supporting Disk "B".
I Shot Hopper with Delivery Tube.
J1 *Bracket to Hold Lower End of Shot Delivery Tube.*
J1 Upper Supporting Bracket Attached to Frame Support "R2".
J2 Lower Supporting Bracket Attached to Frame Support "R2".
J2a Adjustable Guide Arm Attached to "J2".
K Shot Receiver.
L Plunger (12.7 MM. in Diameter).
M Test Bottle.
N Elevating Platform Base.
N1 Platform.
N2 Rack and Pinion Elevating Mechanism.
O Battery Box and Batteries.
P Ordinary Telephone Condenser.
P1 Electrical Connections from Condenser to Contact Points.
P2 Electrical Connections from Condenser to Contact Points.
Q Electrical Switch.
Q1 Electrical Connections from "O" to "C".
Q2 Electrical Connections from "O" to "A".
R1 Base of Gelometer.
R2 Pillar of Gelometer.
S Fine Copper Wire Coil Making Contact Across from Suspended Disk to Binding Post on Support.

rounded to the slightest possible degree. The depth of plunge is exactly 4 mm. as determined by selected Brown and Sharpe gauges.

The time period for the introduction of the shot (used for depressing the plunger) is kept within the limits, 2 to 5 seconds.

*General Description (Refer to figure and legend):*

The instrument is mounted on the base $R_1$ and the pillar $R_2$. The adjustment stand N resting on the base $R_1$ is provided with a platform $N_1$ capable of being raised and lowered by the rack and pinion mechanism $N_2$. Affixed to the upper end of the pillar $R_2$ by the bracket $J_1$, the spring adjusting mechanism G holds the spring F and the plunger L, hanger and pan $H_1$ and $H_2$ respectively. At the upper part of the plunger hanger, the silver contact disc B is set to operate between the contact points $A_1$ and $A_2$. The rod $H_3$ of the plunger hanger works through the adjustable guide $J_{2a}$ which is affixed to the bracket $J_2$.

Affixed to the upper end of the pillar also is the shot hopper I supplying shot through the clamshell cut-off $E$-$E_1$ to the shot receiver K which rests on the pan $H_2$. The automatic shot control mechanism $D$-$D_7$, working on the clamshell cut-off $E$-$E_1$ consists of the electro-magnet C, the soft iron bar $D_7$ carrying the brass dogs $D_1$-$D_5$ respectively, and the brass guide bar D.

The cut-off mechanism consists of the clamshell cut-off $E$-$E_1$, the control rod $E_4$, working on the dogs $D_1$-$D_5$ and the counter-balance weight $E_3$. $E_6$ is the bearing on which the cut-off mechanism turns. The entire cut-off mechanism is adjustable vertically on the pillar $R_2$ by means of the screws $C_2$, this adjustment setting and adjusting the pitch of the clamshell cut-off $E$-$E_1$. This adjustment is made when the machine is assembled and is permanent.

Electric current is supplied to the electro-magnet C through the contact points $A_1$-$A_2$ from the 3 volt dry battery O through the connections $Q_1$, $C_1$, S and $Q_2$. P is a small telephone condenser arranged in shunt circuit by means of the connections $P_1$ and $P_2$.

The test bottle M containing the jelly to be tested rests on the platform $N_1$.

*Operation:*

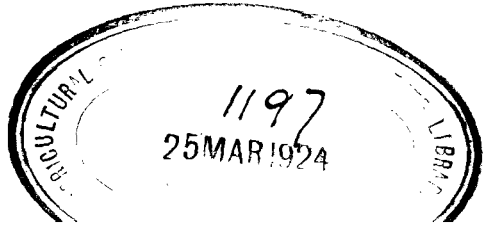
The space between contact points $A_1$ and $A_2$ is adjusted as follows: With the current cut off by means of switch Q and with the silver disc B resting on contact point $A_2$, adjustment is made by means of the adjustment screw $A_5$ so that the distance between the upper face of the silver disc B and the contact point $A_1$ is exactly determined. This determines the depth of plunge. In the case of glue and gelatin jellies, the depth of plunge is exactly 4 mm. as determined by the standard Brown and Sharpe 4 mm. gauge furnished with the instrument.

Adjustment is now made of the silver disc B against contact point $A_2$ by turning the adjustment screw $G_2$ (which acts on the spring F) until the silver disc B is in lightest possible contact with contact point $A_2$. When this point is reached, sparking will be noticed between the point $A_2$ and the disc B and a make and break vibration is set up between the soft iron bar $D_7$ and the core of the electro-magnet C. When this adjustment is once carefully made the machine stays in adjustment for some time but readjustment should be made occasionally.

The glue or gelatin jelly (or the like) prepared in the usual way, or according to standard directions, is placed in the test bottle M and chilled to the test temperature (10° C. for 16 hours, or overnight, in the case of glue and gelatin jellies). The bottle is placed on platform $N_1$ and raised by means of the rack and pinion mechanism $N_2$ until the jelly is in contact with the plunger L and the latter is raised until the silver disc B is brought into light electrical contact with contact point $A_1$. This point is indicated by sparking and make and break vibration between the soft iron bar $D_7$ and the core of the electro-magnet C. The shot receiver K is quickly placed on pan $H_2$ and immediately the lever $E_4$ is raised to the pre-determined position on one of the dogs $D_1$-$D_5$. The height to which lever $E_4$ is raised regulates the velocity of the flow of shot. For weak jellies one of the lower dogs is used, for strong jellies one of the upper dogs. The dog selected should be such as to keep the flow of shot within the prescribed limit of 2-5 seconds. The finest chilled shot obtainable is used, No. 12 or finer. The raising of the lever $E_4$ immediately starts the flow of shot, depressing the plunger L into the jelly until contact is made between the silver disc B and contact point $A_2$. This closes the circuit which acts on the electro-magnet C, moving the soft iron bar $D_7$ and withdrawing the support of the dog from the lever arm $E_4$, which immediately falls, thus cutting off the flow of shot by closing the clamshell cut-off E-$E_1$.

The weight of shot delivered into the shot receiver K plus the weight of the shot receiver itself is the weight required to move the plunger L through the prescribed distance against the resistance of the jelly, and measures the jelly strength. For glues and gelatins this distance is exactly 4 mm. as determined by Brown and Sharpe gauge.

After the combined weight is determined the shot is emptied back into hopper I and the machine is ready for another test.



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