# GELATIN LIQUEFACTION BY BACTERIA

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The liquefaction of gelatin is generally recognized and employed as a fundamental criterion for the differentiation of bacterial species. Unfortunately the methods in vogue for observing this property are crude and unreliable. Usually nutrient gelatin is inoculated by stabbing, kept at a temperature below its gelation point, and compared with controls to detect any liquefaction. Measurement of the rate of liquefaction is sometimes attempted by inoculating the entire surface of a tube of gelatin and recording the depth of liquid formed, after different periods of storage, but comparable results are rarely obtained.

The first step in the liquefaction of gelatin is peptization or transformation from the gel to the sol state; from a very viscous to a more fluid condition. Davis and Oakes (1922), in a recent paper on the physical characteristics of gelatin solutions have shown that the transformation from the gel to the sol state in 4 per cent gelatin solution takes place at 38.03°C. Above this temperature, the viscosity remains constant on ageing or decreases if the temperature is sufficiently high to cause hydrolysis. Below this transition point, viscosity increases with age.

At the meeting of the Society of Bacteriologists in 1919, William M. Clark (1920) reported some observations on proteus gelatinase in which he determined the solidification time by a modification of the method of Palitzsch and Walbum. Unfortunately the complete paper has not been published and the abstract does not give full details.

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We feel that further observations on viscosity changes are much needed and would be very instructive. It will be shown in this paper that in autoclaved gelatin, stored at 22°C., the viscosity may change but little for two or three days, after which it increases markedly for at least a week or ten days. Obviously a gelatinolytic organism may bring about liquefaction quite rapidly when inoculated into freshly prepared gelatin, and much more slowly if introduced into the same medium ten days or two weeks later, for in the latter instance more of the gel form must be transformed to the sol state to make liquefaction evident. Some method which could be employed over a wide range of temperature for measuring the rate of liquefaction of gelatin by bacteria is sorely needed.

In this preliminary paper are recorded some observations on the change in viscosity and formol (Sörenson) titration of gelatin subjected to bacterial decomposition.

## EXPERIMENTS WITH LOW TEMPERATURE ORGANISMS

Seven organisms isolated from milk were employed. These strains grew very well at room temperature, poorly at 37°C., and not at all at 39 to 40°C. The characteristics of the organisms are indicated in the following table, kindly furnished us by Miss Lulu Soppeland of the Iowa Engineering Experiment Station who will report a more detailed study in the near future.

*Medium.* The gelatin medium employed consisted of

| | |
|---|---|
| Peptone (Difco) | 1.0 gram |
| Gelatin (Difco) | 35.0 grams |
| Water (distilled) | 1000 cc. |

This was heated at 60 to 65°C. till dissolved, then further heated in a double boiler for 15 minutes, the reaction adjusted to pH 8.0, loss due to evaporation made up, and heated an additional 15 minutes, then filtered through cotton and flannel, tubed (25 cc. quantities) and sterilized at 15 pounds for fifteen minutes. The medium was allowed to cool at room temperature and the following day (twenty-four hours) inoculations were made.

| LAB. NO. | GENERAL CHARACTERISTICS | S. A. B. GROUP NO. |
|---|---|---|
| 2019 | Gram negative; short rods; singly or short chains; non-motile; spores not found; gelatin not liquefied; greenish pigment on agar | 222.3331433 |
| 2011 | Gram positive; irregular shapes; budding frequent; spores not found, non-motile; gelatin slowly liquefied. (Mold like) | 221.2323913 |
| 2060 | Gram negative; short rods; singly or short chains; spores not found; non-motile; gelatin liquefied slowly; orange pigment | 221.3333633 |
| 2053 | Gram positive; short rods singly or short chains; spores not found; non-motile; gelatin liquefied; orange yellow pigment | 221.2323623 |
| 1021 | Gram positive; short rods singly or short chains; spores not found; motile; gelatin liquefied | 221.1112811 |
| 1059 | Gram positive; short rods; singly or short chains; spores not found motile; gelatin liquefied; yellow pigment | 221.2323512 |
| 1045 | Gram positive; short rods; singly or short chains; non-spore-forming; non-motile; gelatin liquefied; yellow pigment | 221.2323513 |

The inoculum consisted of 1 cc. of a twenty-four-hour broth culture of the test organism and incubation was at 22°C. Control tubes were inoculated with 1 cc. of sterile broth.

Peptone water (0.1 per cent) was similarly inoculated to afford a correction for the peptone in the gelatin medium. Each organism was inoculated into 5 tubes gelatin and peptone water and at stated intervals one tube of each medium was removed from the incubator and examined.

Viscosity measurements were made at 25.6°C. by observing the time of discharge of a given volume in an Ostwald viscosimeter. In the following table the viscosity is expressed in terms of water, at the same temperature, as unity. The time of flow for water was 88.5 seconds.

Formol titrations were carried out in the following manner: To 20 cc. of distilled water in an evaporating dish were added 5 cc. of gelatin (or peptone) medium and the reaction adjusted

TABLE 1

*Effect of period of incubation on formol (Sörenson) titration and viscosity*

| INCUBATION (22°C.) | FORMOL TITRATION | | | | VISCOSITY (25.6°C.) REFERRED TO $H_2O$ |
|---|---|---|---|---|---|
| | Peptone | Peptone gelatin | Gelatin | Increase from gelatin | |
| **Controls** | | | | | |
| *days* | | | | | |
| 0 | 0.13 | 1.46 | 1.33* | | 5.38 |
| 1 | 0.13 | 1.46 | 1.33 | | 5.38 |
| 3 | 0.14 | 1.35 | 1.21 | | 5.14 |
| 5 | 0.14 | 1.29 | 1.15 | | 12.67 |
| 7 | 0.13 | 1.42 | 1.29 | | 19.15 |
| 12 | 0.13 | 1.48 | 1.35 | | † |
| **Organism 2019** | | | | | |
| 0 | 0.13 | 1.46 | 1.28 | | 5.38 |
| 1 | 0.26 | 1.51 | 1.25 | −0.03 | 5.29 |
| 3 | 0.23 | 1.36 | 1.13 | −0.15 | 5.21 |
| 5 | 0.26 | 1.43 | 1.17 | −0.11 | 9.82 |
| 7 | 0.18 | 1.60 | 1.42 | +0.14 | 13.66 |
| 12 | 0.27 | 1.53 | 1.26 | −0.02 | † |
| **Organism 2011** | | | | | |
| 0 | 0.13 | 1.46 | 1.28 | | 5.38 |
| 1 | 0.16 | 1.50 | 1.36 | 0.08 | 3.51 |
| 3 | 0.19 | 1.70 | 1.51 | 0.23 | 2.91 |
| 5 | 0.26 | 1.79 | 1.54 | 0.26 | 2.05 |
| 7 | 0.21 | 2.66 | 2.45 | 1.17 | 1.75 |
| 12 | 0.34 | 2.73 | 2.39 | 1.11 | 1.27 |
| **Organism 2060** | | | | | |
| 0 | 0.13 | 1.46 | 1.28 | | 5.38 |
| 1 | 0.17 | 1.54 | 1.37 | 0.09 | 4.08 |
| 3 | 0.17 | 1.90 | 1.73 | 0.45 | 3.66 |
| 5 | 0.24 | 1.88 | 1.64 | 0.38 | 4.02 |
| 7 | | 2.11 | (1.84) | 0.56 | 3.58 |
| 12 | 0.32 | 4.28 | 3.96 | 2.68 | 1.29 |

* Employed 1.28 as the average of controls for correction.

† Too viscous to ascertain.

Formol titration expressed in terms of N/1 NaOH required per 100 cc. of medium for neutralization after treatment with formaldehyde.

TABLE 1—*Continued*

| INCUBATION (22°C.) | FORMOL TITRATION | | | | VISCOSITY (25.6°C.) REFERRED TO $H_2O$ |
|---|---|---|---|---|---|
| | Peptone | Peptone gelatin | Gelatin | Increase from gelatin | |
| | | | Organism 2053 | | |
| *days* | | | | | |
| 0 | 0.13 | 1.46 | 1.28 | | 5.38 |
| 1 | 0.17 | 1.58 | 1.41 | 0.13 | 2.61 |
| 3 | 0.22 | 1.81 | 1.59 | 0.31 | 2.08 |
| 5 | 0.30 | 1.91 | 1.61 | 0.33 | 1.92 |
| 7 | 0.25 | 2.57 | 2.32 | 1.04 | 1.76 |
| 12 | 0.32 | 2.99 | 2.67 | 1.39 | 1.32 |
| | | | Organism 1021 | | |
| 0 | 0.13 | 1.46 | 1.28 | | 5.38 |
| 1 | 0.36 | 2.24 | 1.88 | 0.60 | 1.80 |
| 3 | 0.37 | 3.61 | 3.24 | 1.96 | |
| 5 | 0.41 | 4.83 | 4.42 | 3.14 | 1.37 |
| 7 | 0.41 | 5.78 | 5.37 | 4.09 | 1.38 |
| 12 | 0.50 | 7.64 | 7.14 | 5.86 | 1.20 |
| | | | Organism 1059 | | |
| 0 | 0.13 | 1.46 | 1.28 | | 5.38 |
| 1 | 0.34 | 2.42 | 2.08 | 0.80 | 1.49 |
| 3 | 0.50 | 5.99 | 5.49 | 4.21 | 1.35 |
| 5 | 0.79 | 9.20 | 8.41 | 7.13 | 1.27 |
| 7 | 0.83 | 13.61 | 12.78 | 11.50 | 1.21 |
| 12 | 0.78 | 18.18 | 17.40 | 16.12 | 1.16 |
| | | | Organism 1045 | | |
| 0 | 0.13 | 1.46 | 1.28 | | 5.38 |
| 1 | 0.27 | 2.16 | 1.89 | 0.16 | 1.54 |
| 3 | 0.40 | 4.16 | 3.76 | 2.48 | 1.34 |
| 5 | 0.62 | 6.45 | 5.83 | 4.55 | 1.34 |
| 7 | 0.72 | 9.48 | 8.76 | 7.48 | 1.33 |
| 12 | 0.74 | 14.00 | 13.26 | 11.98 | 1.17 |

to neutrality to phenolphthalein, 10 cc. of a 50 per cent formalin (neutral to phenolphthalein) was then added, and after ten minutes the sample was titrated with N/50 NaOH. In the tables the formol titration is expressed in terms of cc. N/1 NaOH per 100 cc. of medium or per cent normality of acid liberated by the formaldehyde. Some difficulty was encountered in

determining the exact end point, but in general the differences observed with the various cultures were so great that this end point error (0.1 to 0.15 cc.) may be disregarded.

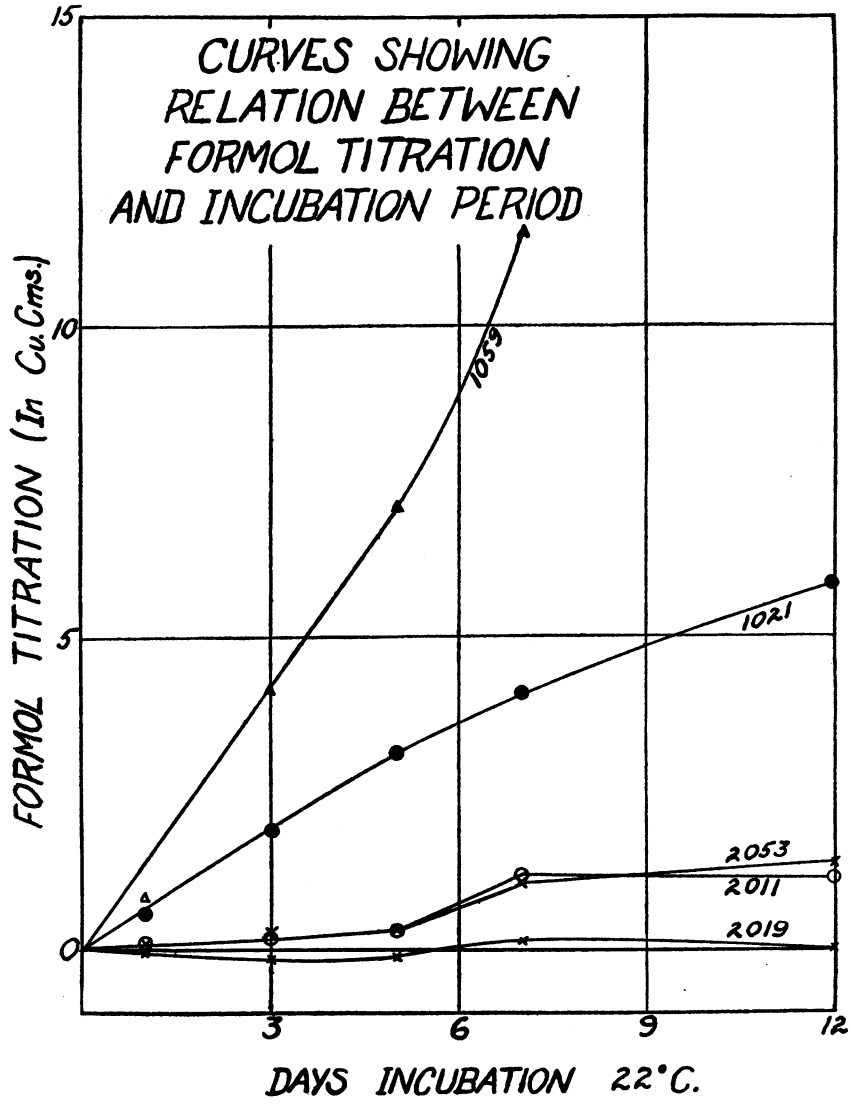


FIG. 1. INCREASE IN FORMOL TITRATION (CC. N/1 NaOH REQUIRED PER 100 CC. MEDIUM)

The results are detailed in table 1, and in figures 1 and 2 are shown the changes in relative viscosity and formol titrations brought about by the growth of the various bacteria studied.

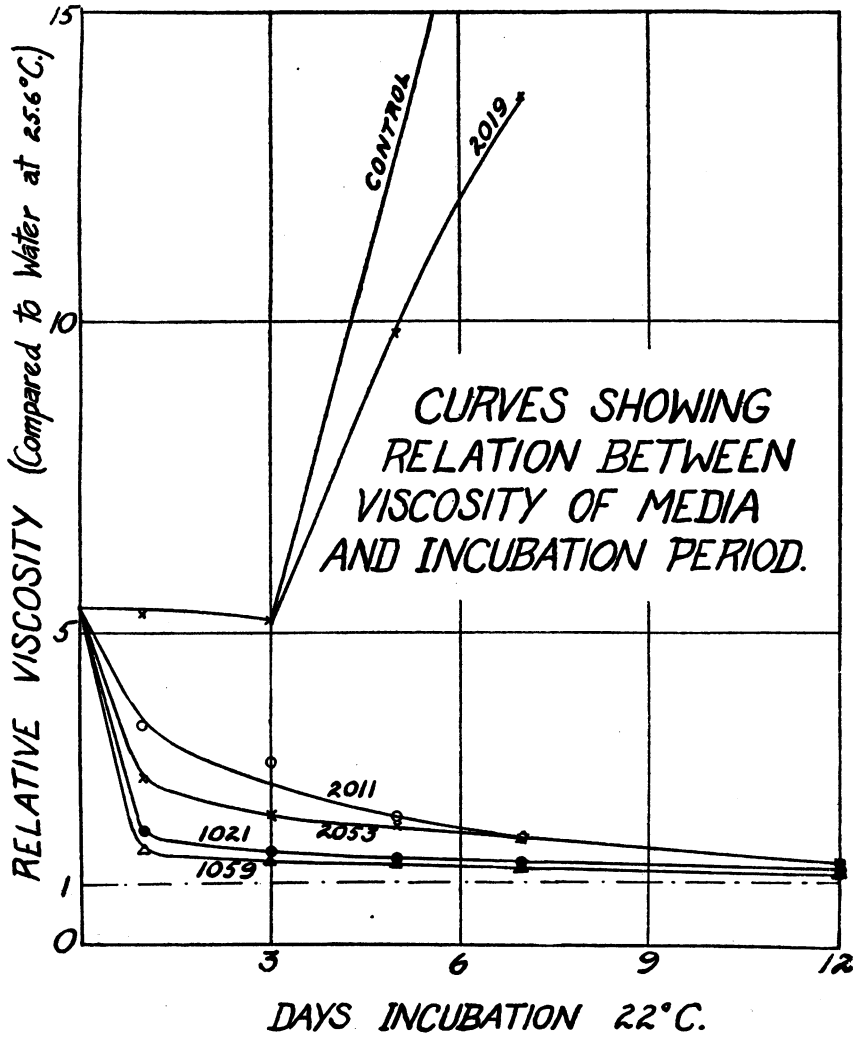


FIG. 2. CHANGE IN VISCOSITY OF PEPTONE-GELATIN

The viscosity and formol titration changes indicate that, with respect to their action on gelatin, bacteria may be subdivided into three groups as follows:

1. Gelatin not hydrolized; liquefaction negative.

2. Gelatin partially hydrolized and liquefied. Subsequent decomposition slow if any; accompanied by slight increase in formol titration.

3. Gelatin more completely hydrolized, and liquefied. Subsequent decomposition, rapid; accompanied by marked increase in formol titration.

As an example of the non liquefying type we have organism 2019. The relative viscosity of the medium (0.1 per cent peptone —3.5 per cent gelatin) increased from 5.38 to 9.38 in five days, to 13.60 after seven days incubation, and after twelve days the gelatin was solidified. There was a slight rise in formol titration, but after correcting for the peptone, the increase, which may be ascribed to the gelatin proper, became a negative quantity. This is due probably to the fact that in peptone water the organism grew throughout the medium whereas in the peptone-gelatin medium growth was restricted to the upper layers.

Organisms 2011, 2053 and 2060 are representative of the second type. There is a distinct drop in the viscosity twenty-four hours after inoculation (from 5.38 to 3.51, 4.08 and 2.62 respectively) with practically no increase in the formol titration. On further incubation the viscosity continues to decrease and the formol titration rises slowly.

With organisms 1021, 1059 and 1045, the change in viscosity is much more marked falling to 1.86, 1.49 and 1.54 respectively in twenty-four hours. The rise in the formol titration is relatively rapid, the increase in one day being as great as was observed in five to seven days among the strains of the previously described liquefying group.

The results are quite in accord with the statement of Berman and Rettger (1918) that "the power of an organism to liquefy gelatin is not necessarily accompanied by the ability to decompose the gelatin and seize upon it as food."

To ascertain whether the liquefaction is independent of the presence of living microörganisms, the following experiment was carried out.

Eight tubes of the above batch of peptone-gelatin which had solidified were heated for thirty minutes at 55°C. to convert to the sol form, and then cooled to 22°C.

To each of four tubes were added 2.5 cc. of 5 per cent phenol (giving a concentration of 0.5 per cent phenol) and to each of the remaining 4 gelatin tubes were added 2.5 cc. of broth. Two of the phenolated and two of the ordinary gelatin tubes were inoculated with 1 cc. of a forty-eight-hour broth culture of organism 1059, and to the remaining (control) tubes was added 1 cc. of broth so as to maintain the concentration of gelatin as equal as possible. All were incubated at 22°C. The results are indicated in table 2.

TABLE 2

*Effect of period on incubation and presence of phenol (0.5 per cent) on formol titration and viscosity of peptone gelatin*

| ORGANISM | INCUBATION (22°C.) | NOT PHENOLATED | | PHENOLATED | |
|---|---|---|---|---|---|
| | | Formol titration | Viscosity | Formol titration | Viscosity |
| | *days* | | | | |
| None | 1 | 1.52 | 4.95 | 1.56 | 3.34 |
| | 6 | 1.54 | 6.58 | 1.56 | 4.90 |
| 1059 | 1 | 3.04 | 1.50 | 1.65* | 1.62 |
| | 6 | 10.44 | 1.18 | 1.71 | 1.31 |

* Corrected for formol titration of 1 cc. broth inoculum (0.17 cc.)

There was no evidence at any time of growth in the phenolated medium. The liquefaction was therefore due to the presence of enzymes, secreted by the microörganisms in question. Considering the formol titration it is clearly evident that in the absence of bacterial growth (phenolated gelatin) there was practically no increase, whereas the increase was very considerable in the non phenolated tube. These results are in accord with those obtained by Kendall and his associates (1922) on studies with *Proteus*. They observed that liquefaction of gelatin by the bacteria-free enzyme was accompanied by a slight increase in ammonia and amino acids (formol titration), whereas in the presence of the viable bacteria, the ammonia content rose very markedly.

The slight increase in the formol titration observed in our experiments with phenolated gelatin is taken to represent the increase in ammonia and amino acids accompanying the enzymic

hydrolysis of gelatin. The marked rise obtained with the actively growing bacteria is most likely due to liberation of ammonia due to intracellular deaminization. Further work on this point is contemplated.

## SUMMARY

The change in viscosity of a gelatin medium and simultaneous rate of increase of the "formol titration" was observed with 7 organisms.

The viscosity was found to drop before the formol titration begins to rise. The rate of increase in formol titration serves to distinguish two types of gelatin liquefiers.

A standardized method for ascertaining the change in viscosity of gelatin culture media should be far superior to the present methods of detecting gelatin liquefaction. A temperature of 40°C. which is slightly above the gelation point is suggested as desirable for this purpose and is now under investigation.

## REFERENCES

BERMAN, N., AND RETTGER, L. F. 1918 Jour. Bact., **3**, 367–89.
CLARK, WM. M. 1920 Abs. Bact., **4**, 2.
DAVIS, C. E., AND OAKES, E. T. 1922 Jour. Amer. Chem. Soc., **40**, 464–79.
KENDALL, I. A., CHEETHAM, H. C., AND HAMILTON, C. S. 1922 Jour. Inf. Dis., **30**, 251–58.