THE OSTWALD VISCOSIMETER FOR THE DETERMINATION OF THE LIQUEFACTION OF GELATIN BY BACTERIA

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In a recent paper by Levine and Carpenter, there is a plea for a standardized method for ascertaining the change in viscosity of gelatin culture media. They suggest the use of Ostwald's viscosimeter, and that the readings be made at 40°C. It was later shown by Levine and Shaw, at the meeting of the Detroit Section of the Society of American Bacteriologists, October 5, 1923, that within certain limits of time and temperature of storage the viscosity of gelatin will give a constant reading at 40°C., irrespective of the history of the gelatin.

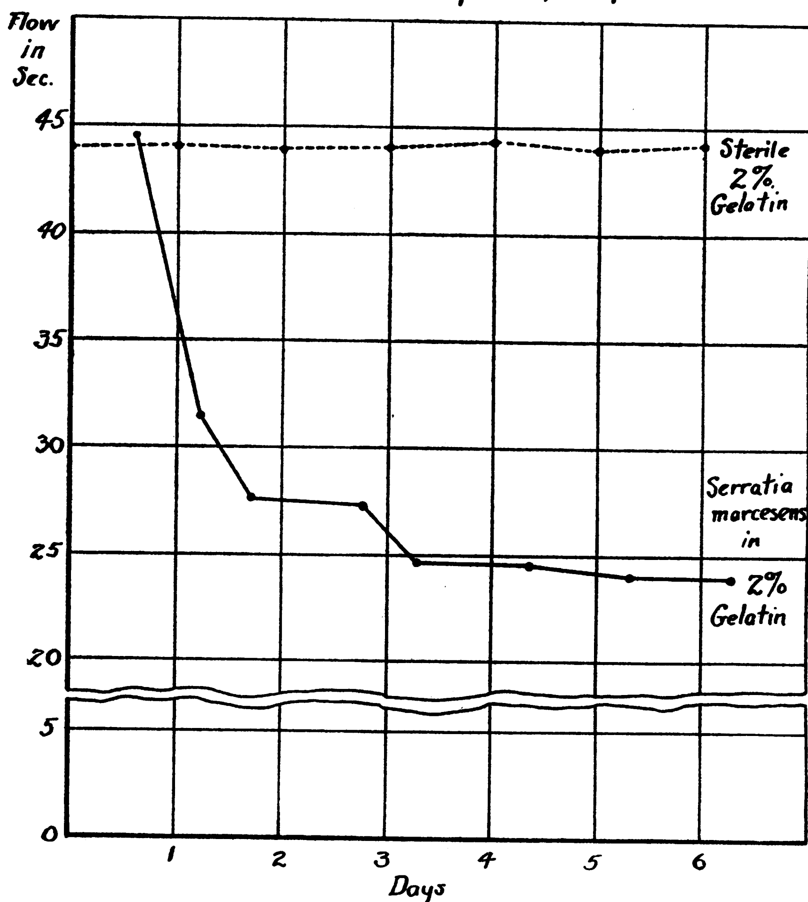
The work cited above was done by the use of ordinary culture tubes. The viscosity readings were preformed by pouring the gelatin solution or culture into an Ostwald viscosimeter. This method caused the gelatin culture to be useless after one reading, and it also caused contamination of the gelatin cultures which interferes with long readings.

Direct inoculation of sterilized gelatin culture media in Ostwald's viscosimeters was tried, by the writer, for a period of three months and found to give satisfaction. The longest continuous reading for any one viscosimeter was twenty days. Readings were made at least once each day, and at times several readings were taken in a day without contamination of the gelatin.

Care must be exercised in the selection of the viscosimeter. A viscosimeter whose bore is too small, should not be used as such an instrument is apt to become clogged with a small clump of organisms, or with a small piece of cotton from the plugs.

PREPARATION OF THE VISCOSIMETERS

Pour 4 cc.[1] of the gelatin culture medium into Ostwald viscosimeter of about twenty-five seconds flow for water. This



gelatin culture should be of about two per cent gelatin of good quality. Plug the two openings with non-absorbent cotton for a distance of about 2 cm. Care should be used in fitting the

[1] This amount varies in different viscosimeters.

plugs, because if these are fitted too tightly, the flow of gelatin in the viscosimeter will be retarded and a false reading obtained in the faster viscosimeters. Sterilize in the usual manner at 15 pounds for ten minutes. Remove from the sterilizer, cool to 40°C. and read. Keep the tubes at 40° until a constant reading is obtained. The flow of distilled water between the marks on the viscosimeter at 40°C. should have been previously taken and recorded. This need be taken but once for each instrument at the desired temperature. Inoculate through the reservoir side of the viscosimeter, in the usual way. The cotton should be kept dry as liquid in the cotton will obstruct the passage of air.

For the reading of cultures which have been incubated at 37° or higher, it is not necessary to heat at 50° for ten or fifteen minutes as it is with cultures which have incubated at lower temperatures. It should be understood that the cultures are placed in the 40° water bath at once on removal from the incubator. Readings at 40°, made on the cultures removed from the 37° incubator and placed immediately in the 40° water bath, give constant readings in less than fifteen minutes.

Mixing of the gelatin culture may be done by sending a bubble or two of air through the gelatin via the capillary side of the viscosimeter. Bubbles which have formed on the top of the gelatin, on the capillary side, are generally due either to too rapid income of gelatin into the measuring chamber, or to air in the capillary tube between small amounts of gelatin. These bubbles can be removed by gently blowing down the capillary tube as above described. There should be no bubbles in the capillary tube at the time of reading.

The water bath used by the writer, is a De Khotinsky, electric heated, with thermo-regulator. Some difficulty was experienced, at first, with the reading of the viscosimeters because the sides of the water bath do not admit light. This was overcome by using a microscope lamp having a frosted bulb and a sheet metal housing. After removing the stand, the lamp is suspended above the center of the bath with the lower portion

TABLE 1

Viscosimeter incubated at 37°C.

	TIME AFTER REMOVAL FROM INCUBATOR	VISCOSITY FLOW (40°C.)
	minutes	*seconds*
Tube 4	2	7.2
	15	7.2
	30	7.2
	45	7.2
	60	7.2
	120	7.2
	240	7.2
Tube 7	2	8.4
	15	8.4
	30	8.4
	45	8.4
	60	8.4
	120	8.4
	240	8.4
Tube 3	2	21.2
	15	21.2
	30	21.2
	60	21.2
	120	21.2
	240	21.2

TABLE 2

Viscosity readings with unplugged, loosely plugged, tightly plugged tubes. Time of flow in seconds

TUBE NUMBER	UP	LP	TP
4	7.0	7.2	7.8
7	8.2	8.4	9.0
3	21.2	21.2	21.2
1	115.4	115.4	115.4

Symbols: UP, viscosimeters without cotton plugs; LP, viscosimeters with lightly placed cotton plugs; TP, viscosimeters with tightly placed cotton plugs.

of the lamp within about 3 cm. of the surface of the water. This arrangement gives satisfaction, as the lines on the viscosimeter may be plainly seen by the dispersion of the light through the water.

TABLE 3

(Graphic chart I)

Observations of sterile 2 per cent gelatin pH 7.8 using the viscosimeter as a culture tube. Temperature 36°

TIME	VISCOSITY FLOW
	seconds
At beginning	44.0
End of first day	44.2
End of second day	44.0
End of third day	44.2
End of fourth day	44.4
End of fifth day	44.0
End of sixth day	44.4

TABLE 4

(Graphic chart I)

Observations of Serratia marcesens in 2 per cent gelatin, pH 7.8, using the viscosimeter as a culture tube. Temperature 36°

TIME	VISCOSITY FLOW
hours	*seconds*
14	44.6
29	31.6
41	27.6
66	27.4
77	24.8
104	24.5
127	24.0
150	24.0
210	24.0

Note the constant viscosity at 41 and 66 hour readings and the second drop between 66 and 77. This phenomenom may be seen in table 1 of Levine and Carpenter's paper.

It will be noticed in table 2, that the difference in the readings between the three methods, viz., without plug, with plug loosely packed, and with plug tightly packed is within the experimental error in tubes of twenty seconds or more. A comparison

of these readings will show that the cotton plugs in the viscosimeters do not interfere too greatly, for practical purposes, with the flow of the gelatin.

COMMENT

The readings of gelatin liquefaction by the former methods have been almost worthless for comparative purposes. Attempts have been made to prepare gelatin culture media of specific per cent gelatin. This is the method used in all of the laboratory manuals with which the writer is familiar. This is nearly impossible because of the many factors entering into the composition of gelatin. Some brands of gelatin contain more gel than others. In the writer's laboratory, one brand of 4 per cent gelatin solution became solid over night at 22°C., while a 15 per cent solution made from gelatin of another brand did not gel in a week at the same temperature. The time that the gelatin is in the autoclave has its effects on the gelation of the gelatin. These conditions are so general that it seems that a viscosity standard should be worked out to replace the per cent gelatin standard now in use.

REFERENCES

Levine and Carpenter 1923 Gelatin liquefaction by bacteria. Jour. Bact., **8**, 297.

Torrey, John C. 1910 The viscosimeter as an aid in the detection of liquefying bacteria. Jour. Med. Res., **23**, 377.