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United States Department of the Interior, J. A. Krug, Secretary
Fish and Wildlife Service, Albert M. Day, Director

Fishery Leaflet 307

Washington 25, D. C. May 1948

SYNERESIS OF AGAR GELS

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The word syneresis (from the Greek word meaning a drawing together) was first applied by Graham in 1864 to the phenomenon of breaking up of jellies on long standing or when disturbed. Since then it has come to mean the separation of any free liquid from a gel regardless of the quantity or the cause. The quantity ranges from 90 percent of the initial volume with some silicic acid gels (Holmes, et al, 1919) to a fraction of a percent for most agar gels. The cause is still a matter of conjecture but the preponderance of evidence leads to the view that contraction of the gel due to internal forces drives out the liquid (Arsem, 1926; Kunitz, 1928; Liepatoff, 1927; and Mukoyama, 1927). It can readily be demonstrated that external sources of pressure achieve the same effect.

Syneresis of agar gels is of particular interest to the bacteriologist because excessive amounts of fluid induce the spreading of colonies and cause slipping of the gels in inverted petri dishes. A means of measuring the extent of this phenomenon, therefore, would be valuable in setting tolerances for agar to be used for bacteriological media.

When the volume of liquid synerized is considerable, it may be measured by pouring it off into a graduated cylinder as has been done by Liepatoff (1927), Mukoyama (1927), and Prakash and Dahr (1930). When the volume of liquid to be measured is small, such procedures do not suffice. Pijper and Kraan (1921) measured the syneresis of agar gels by placing columns of the gel above funnels to catch and measure the drip. Aside from the obvious mechanical difficulties in handling small amounts of liquid in this manner, the method is impractical because of the abnormal position in which the gel is placed. The unsupported column of gel creates considerable pressure against itself. The effect of this pressure was clearly demonstrated in the following experiment: Two gels were compared by the funnel method. One of these gels exhibited syneresis 10 to 20 times greater than the other under conditions normally

encountered when gels are supported in the containers in which they are formed. When tested by the funnel procedure of Pijper and Kraan the amounts of water exuded were much greater than encountered when the gels were supported in their containers and were exactly the same for both gels.

A technique has been devised whereby the adhering free water can be removed from a gel supported in its container without undue disturbance. The gel is formed in a flask, care being taken to prevent the formation of condensed water by pouring the agar sol at as low a temperature as possible. After the gel has set the flasks are stoppered to prevent evaporation. The free moisture can be determined at any time by washing it out of the flask with a heavier, immiscible liquid, such as carbon tetrachloride (saturated with water), into any suitable vessel where the volume of water can be measured. From five to six portions of carbon tetrachloride of 10 to 15 ml. each are used; each portion is swirled over the gel and poured into the measuring container. Pear shaped, graduated centrifuge tubes of 125 ml. capacity have been found satisfactory (Figure 1.). These are almost completely filled with additional carbon tetrachloride after the washings from the gel have been introduced, then are stoppered and centrifuged in the inverted position until the water is completely separated. The water, which now should be in the nipple of the tube, can be measured from the graduations. The efficacy of this procedure was determined by recovery of water added to gels which had been washed free of liquid. The data in Table 1 show that in half the experiments complete recovery was accomplished, while on the average all but 0.03 ml. was recovered.

Table 1. Recovery of water added to gels by washing with carbon tetrachloride

Added	Recovered	Difference
milliliters	milliliters	milliliters
0.10	0.07	0.03
0.10	0.10	0.00
0.20	0.15	0.05
0.20	0.20	0.00
0.30	0.20	0.10
0.30	0.23	0.07
0.40	0.39	0.01
0.40	0.40	0.00
1.00	1.00	0.00
1.00	1.00	0.00
	Average	0.03

The factors that might affect the extent of syneresis were studied by means of the above technique. These factors are time of storage, agar concentration, gel surface area and gel volume, the presence of other solutes and the rate at which the gel sets. The precision of the method was also determined.

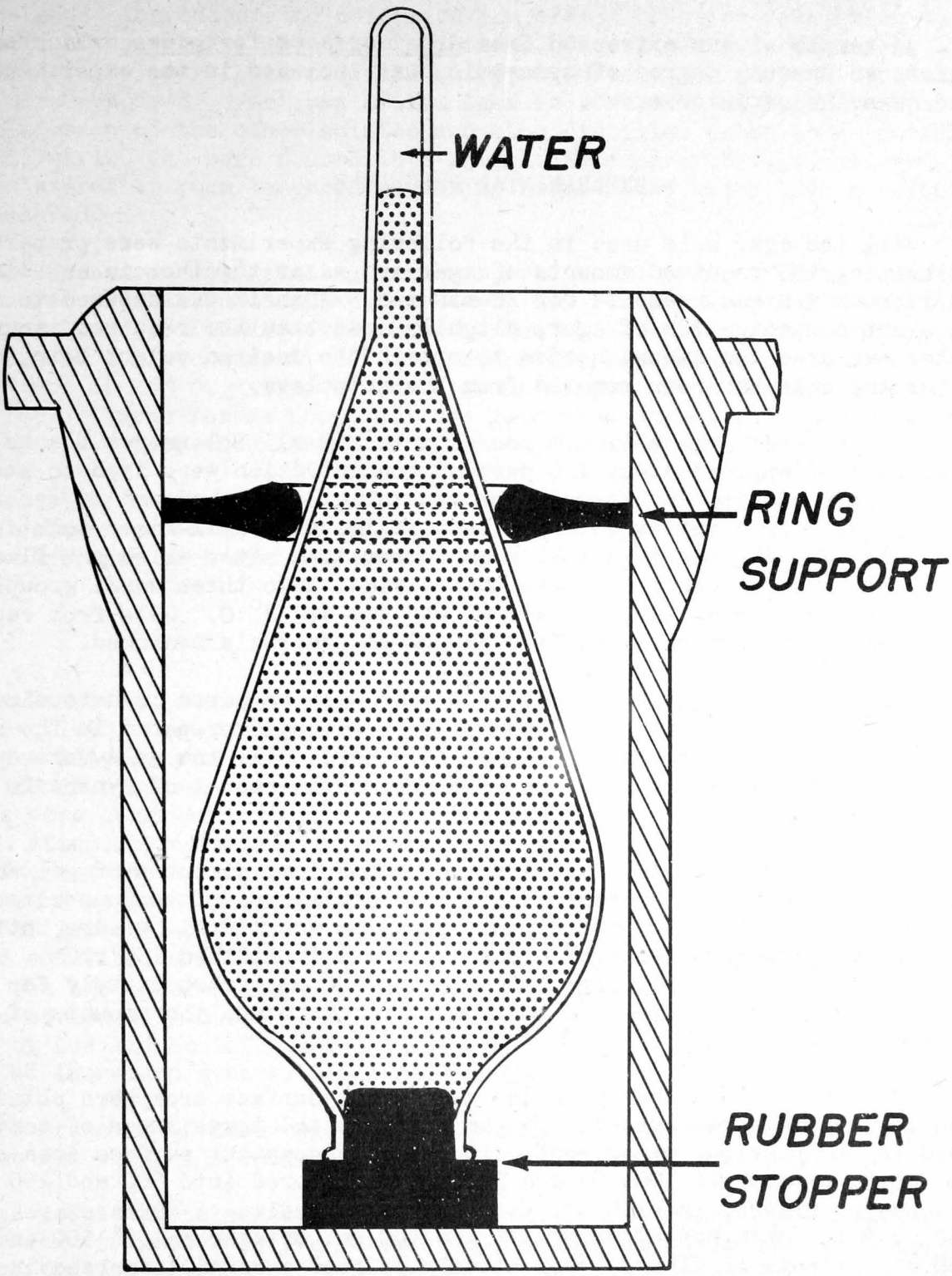

FIGURE 1. SECTIONAL VIEW OF PEAR-SHAPED CENTRIFUGE TUBE IN CENTRIFUGE BASKET, SHOWING HOW IT IS USED TO MEASURE WATER WASHED FROM GELS WITH CARBON TETRACHLORIDE.

A sample of gum extracted from Gracilaria confervoides, which exhibits an unusual degree of syneresis, was included in the experiments to increase the range observed.

EXPERIMENTAL PROCEDURE

All the agar sols used in the following experiments were prepared by heating the required amounts of agar and water together in an autoclave at 15 pounds steam pressure for 20 minutes. When it was desired to obtain an exact concentration of agar, slightly less than the required amount of water was used the amount needed to obtain the desired weight being added after the sols had been removed from the autoclave.

One hundred gram aliquots poured into 250 ml. Erlenmeyer flasks from agar sols of approximately 1.0 percent concentration were used to study the effects of time and temperature of storage on the amount of syneresis. All aliquots in a series were portions of the same well-mixed solution. The gels were allowed to set at room temperature after which the flasks were stoppered. Each series was then divided into three equal groups, one of which was held at 20°, one at 30° and one at 37° C. Gels from each group were removed periodically and the amount of syneresis measured.

Gels of 1.0, 1.5 and 2.0 percent agar were compared to determine the effect of concentration. One hundred gram aliquots prepared in the same manner as in the previous experiment were used. All the gels were held at room temperature for 24 hours after which the amount of syneresis was measured.

Variations in surface area for 100 and 70 gm. aliquots of gel were obtained by pouring the original sol into containers of various size. Erlenmeyer flasks of 500 and 250 ml. capacity and 150 ml. square bottles were used, giving surfaces of approximately 165, 150 and 135 $cm.^2$ respectively for 100 gm., and 150, 135 and 100 $cm.^2$ respectively for 70 gm. of agar gel. All gels were held at room temperature and the amounts of syneresis measured after 24 hours.

Variations in volume with the same total surface area were obtained by pouring the required amounts of agar sol into the three sizes of containers used in the previous experiment. To obtain a constant surface area of 135 $cm.^2$, amounts of 100, 70 and 30 gm. were poured into 500 and 250 ml. Erlenmeyer flasks, and 150 ml. bottles respectively. A surface area of 150 $cm.^2$ was obtained by using 130 and 100 gm. of agar sol in 500 and 250 ml. Erlenmeyer flasks, respectively. These were all immersed in a water bath at 45° C. and allowed to cool to room temperature with the bath so that all the gels set at the same rate. Syneresis was measured after 24 hours.

Cane sugar, Bacto peptone and calcium acetate were used as typical culture media ingredients to determine the effect of other solutes on the syneresis of agar gels. Separate solutions of each of these materials and each of the agars examined, all at approximately 2.0 percent concentration, were prepared. Portions of the agar sols were mixed with an equal weight of each of the other solutes and also distilled water as a control. Aliquots of 100 gm. were poured into 250 ml. Erlenmeyer flasks, allowed to set and stored at room temperature for 24 hours after which time syneresis was measured.

Different rates of setting were compared as to their effect on syneresis. One hundred gram aliquots of approximately 1.0 percent agar solutions in 250 ml. Erlenmeyer flasks were cooled at the rates attained in a refrigerator at 5° C., in an incubator at 20° C., and on the laboratory bench at a temperature of 25° C. After having a group from each series set at each of these temperatures for an hour, all the gels were stored at room temperature for 24 hours before the amounts of syneresis were measured.

To determine the precision of the procedure to be recommended, groups of six gels prepared on three successive days from 1.50 percent solutions of the same agar were analyzed according to that procedure.

RESULTS

It has been noted by Liepatoff (1927), Mukoyama (1927) and Prakash and Dahr (1930) that the syneresis of a number of different gels reaches a maximum after a period of time. They found that this maximum is greater and is reached sooner, the higher the temperature of storage. Similar results were observed with agar gels (Table 2) where syneresis reaches a maximum after approximately 24 hours at temperatures of 30° and 37° C. and is greater at these temperatures than at 20° C. Between 30° and 37° C. the temperature apparently has little effect on the amount of syneresis. A sample of gum extracted from Gracilaria confervoides (North Carolina) had not reached equilibrium with regard to syneresis within the 48 hour observation period but the rate of increase had been considerably reduced. A more extensive series of samples from Gracilaria confervoides (North Carolina) tested by Lee and Stoloff (1946) indicated that gels made from this extractive exhibited increased syneresis for periods up to 72 hours.

The presence of cane sugar, Bacto peptone or calcium acetate in solution was without effect on the degree of syneresis (Table 3).

The result of increased agar concentration was to decrease the degree of syneresis (Figure 2). The decrease was proportional to the increase in concentration between one and two percent agar and was of approximately the same magnitude for the five agars studied. The result of increasing the concentration of Gracilaria gum included in the study was ten times that experienced with agar, and was roughly in proportion to the tendency of each to synerize.

Table 2. Syneresis of gels held at different temperatures for different lengths of time

Temperature in °C.:		Syneresis in milliliters		
		20	30	37
Sample	Time in hours			
A	6	2.6	2.6	2.8
	18	4.0	5.2	4.0
	24	3.6	4.9	5.1
	48	4.5	6.2	5.4
C	18	.33	1.02	1.06
	24	.44	1.03	1.05
	48	.53	1.08	1.12
D	6	.23	.20	.21
	18	.37	.73	.67
	24	.46	.84	.82
	66	.40	.85	.80
H	18	.23	1.00	1.20
	24	.24	1.07	1.45
	66	.63	1.10	1.16

Note: The values in this and subsequent tables and figures are the averages of from 6 to 12 determinations. Sample A is a gum extracted from Gracilaria confervoides.

Table 3. Syneresis of gels when other solutes are added

Added Ingredient:	Syneresis in milliliters			
	Control	Calcium Acetate	Cane Sugar	Peptone
Sample				
A	2.6	2.4	2.8	2.6
C	.28	.30	.31	.31
D	.22	.26	.20	.20

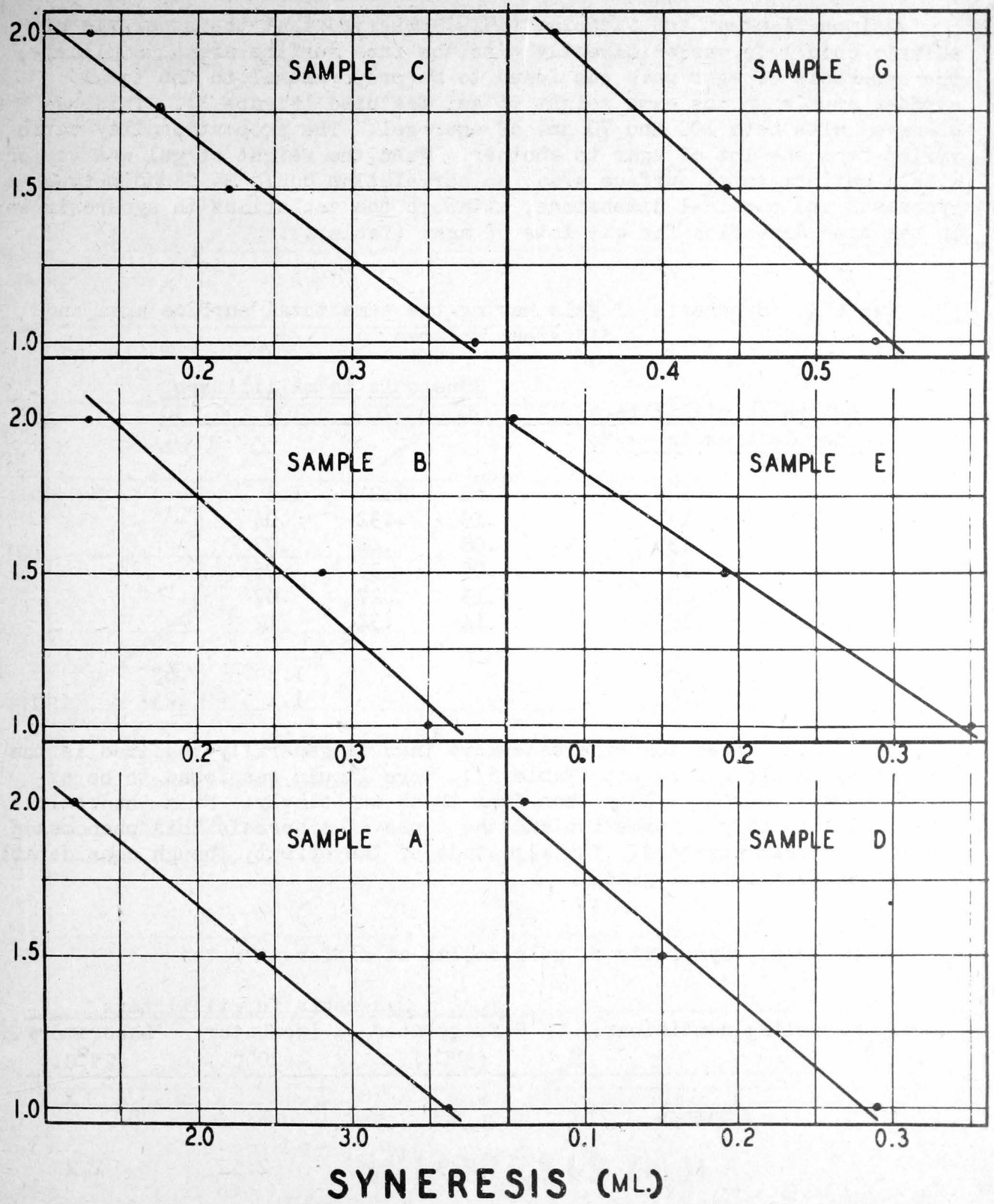

FIGURE 2. AMOUNTS OF SYNERESIS OF VARIOUS SAMPLES AT CONCENTRATIONS OF 1.0, 1.5 AND 2.0 PERCENT; PREPARED AND AGED UNDER IDENTICAL CONDITIONS. SAMPLE A IS A GUM EXTRACTED FROM GRACILARIA CONFERVOIDES.

Holmes, Kaufman and Nicholas (1919) observed that the syneresis of silicic acid gels varied directly with the free surface area. Similarly, the syneresis of agar gels was found to be proportional to the total surface area when the same weight of gel was used (Figure 3). This was observed with both 100 and 70 gm. of agar gel. The proportionality ratio varied from one lot of agar to another. When the weight of gel was varied with a uniform total surface area, no correlation could be found between syneresis and physical dimensions, although the variations in syneresis were in the same direction for all lots of agar (Table 4).

Table 4. Syneresis of gels having the same total surface area and different volumes

Volume in milliliters:		Syneresis in milliliters			
Sample	Area in $cm.^2$	30	70	100	130
A	135	.63	2.2	1.5	-
B	135	.19	.32	.04	-
C	135	.08	.48	.07	-
D	135	.05	.33	.05	-
F	135	.13	.27	.07	-
G	135	.14	.34	.12	-
D	165	-	-	1.3	.63
E	165	-	-	1.1	.23

One factor affecting syneresis more than is generally realized is the rate at which the gel is set (Table 5). More liquid was found to be expelled from gels set rapidly than from those set slowly. From theoretical considerations of gel formation and the cause of syneresis this phenomenon should have been expected. The magnitude of the effect, though considerable, was not uniform for all gels.

Table 5. Syneresis of gels cooled at different rates

Cooling conditions:	Syneresis in milliliters		
	Refrigerator (5°C.)	Incubator (20°C.)	Laboratory (25°C.)
Sample			
A	2.9	2.5	2.2
D	.50	.35	.28
G	.85	.44	.26

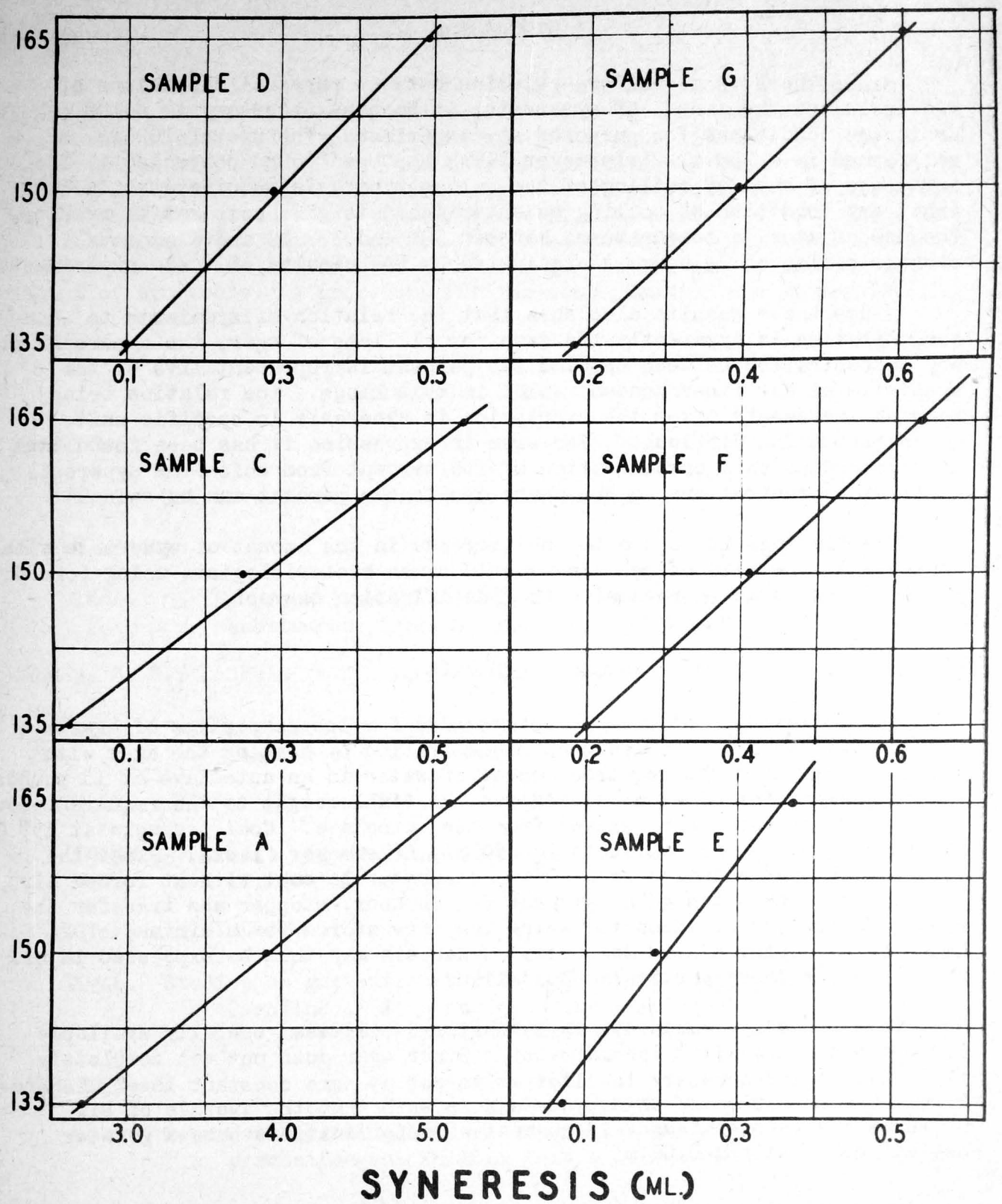

FIGURE 3. AMOUNTS OF SYNERESIS OF VARIOUS SAMPLES MADE INTO GELS HAVING THE TOTAL SURFACE AREAS INDICATED AND ALL WEIGHING 100 GRAMS; PREPARED AND AGED UNDER IDENTICAL CONDITIONS; ALL GELS IN A SERIES PREPARED FROM THE SAME SOLUTION. SAMPLE A IS A GUM EXTRACTED FROM GRACILARIA CONFERVOIDES.

DISCUSSION

Since there is no uniform relation between physical dimensions of agar gels and the amount of syneresis, it becomes necessary to establish arbitrary conditions for purposes of comparison. The use of 100 gm. of gel formed in a 250 ml. Erlenmeyer flask has been found convenient. The influence of rate of setting of the gel on syneresis requires that some arbitrary condition of cooling be established to give comparative results. The use of storage temperatures between 30° and 37° C. and a minimum storage period of 24 hours is indicated by the results of these experiments.

Since these results also show that the relation of syneresis to agar concentration is apparently the same for all lots of agar, the syneresis at any concentration between one and two percent is representative of the syneresis at any other concentration in this range. The relation being between increments makes the expression in syneresis in specific units of concentration difficult. For ease in expression it has been found best to standardize on a concentration of 1.5 percent from which the syneresis at other concentrations in the range one to two percent can be calculated.

The findings in regard to the increase in the amount of syneresis with increase in the rate of gelation should cause bacteriologists using forced cooling procedures to reexamine the justification thereof.

RECOMMENDED METHOD

The following procedure is recommended for the comparison of agars: Prepare agar sols of 1.50 percent concentration by heating the agar with slightly less than the required amount of water in an autoclave at 15 pounds steam pressure for 20 minutes. Adjust the final weight to the required amount after the sols have been removed from the autoclave. Cool the sols at 45° C. before placing 100 gm. aliquots in 250 ml. Erlenmeyer flasks. Place the flasks with agar solution in a 20° C. incubator to cool without forced air circulation. When the gels have set for an hour, stopper and transfer the flasks to the 37° C. incubator where they are stored for a minimum of 24 hours before syneresis is measured. Syneresis may then be expressed in ml. of liquid for these particular conditions.

This procedure makes use of incubator facilities generally available in most bacteriological laboratories. Since agar does not set completely at 37°C., it is necessary to allow it tc set at some constant lower temperature. The precision of this procedure is such that the average of six measurements is significant (5 percent of determinations have a greater deviation) to within 0.08 ml.

SUMMARY

A technique is described for determining small amounts of liquid of syneresis. This method was applied to a study of agar gels. The syneresis of agar gels reaches a maximum after approximately 24 hours at temperatures of 30° to 37° C. and is greater at these temperatures than at 20° C. Between 30° and 37° C. the temperature apparently has little effect on the amount of syneresis. The presence of cane sugar, Bacto peptone or calcium acetate was without effect. Concentration of agar, total surface area of the gel and the rate at which the gel is set all influence the amount of syneresis. A procedure for the comparison of the syneresis of gels is recommended.

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