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STRENGTH MEASUREMENT OF AGAR GELS

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INTRODUCTION

The following study was conducted because of the paucity of work on the factors affecting stress-strain measurements on gels and the general lack of any work relative to agar gels. The term "gel strength" has been interpreted in different ways by different investigators. The one thing all have agreed upon is that a stress and a strain have to be measured. Some investigators measured the strain produced by a given stress; some the stress produced by a given strain. Others have measured the stress necessary to produce an irreversible strain, that is, rupture of the gel. Some workers have established the stress-strain curve within the elastic limits of the gel. It was also suggested that the work required to rupture the gel was an appropriate measurement.

REVIEW OF LITERATURE

Sheppard and Sweet (1921, 1923), Hatschek (1932), Lampitt and Norris (1934) and Saxl (1938) have demonstrated that the stress-strain relation of gelatin and glue gels follows Hooke's law for a considerable portion of its course. Assuming that all gels have this elastic property, it becomes evident that a true consideration of gel strength involves breaking load (the force required to break the gel), breaking strain (the displacement of the gel at the elastic limit which is the point of rupture) and elastic modulus (the displacement of the gel per unit of force). When the term "gel strength" is used hereafter it will be understood to include these three components. The work accomplished in breaking the gel may be computed from the breaking load and strain.

The variety of instruments devised to measure stress and strain of gels is almost equal to the number of investigators. Lipowitz (1861) used a simple plunger topped with a funnel into which shot was poured by hand until the gel broke. Plunger, funnel and shot were then weighed. Valenta (1909) modified the Lipowitz tester by using a controlled flow of mercury to load the plunger. Tracey (1928) used shot instead of mercury, controlled the flow by means of a shutter and added a tilting mirror to the plunger to indicate displacement. Scott (1907) measured the breaking force directly by placing the gel sample on

a spring balance. A plunger was driven against the gel by a gear and screw arrangement. A maximum indicator on the balance gave the breaking force. The Forest Products Laboratory (1919) added a linear scale and indicator to the Lipowitz tester to indicate displacement under a given load of shot. Richardson (1923) describes the Bloom gelometer which has been accepted as the standard instrument by the National Association of Glue Manufacturers (1924). The plunger and loading basket are suspended from a spring. Shot is fed into the basket from a hopper controlled by a gate. An arm attached to the plunger moves between two spaced electric contacts. When the lower contact is reached an electric impulse closes the shot gate. Greiner (Verschuur, 1932) and Goebel (1931) each had their own device for adding known metal weights to a plunger. Both measured displacement by translating the linear motion of the plunger to the rotary motion of a revolving dial.

A number of workers have attached a plunger to one arm of a laboratory suspension balance and have counterbalanced it with weights on the opposite arm. The balance pointer then magnifies the displacement of the plunger. Oakes and Davis (1922) loaded the plunger with water discharged from a burette and computed the load from the volume of water used. Sumner (1938) used mercury instead of water. Sheppard and Sweet (1921) loaded the plunger arm of the balance by means of a calibrated chain. Lampitt and Norris (1934) weighed the load of sand delivered from a hopper through a controlled gate. Saxl (1936) used a chain loading device similar to that of Sheppard and Sweet but obtained the displacement by the distance the gel had to be lifted to bring the balance indicator to its original position. Saxl (1938) later employed this same principle in an apparatus which utilized a standard laboratory triple beam balance having sliding weights. Smith (1909) made use of an entirely different principle, that of volume displacement. An elastic membrane, stretched over the mouth of a thistle tube, was placed against the surface of the gel. Pressure was applied to the membrane through a hydraulic system activated by compressed air supplied by a rubber bulb. The pressure was measured on a manometer and volume displacement by displacement of the liquid-air interface in the hydraulic system. This apparatus was modified slightly by Hulbert (1913) and the technique of operation, by Low (1920).

The hydraulic principle was used by Tarr (1926) and Baker (1926) to apply pressure to the plunger of a syringe, the role of the syringe being reversed from the usual. Pressure was created by running water from a tap into a closed system and was measured on a manometer. Baker (1938) later replaced the tap water with mercury delivered from a constant head.

All the above methods apply a force to a portion of the gel surface. The stress-strain relations of a cylinder of gel can also be measured. Alexander (1908) patented a press which applied a load to a standard size cylinder of gel. An electric contact signalled a given displacement. Lockwood and Hayes (1931) reduced this system to its simplest form by measuring the decrease in height of a standard jelly column under its own weight. Hatschek (1932) employed the simple press as used by Alexander but measured displacement with a cathetometer. Absolute physical constants were obtained by Sheppard, Sweet and Scott (1920) by applying a torsional force to a cylinder of gel. The

sample was mounted between two parallel plates, one attached to a turntable which could be activated by hand or motor, the other to a turntable attached by a pulley to a weighted lever arm which applied an increasing force as it was tilted from the vertical. The turntables were marked to indicate their rotational displacement to each other and the lever arm was equipped with a pall to maintain it at the maximum load. A thermostatic chamber housed the turntables.

Several techniques have been used which are devoid of any simple physical interpretation. A method which applies only to strong gels was used by Smith (1920). A truncated cone of gel was formed within a funnel on a mercury surface. After the gel had set, the mercury was removed and suction applied to the outlet of the funnel. The displacement of the center of the gel under a definite pressure was measured. Briefer and Cohen (1928) measured the penetration into the gel of uniform weight shot dropped from a standardized height. A modified stopcock was used to pick up and drop the shot. Buston and Nanji (1932) used the force required to pull a perforated plate through the gel at a predetermined speed. The gel was permitted to set in a vessel equipped with inside fins to hold the gel in place and the perforated plate was inserted before the gel was set. The force was applied by placing weights on a pan attached to the perforated plate through a pulley. These last two mentioned methods were suitable for only weak gels.

EXPERIMENTAL PROCEDURE

Apparatus Used. - For the following studies an instrument was devised (Fig. 1) based on those of Scott (1907) and the Forest Products Laboratory (1919). A mechanical system that worked to depress the plunger smoothly was obtained by overweighting the plunger and releasing it through a series of pullies by means of a cord wound around a spindle. The spindle was turned by a motor equipped with a variable speed governor. A hand centrifuge drive was attached to the motor to rewind the spindle. This is an improvised system and may be modified in many ways without affecting the essential characteristics of the instrument.

Most investigators who used a plunger type of apparatus chose between a flat and a hemispherical ended plunger as fancy dictated. However, Sheppard and Sweet (1923) found that the load-displacement curve followed a straight line if a flat ended plunger was used, but that hemispherical ended plungers gave a linear curve only after penetration had afforded a nearly uniform surface area. These observations were confirmed by Lampitt and Norris (1934). Tracey (1928) noted that a hemispherical ended plunger gave a more definite break than a flat one but observations by the author do not confirm this statement. A flat ended plunger was used in these experiments because a linear load-strain relation can be obtained with it and because of the simple force-load relation that exists.

In operation a sample was placed on the balance pan and tared to zero weight by means of the adjustable scale. The plunger was then operated manually to bring it into contact with the gel surface. By retracting the

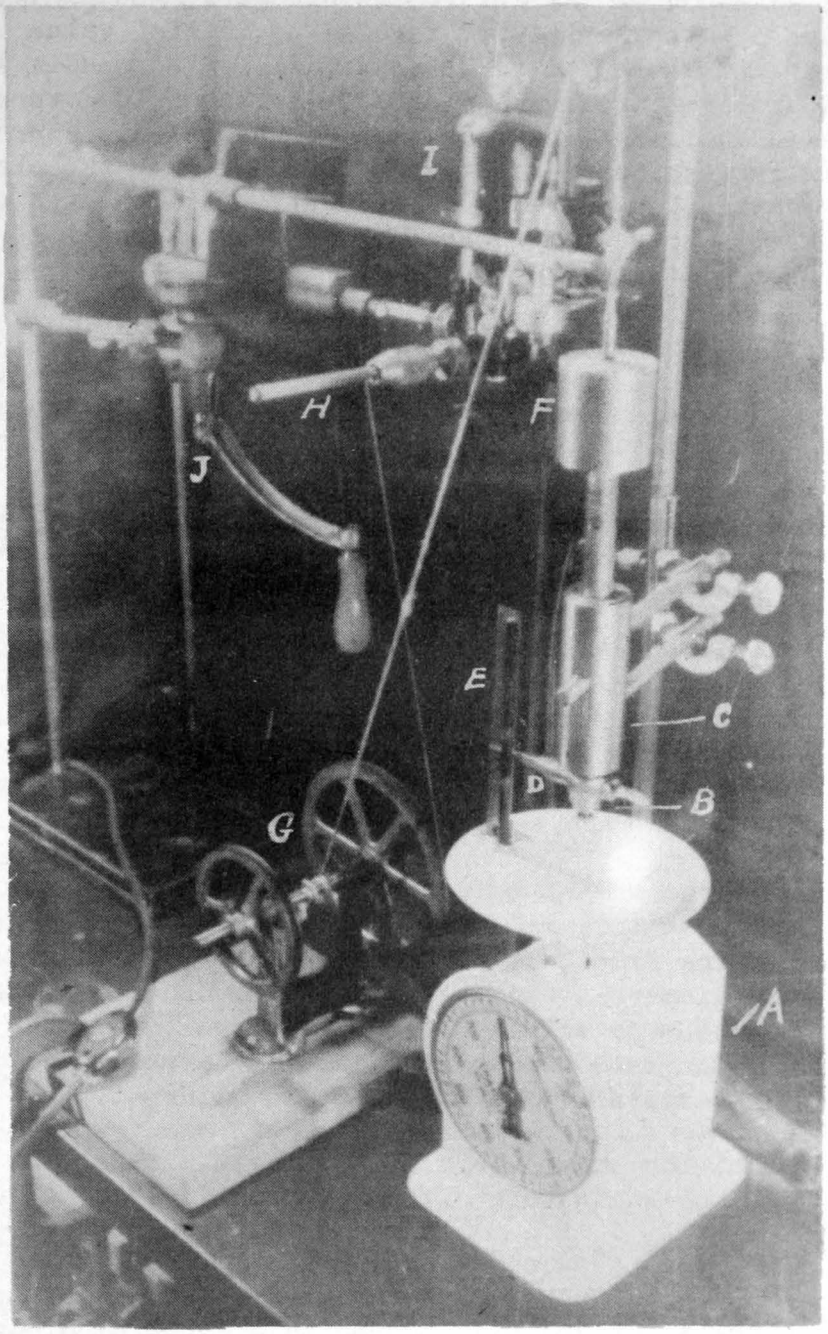

Figure 1. Apparatus used for gel strength studies. A, 1 kg. capacity spring balance; B, removable plunger head; C, plunger support; D, displacement pointer; E, scale divided in 0.5 mm.; F, weight; G, reducing pulley; H, spindle; I, variable speed motor; J, hand centrifuge drive.

plunger slightly the balance pointer was made to register a slight negative value because of adhesion of the gel to the plunger. The plunger was again advanced until the pointer registered zero. The initial value of the pointer on the plunger arm was noted (a 5X lens facilitated reading) and a force was then applied by lowering the plunger. A series of load versus strain readings were taken until the elastic limit of the gel was approached as indicated by a slow regression of the balance pointer when the plunger was stopped. The force was then applied continuously until the gel surface broke, evidenced by a sharp snapback of the balance. The maximum load applied was noted by close observation of the balance pointer.

COMPUTATIONS

If a gel obeys Hooke's law, the values noted for the stress and strain should plot on a straight line. If this line is extrapolated to the observed breaking load, the breaking strain may be obtained. The work accomplished in breaking the gel may be computed from the relation $W = \frac{1}{2}BS$ (W-work, B-breaking load and S-breaking strain) and the elastic modulus may be obtained from the slope of the curve.

The agar gels with which this work was conducted follow Hooke's law for a considerable portion of the stress-strain curve. A typical curve is shown in Figure 2. By assuming a straight line function for the entire curve, an approximation may be obtained for the breaking strain and the work accomplished in breaking the gel. The slope of the linear portion of the curve is taken as the elastic modulus.

Effect of Gel Dimensions. - Agar solutions of approximately one percent concentration in distilled water, made from three agars selected at random, were poured to depths ranging from 2 to 6 cm. in cylindrical containers. These cylinders had inside diameters ranging from 3.0 to 8.6 cm. All samples in a series, aliquots of the same solution, were immersed in a water bath heated initially to 45° C. The rate of cooling from 45° C. was then controlled by the bath. The final temperature of the bath was adjusted to 20° C. and maintained at that temperature for 17 hours. Strength measurements of the gels at 20° C. were made at the end of this time using a plunger 1.1 cm. in diameter and maintaining a constant rate of application of force of approximately 50 gm. per second.

Effect of Diameter of Gel and Diameter of Plunger. - Gels were prepared as before except that the solutions were poured to a depth of 4.5 cm. in all containers. The gels in each series were divided into three groups, each having the same variety of diameters. Strength measurements were made as before using plungers having diameters of 0.8, 1.1 and 1.4 cm., a different plunger being used for each group of gels.

Effect of Diameter of Plunger. - Gels were prepared as before except crystallizing dishes 7.0 cm. in diameter by 5.0 cm. high were used. The previous experiments had indicated these gel dimensions to be desirable. The solutions were divided into three equal groups for each series. Strength measurements were made as before using plungers 1.1, 1.4 and 1.7 cm. in diameter, a

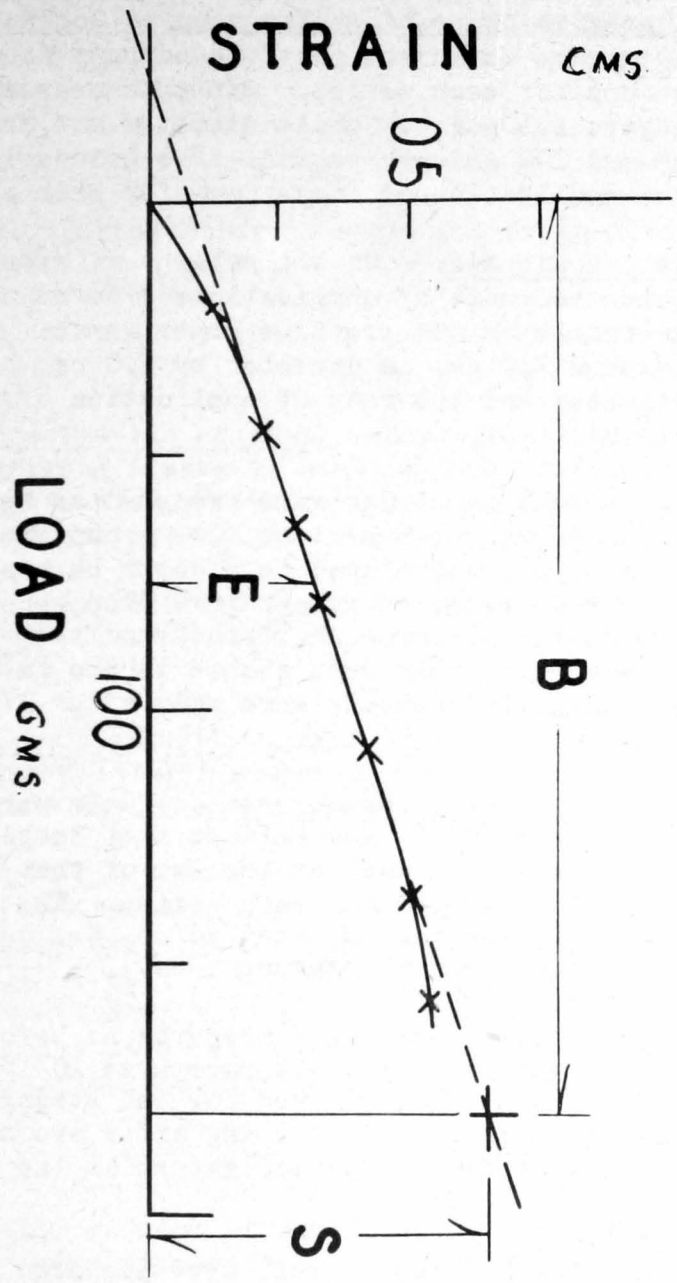

Figure 2. A typical stress-strain curve for agar gels. (B, breaking load; S, breaking strain; E, elastic modulus cms. 100 gms.)

different plunger being used for each group of gels.

Effect of Diameter of Plunger and Rate of Application of Force. - Gels were prepared in crystallizing dishes as in the previous experiment. These were divided into six equal groups for each series. Strength measurements were made as before using plungers 1.1 and 1.4 cm. in diameter and rates of application of force of 34, 58 and 100 gm. per second. Two groups in each series were used for each rate of application of force, one for each plunger size.

Standard Measuring Conditions Adopted. - In all subsequent experiments the gel dimensions, plunger size and rate of application of force employed were those shown to be most desirable by the previous experiments. The containers used were crystallizing dishes 7.0 cm. in diameter by 5.0 cm. high. The plunger was 1.1 cm. in diameter and the rate of application of force was approximately 50 gm. per second.

Effect of Rate of Cooling. - Agar solutions were prepared as before and were divided into three equal groups for each series. One group was placed in a refrigerator to cool and one group was placed in a water bath at 45° C. to cool with the bath. The average rates of temperature drop were 0.43°, 0.18° and 0.05° C. per minute respectively for each of the conditions. When all groups had reached room temperature they were placed in the bath which was brought to 20° C. Gel strength measurements were made after 17 hours of storage in the bath.

Effect of Temperature of Gel at Time of Measurement. - Gels were prepared as before, cooled in a water-bath at 20° C. and held at that temperature for 17 hours. Gel strength measurements were made at the end of that time. The gels were divided into four equal groups for each series. The temperature of one group was left at 20° C., one was adjusted to 25° C., one to 30° C. and one to 35° C., before making strength measurements.

Effect of Length of Time Gel Ages. - Gels were prepared as before. One series for each agar was cooled and held in a water-bath at 10° C., one series at 20° C. and one at 30° C. Groups were removed for gel strength measurements from each series at regular time intervals starting after two hours and ending at 28 hours. Measurements were made at the temperature of the bath for each series.

Effect of Temperature of Gel While Aging. - Gels were prepared as before. Each series was divided into three groups. One group was stored at 10° C., one at 20° C. and one at 30° C. The next day the temperature of all groups was brought to 30° C. and gel strength measurements were made at this temperature.

Effect of pH of Gel. - Agar solutions of approximately two percent concentration, in distilled water, were prepared from three agars selected at random. For a series, four aliquots of the same solution were mixed with an equal weight of citrate-phosphate buffers adjusted to pH 4.0 for one and pH 5.0,

6.0 and 7.5 for each of the others. The buffered solutions were poured into standard crystallizing dishes, cooled and held in a water-bath at 30° C. Gel strength measurements were made the following day at this temperature. The pH values of the gels were determined and found to be the same as the added buffer solutions.

Effect of Solutes Other than Agar. - Agar solutions of approximately two percent concentration, in distilled water, were prepared from three agars selected at random. For a series, one aliquot of agar solution was mixed with an equal weight of distilled water and another aliquot with an equal weight of the desired solute in water solution. The solutes used were cane sugar, Bacto-peptone and calcium acetate in final concentrations of 2, 1 and 1 percent respectively.

Effect of Concentration of Agar. - Agar solutions in distilled water were prepared in weighed flasks by heating in an autoclave for 20 minutes at 15 pounds steam pressure. The solutions were adjusted by the addition of distilled water to the net weights required to give agar concentrations (dry-weight basis) of 0.50, 1.00, 1.25, 1.50 and 2.00 percent. After mixing, the solutions were poured into standard crystallizing dishes and allowed to set overnight at 30° C. Gel strength measurements were made the next day at this temperature.

Reproducibility of Results. - Agar solutions of 1.00 percent (dry-weight basis) concentration were prepared, gels were formed and strength measurements made in the same manner as in the previous experiment. Four agars were tested together in this manner on five separate days.

The time and temperature of heating must be controlled because of the tendency of agar to lose strength by hydrolysis. Although hydrolysis of agar was not studied at this time it is known to be of significant magnitude for the conditions ordinarily employed to prepare agar media. The time and temperature generally used for the sterilization of bacteriological media were selected as practical heating conditions on which to standardize.

INTERPRETATION OF DATA

The validity of many of the following observations and the significance of the results obtained depend on a knowledge of the variability of the strength of gels as prepared for these experiments (table 11). Absolute variations in determined strengths of equivalent gels were about the same for four different agars although the magnitude of the breaking loads of the agars covered a wide range. The standard error of the means of five identical groups of six gels each averaged for the four agars, 9 gm. for breaking loads ranging from 237 to 618 gm., 0.02 cm. for breaking strains ranging from .57 to .69 cm. and 0.004 cm. per 100 gm. for elasticities ranging from .10 to .23 cm. per 100 gm. These values were obtained with a 1.1 cm. diameter plunger which was used for most of the work.

The apparent strength of agar gels is practically independent of the

dimensions of the container above limiting values not related to the strength of the gel (tables 1 and 2). This is in agreement with the work of Sheppard and Sweet (1923) on gelatin gels. The limiting height is about 4 cm. regardless of the gel diameter and the limiting diameter is 6.5 cm. regardless of the gel height. These figures hold for the sizes of plungers tested which ranged from 0.8 to 1.7 cm. in diameter (tables 2 and 3). Only the elasticity appears unaffected by gel height (range 2 to 6 cm.).

The apparent breaking load and elasticity of agar gels are related to a linear dimension of the plunger, most probably the circumference (tables 2, 3 and 4), which finding is in agreement with the work of Tracey (1928) on gelatin gels. The breaking strain, however, is about the same for most plunger sizes. These relations hold only for plungers having diameters of 1.1 cm. and larger.

An interesting observation which may have a bearing on all the foregoing relations is that the break in the gel takes the form of a cone with its base coinciding with the base of the plunger.

The rate at which the force is applied to a gel has a slight influence on the apparent gel strength (table 4). This influence is so slight that control of the rate can be maintained easily with sufficient accuracy by hand manipulation. The tendency is for an increase in breaking load and strain, and decrease in elasticity with increase of rate of application of force, evidently independent of plunger size.

Large differences in the rate at which agar solutions were cooled produced no effect on any of the gel strength components (table 5).

The temperature at which the determination is made has a slight effect on gel strength measurements (table 6). Breaking load decreases an average of 0.8 gm. per cm. of plunger circumference, breaking strain decreases about 0.002 cm. and the elasticity increases about 0.003 cm. per 100 gm. per cm. plunger circumference for each 1.0° C. rise in temperature within the range 20° to 35° C.

Breaking load and strain of agar gels increased to constant values after approximately six hours of storage regardless of the temperature within a range of 10° to 30°C. (table 7). The first determinations were made after two hours storage at which time constant elasticity appears to have been attained.

The temperature at which the gel is aged seems to bear little, if any, relation to the gel strength (table 8). At any rate, the gel strength should not be affected by variations in room temperature normally encountered.

Gel strength was unchanged by differences in pH between 4.0 to 7.5 (table 9) or the presence of cane sugar or Bacto-peptone, but the presence of calcium acetate increased the breaking load considerably and decreased elasticity (table 10). The fact that some substances can cause changes in

gel strength makes the use of distilled water advisable in preparing gels for test. The loss of strength of agar on heating which is accentuated by low pH should not be confused with this study since all pH adjustments were made after heating.

Carpenter et al. (1928) and Sheppard and Sweet (1921) have found the relation between concentration and strength of gelatin gels to be a function of the type $S = KC^n$ (S, strength; C, concentration) where K and n are constants dependent on the gelatin. A similar relation was noted in these studies (table 12, figure 3) for the breaking load of agar gels where the function takes the form $B = A + KC^n$ (B, breaking load; C, concentration) where A is a constant probably denoting the minimum concentration at which the agar will gel. The values for n were found to fall between 1 and $\frac{1}{2}$, and the values for A between 0.15 and 0.43 percent by weight. For practical purposes n can be assumed to be 1, making the relation $B = A + KC$.

All the gels studied had about the same breaking strain for the same agar concentration, the breaking strains being more nearly the same the greater the concentration (tables 11 and 12). The average breaking strains for the gels studied were found to be related to the agar concentrations by the function $S = 0.79 - 0.18C$ (S, breaking strain in gm.: C, concentration in gm. per 100 gm.).

There is evidently a relation between the breaking load and elasticity of the type $BE = K$ (B, breaking load; E, elasticity) (table 12, figure 4) where K is a constant whose average value for all the gels examined in the complete series of experiments is 55 with a standard deviation of $\pm$ 11.

Since it appears that the breaking strain is nearly identical for all agar gels of the same concentration, particularly in the range of concentrations most frequently used, consideration of the strength of agar gels becomes limited to the breaking load and elasticity. These are related by a practically constant ratio so that but one need be studied. The breaking load can be determined most rapidly and simply.

For practical purposes, the breaking load of any agar gel is related to the concentration of agar by the linear function $B = A + KC$. When the variation in A is small with regard to KC, K becomes the value characteristic of any particular agar. By making C constant, that is by making all measurements at the same agar concentration, B becomes proportional to K and therefore characteristic of the agar. Although a curvilinear relationship is indicated for certain samples of agar, the data in figure 3 demonstrate that these assumptions and simplifications are workable for the agars studied at concentration greater than approximately one percent. The value of A is probably a measure of impurities so that variations greater than those observed would not be expected from the usual supply of commercial and bacteriological agars.

A sample of gum extracted from Gracilaria confervoides, sometimes known as Atlantic agar, showed stress-strain relations similar to the agars. From the findings of previous investigators with gels from other materials it is probable that these relations hold true for all gels.

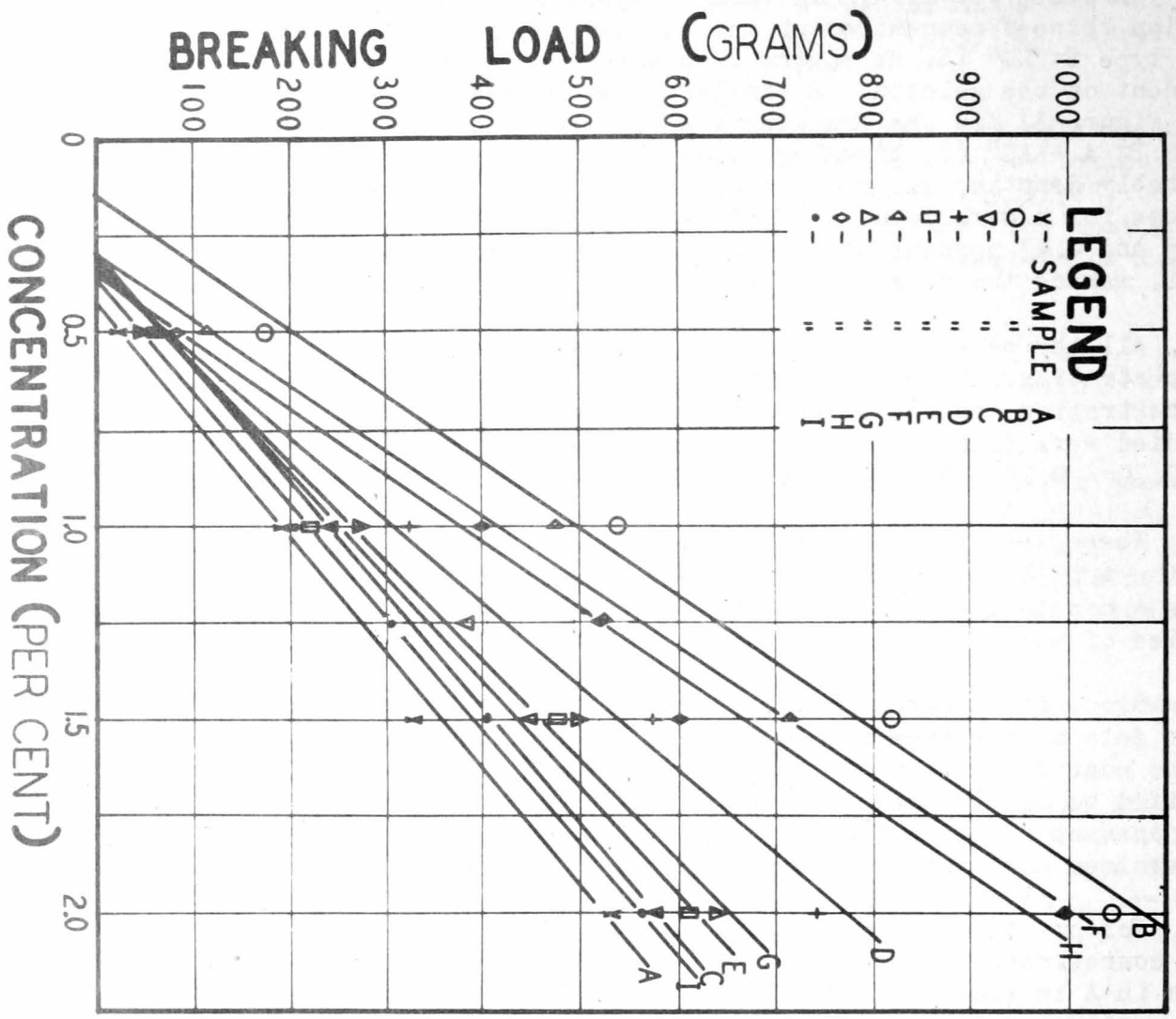

Figure 3. The breaking load of agar gels of graded concentration.

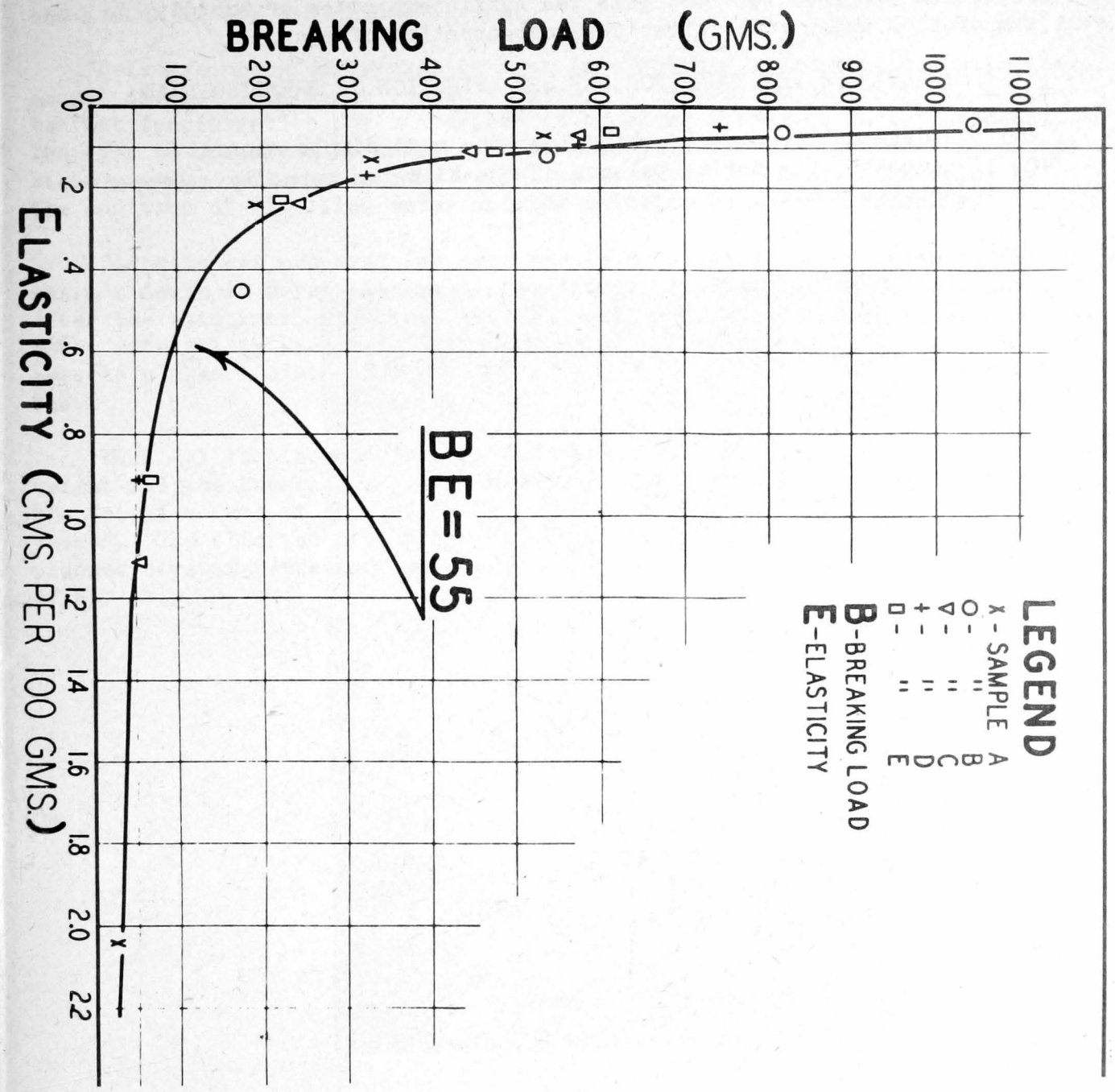

Figure 4. Breaking load and elastic modulus of agar gels.

It may be observed that no mention has been made concerning protection of the gel during storage. Oakes and Davis (1922) have demonstrated that protection of gelatin gels from contact with air had no effect on gel strength. The same has been assumed to be true for agar gels since skin formation is not experienced except under the most severe drying conditions. All the protection required for test gels was sufficient cover or humidity to prevent evaporation which would alter the concentration of agar.

A RECOMMENDED PROCEDURE FOR THE DETERMINATION OF BREAKING LOAD

For the determination of breaking load the most simple apparatus (figure 5) consists of a spring balance of one kilogram capacity equipped with an adjustable scale.

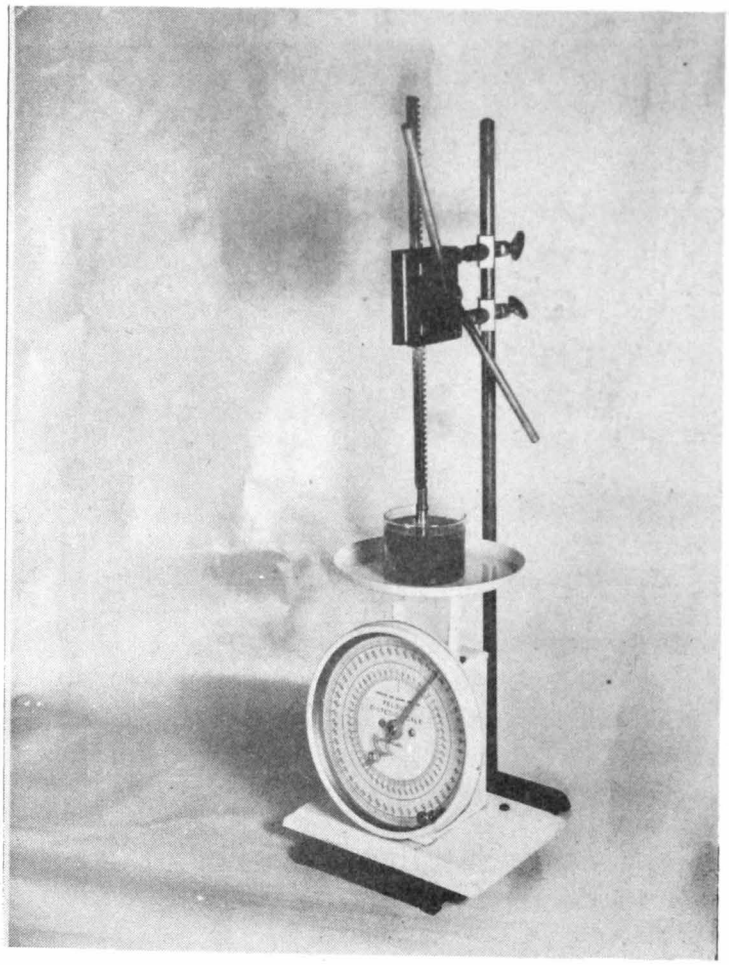

Figure 5. Recommended simplified apparatus for determining breaking load of gels.

Above this is attached a support and guide for the plunger. The plunger may be any convenient piece of cylindrical rod, faced off squarely at one end with the edge rounded slightly to prevent cutting of the gel. A plunger approximately 1.1 cm. in diameter will cover the range of breaking loads which are ordinarily encountered. The plunger may be activated by hand or through the medium of any mechanical device.

Gels are prepared by placing agar in a weighed flask together with the amount of distilled water needed to give the desired concentration. A convenient concentration for comparison of breaking strengths is 1.5 percent. The agar is dissolved by heating in an autoclave for 20 minutes at 15 pounds steam pressure after which the weight is adjusted to the correct value by the addition of distilled water and the solution is mixed thoroughly.

Six gels are prepared for each sample by pouring the solution to the maximum depth in crystallizing dishes 7.0 cm. in diameter by 5.0 cm. deep. After the solutions have cooled and the gels set they are covered by any suitable material to keep off dust and prevent excessive evaporation. When the gels have aged a minimum of six hours at room temperature they are ready for test.

Each gel is placed on the pan of the balance which is tared to zero weight and the breaking load is determined by pressing the plunger against the gel at a rate of approximately 50 gm. per second until a rupture is obtained. The observed breaking loads are calculated in terms of gm. per cm. plunger circumference and reported to the nearest 10 gm. unit for the average of six gels.

SUMMARY

Some factors were studied which affect the measurement of gel strength. A flat plunger type of instrument was used. Factors having little or no effect on apparent gel strength are the rate at which the force is applied, the rate at which the gel is cooled, the temperature of the gel while aging, pH of the gel and the presence of sugar or peptone in the gel. The dimensions of the gel, above certain limiting values, do not affect apparent strength. The same is true for the length of time the gel ages.

The concentration of agar temperature of the gel and the presence of calcium salts all influence gel strength.

The breaking load and elasticity are related to a linear dimension of the plunger, probably circumference, but the breaking strain is approximately the same for all plunger sizes. The breaking strain is also approximately the same for all agar gels of the same concentration. The breaking load, for practical purposes, bears a linear relation to agar concentration and is related to elasticity by a constant inverse ratio.

The measurement of the breaking load of agar gels of a fixed concentration is the most practical method for comparing gelation of agars.

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1932 Les appareils pour la mesure de la force superficielle de matieres gelatineuses.
Chim. et Indus. 28:180S, Special no. March.

Table 1. The strength of agar gels of graded height and diameter.

Agar Sample	Diameter of Container centimeters	Height of gel in centimeters 2	3	4	5	6
		Breaking load in grams:				
	3.0	252	290	291	-	-
	4.9	169	277	287	302	-
A	6.5	-	299	345	318	325
	7.5	-	340	353	357	369
	8.6	-	-	379	347	362
	3.0	104	86	120	-	-
	4.9	104	135	135	163	154
B	6.5	130	152	144	195	208
	7.5	149	160	186	235	186
	8.6	142	158	160	155	128
	3.0	290	300	284	-	-
	4.9	290	290	281	260	257
C	6.5	226	318	303	312	308
	7.5	244	238	261	254	250
	8.6	139	234	248	230	244
		Breaking Strain in centimeters:				
	3.0	.44	.45	.44	-	-
	4.9	.42	.69	.66	.67	-
A	6.5	-	.75	.81	.79	.81
	7.5	-	.94	.83	.89	.89
	8.6	-	-	.90	.86	.93
	3.0	.30	.18	.21	-	-
	4.9	.32	.43	.38	.44	.44
B	6.5	.40	.45	.47	.59	.55
	7.5	.43	.48	.57	.68	.55
	8.6	.38	.50	.53	.52	.51
	3.0	.58	.55	.52	-	-
	4.9	.71	.82	.77	.75	.76
C	6.5	.56	.90	.95	.98	.99
	7.5	.86	.90	.92	.97	.96
	8.6	.64	.93	.97	1.03	1.02

Table 1. Continued.

Agar Sample	Diameter of Container centimeters	Height of gel in centimeters 2	3	4	5	6
		Elastic modulus in centimeters per 100 grams:				
A	3.0	.17	.17	.15	-	-
	4.9	.19	.22	.22	.22	-
	6.5	-	.25	.23	.25	.25
	7.5	-	.27	.24	.24	.25
	8.6	-	-	.27	.25	.25
B	3.0	.25	.19	.17	-	-
	4.9	.27	.30	.26	.27	.27
	6.5	.29	.28	.31	.28	.24
	7.5	.28	.26	.27	.27	.28
	8.6	.23	.29	.30	.30	.38
C	3.0	.18	.16	.17	-	-
	4.9	.23	.27	.25	.27	.26
	6.5	.21	.26	.35	.34	.30
	7.5	.35	.35	.34	.37	.35
	8.6	.27	.31	.32	.34	.32

Agar gels used were at approximately one percent concentration and at a temperature of 20° C. The diameter of the plunger was 1.1 cm. and the rate of application of force approximately 50 gm. per second. All values are averages of from four to eight determinations.

Table 2. The strength of agar gels of graded diameters as determined with plungers of graded diameters.

Agar sample	A			B			C		
Diameter of plunger in centimeters	0.8	1.1	1.4	0.8	1.1	1.4	0.8	1.1	1.4
Diameter of container in centimeters	Breaking load in grams:								
3.0	77	153	215	116	250	342	175	280	497
4.9	86	151	227	163	248	353	207	390	598
6.5	82	150	223	159	267	377	224	385	574
7.5	88	169	234	123	257	395	223	396	602
8.6	95	184	275	135	252	308	212	430	570
Average:	88	159	235	145	255	354	217	397	553
Average breaking load in grams per cm. of plunger circumference:	35	46	53	58	74	80	87	115	125
Diameter of container in centimeters	Breaking strain in centimeters:								
3.0	.25	.29	.25	.53	.54	.44	.43	.43	.38
4.9	.31	.32	.38	.83	.66	.70	.54	.69	.61
6.5	.37	.43	.48	.83	.84	.94	.68	.76	.79
7.5	.41	.45	.49	.64	.90	.99	.73	.78	.84
8.6	.46	.56	.57	.73	.81	.76	.73	.86	.84
Average:	.41	.51	.51	.74	.85	.89	.71	.79	.82
Diameter of container in centimeters	Elastic modulus in centimeters per 100 grams:								
3.0	.30	.16	.10	.43	.18	.11	.25	.14	.07
4.9	.36	.21	.16	.47	.22	.17	.25	.17	.10
6.5	.45	.28	.20	.49	.29	.23	.29	.20	.14
7.5	.44	.26	.20	.47	.32	.23	.33	.19	.14
8.6	.46	.25	.21	.53	.31	.24	.29	.18	.15
Average:	.45	.26	.20	.50	.30	.23	.30	.18	.14
Average modulus in centimeters per 100 grams per centimeter of plunger circumference:	1.13	.90	.88	1.26	1.04	1.01	.75	.62	.62

Agar gels used were at approximately one percent concentration and at a temperature of 20° C. The force was applied at a rate of approximately 50 grams per second. All values are the averages of two determinations.

Table 3. The strength of agar gels as determined with plungers of graded diameters.

Agar sample	A			B			C		
Diameter of plunger in centimeters	1.1	1.4	1.7	1.1	1.4	1.7	1.1	1.4	1.7
Breaking load in grams:	271	346	428	114	175	209	177	266	318
Breaking load in grams per cm. of plunger circumference:	79	79	80	33	40	39	51	60	60
Breaking strain in centimeters:	.52	.60	.64	.59	.58	.57	.40	.43	.45
Elastic modulus in cm. per 100 gms:	.19	.15	.14	.42	.30	.25	.20	.16	.15
Elastic modulus in centimeters per 100 gms. per cm. of plunger circumference:	.66	.66	.75	1.45	1.32	1.34	.69	.71	.80

Agar gels used were at approximately one percent concentration and at a temperature of 20° C. The force was applied at a rate of approximately 50 gms. per second. All values are the averages of four determinations.

Table 4. The strength of agar gels as determined with plungers of graded diameters used at graded rates of application of force.

Agar sample	A		B		C	
Diameter of plunger in centimeters	1.1	1.4	1.1	1.4	1.1	1.4
Rate of application of force in grams per second	Breaking load in grams per centimeter of plunger circumference:					
34	55	64	93	106	125	141
58	63	68	97	104	131	142
100	68	75	101	110	143	148
	Breaking strain in centimeters:					
34	.48	.54	.76	.83	.77	.73
58	.50	.53	.69	.79	.73	.79
100	.53	.57	.79	.81	.72	.77
	Elastic modulus in centimeters per 100 grams per centimeter of plunger circumference:					
34	.82	.81	.75	.75	.54	.49
58	.78	.74	.60	.70	.52	.50
100	.75	.74	.67	.69	.50	.47

Agar gels used were at approximately one percent concentration and at a temperature of 20° C. All values are the averages of three determinations.

Table 5. The strength of agar gels cooled at different rates.

Agar sample	A	B	C
Average rate of temperature. Decrease in degrees C. per minute.		Breaking load in grams:	
.05	555	333	321
.18	577	389	333
.43	557	338	280
		Breaking strain in centimeters:	
.05	.73	.51	.67
.18	.65	.54	.67
.43	.65	.48	.58
		Elastic modulus in centimeters per 100 grams:	
.05	.12	.15	.20
.18	.11	.13	.19
.43	.11	.13	.20

Agar gels used were at approximately one percent concentration and at a temperature of 20° C. The force was applied with a 1.1 cm. diameter plunger at a rate of approximately 50 gm. per second. All the values are the averages of six determinations.

Table 6. The strength of agar gels determined at different temperatures.

Agar sample	A	B	C
Temperature in degrees C.		Breaking load in grams:	
20	170	181	215
25	156	168	199
30	142	171	196
35	131	142	174
		Breaking strain in centimeters:	
20	.51	.68	.52
25	.50	.65	.55
30	.45	.66	.52
35	.45	.59	.47
		Elastic modulus in centimeters per 100 grams:	
20	.28	.34	.23
25	.30	.35	.25
30	.29	.35	.26
35	.31	.35	.26

Agar gels used were at approximately one percent concentration. The force was applied with a plunger of 1.1 cm. diameter at a rate of approximately 50 gms. per second. All values are the averages of six determinations.

Table 7. The strength of agar gels aged for different lengths of time.

Agar sample	A			B			C		
Holding temperature in degrees C.	10	20	30	10	20	30	10	20	30
Length of storage in hours				Breaking load in grams:					
2	170	135	-	122	135	-	158	158	-
3	-	-	130	-	-	186	-	-	155
4	162	149	-	128	158	-	162	172	-
6	174	161	138	136	152	191	172	175	170
10	181	161	138	135	170	190	165	167	183
22	-	150	138	-	172	185	-	186	182
28	-	166	134	-	-	-	-	-	-
				Breaking strain in centimeters:					
2	.52	.45	-	.54	.55	-	.37	.43	-
3	-	-	.43	-	-	.77	-	-	.46
4	.51	.47	-	.57	.63	-	.37	.44	-
6	.51	.51	.43	.63	.65	.78	.39	.45	.48
10	.55	.52	.44	.64	.74	.78	.37	.39	.52
22	-	.48	.45	-	.76	.78	-	.45	.51
28	-	.55	.43	-	-	-	-	-	-
				Elastic modulus in centimeters 100 grams:					
2	.27	.30	-	.39	.36	-	.22	.25	-
3	-	-	.30	-	-	.37	-	-	.27
4	.27	.29	-	.41	.36	-	.21	.24	-
6	.26	.30	.30	.42	.37	.38	.22	.24	.26
10	.27	.30	.29	.42	.40	.37	.21	.21	.26
22	-	.29	.29	-	.42	.38	-	.22	.26
28	-	.30	.29	-	-	-	-	-	-

Agar gels used were at approximately one percent concentration, and at the same temperature as that at which they were stored. The force was applied with a plunger of 1.1 cm. diameter at a rate of approximately 50 gms. per second. All values are the averages of six determinations.

Table 8. The strength of agar gels aged at different temperatures.

Agar sample	A	B	C
Temperature in degrees C.		Breaking load in grams:	
10	435	453	462
20	486	430	475
30	462	453	442
		Breaking strain in centimeters:	
10	.52	.48	.59
20	.61	.48	.62
30	.59	.62	.61
		Elastic modulus in centimeters per 100 grams:	
10	.11	.10	.11
20	.12	.11	.11
30	.13	.11	.12

Agar gels used were at approximately one percent concentration and at a temperature of 30° C. The force was applied with a plunger of 1.1 cm. diameter at a rate of approximately 50 gms. per second. All values are the averages of six determinations.

Table 9. The strength of agar gels at different pH values.

Agar sample	A	B	C
pH of agar gel	Breaking load in grams:		
4.0	177	172	282
5.0	157	175	296
6.0	176	170	277
7.5	172	168	287
	Breaking strain in centimeters:		
4.0	.87	.66	.77
5.0	.83	.64	.77
6.0	.85	.66	.79
7.5	.87	.65	.78
	Elastic modulus in centimeters per 100 grams:		
4.0	.42	.32	.25
5.0	.46	.33	.24
6.0	.43	.34	.25
7.5	.46	.31	.24

Agar gels used were at approximately one percent concentration and at a temperature of 30° C. The force was applied with a plunger of 1.1 cms. diameter at a rate of approximately 50 gms. per second. All values are the averages of six determinations.

Table 10. The strength of agar gels containing added solutes.

Agar sample	A	B	C
Solute	Breaking load in grams:		
Cane sugar at 2 percent concentration:	128	85	250
Control:	148	81	255
Bacto-peptone at 1 percent concentration:	240	112	319
Control:	230	112	334
Calcium acetate at 1 percent concentration:	257	196	366
Control:	215	177	299
	Breaking strain in centimeters:		
Cane sugar at 2 percent concentration:	.54	.80	.68
Control:	.61	.75	.65
Bacto-peptone at 1 percent concentration:	.68	.70	.62
Control:	.64	.76	.66
Calcium acetate at 1 percent concentration:	.61	.63	.71
Control:	.62	.81	.64
	Elastic modulus in centimeters per 100 grams:		
Cane sugar at 2 percent concentration:	.38	.82	.24
Control:	.36	.76	.22
Bacto-peptone at 1 percent concentration:	.26	.53	.18
Control:	.24	.60	.18
Calcium acetate at 1 percent concentration:	.20	.29	.18
Control:	.25	.41	.19

Agar gels used were at approximately one percent concentration and at a temperature of 30° C. The force was applied with a plunger of 1.1 cm. diameter at a rate of approximately 50 gms. per second. All values are the averages of four determinations.

Table 11. The strength of agar gels of graded concentration.

Agar sample	A	B	C	D	E	F	G	H	I
Concentration in percent by weight		Breaking load in grams:							
0.50	21	176	54	52	62	108	44	85	45
1.00	192	539	244	325	222	478	274	400	203
1.25	-	-	-	-	-	525	384	521	307
1.50	330	818	448	576	477	728	498	604	407
2.00	532	1044	577	742	613	1003	638	996	562
		Breaking strain in centimeters:							
							Average		
0.50	.51	.93	.78	.62	.68	-	.70	-	-
1.00	.54	.70	.65	.62	.59		.62		
1.50	.48	.50	.51	.56	.53		.52		
2.00	.41	.44	.44	.45	.43		.43		
		Elastic modulus in centimeters per 100 grams:							
0.50	2.04	.45	1.11	.91	.91				
1.00	.24	.12	.24	.17	.23				
1.50	.13	.06	.11	.09	.11				
2.00	.07	.04	.07	.05	.06				

Agar gels used had been stored overnight and adjusted to a temperature of 30° C. The force was applied with a plunger of 1.1 cm. diameter at a rate of approximately 50 gms. per second. All values are the averages of six determinations.

Table 12. Reproducibility of results.

Agar sample	A	B	C	D
Determination		Breaking load in grams:		
1	254	592	264	346
2	213	616	235	363
3	251	642	214	388
4	251	642	214	343
5	214	615	246	354
Average:	237	618	241	359
Standard error:	±10	±8	±8	±8
		Breaking strain in centimeters:		
1	.67	.65	.58	.66
2	.59	.68	.58	.68
3	.66	.67	.58	.68
4	.70	.73	.53	.63
Average:	.64	.69	.57	.66
Standard error:	±.02	±.02	±.01	±.01
		Elastic modulus in centimeters per 100 grams:		
1	.22	.10	.19	.17
2	.23	.09	.22	.16
3	.22	.10	.22	.15
4	.24	.10	.23	.16
Average:	.23	.10	.21	.16
Standard error:	±.006	±.000	±.007	±.001

Agar gels used were at 1.00 percent concentration and at a temperature of 30° C. after overnight storage. The force was applied with a plunger of 1.1 cm. diameter at a rate of approximately 50 gms. per second. All values are the averages of six determinations