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ON THE ETHEREAL SULPHATE, THE ESSENTIAL CONSTITUENT OF AGAR-AGAR

By

E. Takahashi & K. Shirahama

The principal constituent of agar-agar is known as "Gelose" or "δ-galactan." In former days it was believed to be similar to pectin (Payen(19), Marpmann(15)), or Gum (Morin(16), Biot and Persoz(2)), or sometimes as a substance like lichenin or tunisin (Poranburn(20)). As it yields a great deal of galactose by hydrolysis (Greenish(7), Bauer(3), Gran(8), König and Bettels(11), Tollens(25), Takao(26), Matsui(17), Furuichi(5), Lüdtke(14), Takahashi(27)) it is regarded as a galactan by many scholars. Further, the presence of pentose such as arabinose or xylose (Reichardt(21), Czapek(4), Seber(23), Takao(26), Matsui(17), Furuichi(5)), fructose (Takao(26), Matsui(17), Furuichi(5)), amidon (Rouvier(22)) and uronic acid (Furuichi(5)) has been reported. Lüdtke(14) recently remarked the presence of a reducing substance other than fructose, and an organic acid other than laevulinic acid or uronic acid. Haas(9), investigating the quantitative relation of calcium and sulphuric acid in sea-weed, gave the explanation that sulphuric acid in sea-weed occurs in the form of ethereal sulphate.

Neuberg and Ohle(18) having detected the appearance of H_2SO_4 when agar-agar was subjected to hydrolysis, thought that the ether combination of sulphuric acid must probably be contained in agar. Samec and Isajevič(24) reported that the sulphuric ester in agar-agar would bear an important relation to its jelly formation similar to the action of the phosphoric combination in starch. Afterwards, by Fairbrother and Mastin(6) as well as Hoffman and Gortner(10), so called free agar acid was liberated, and they explained that agar is nothing more than a calcium salt of this organic acid. We have made further investigations on the nature of various intermediate products obtained by the partial hydrolysis of agar-agar.

[Jour. Facul. Agr., Hokkaido Imp. Univ., Sapporo, Vol. XXXV, Pt. 2, March, 1934]

I. Hot-water-hydrolysis of agar-agar.

SAMEC and ISAJEVIČ'S(24) experiments show that when agar-agar is mixed with water and heated under pressure it undergoes hydrolysis and separates sulphuric acid. FAIRBROTHER and MASTIN(6) demonstrated this decomposition by the following equation:

$$2H_2O + (R{\cdot}O{\cdot}SO_2{\cdot}O)_2Ca = 2ROH + CaSO_4 + H_2SO_4$$

(A) Solubility of agar in hot water.

To test the solubility of agar-agar, 1 g of square-agar was put in different quantities of hot water at 90°, and, stirring frequently, its dissolving condition was observed.

TABLE I.

Solubility of agar-agar in hot water.

Quantity of agar g	Quantity of hot water c.c.	Round concentration of the solution %	Time for Boiling	
			30 minutes	60 minutes
1	50	2	Not dissolved.	Almost not diss.
1	100	1	Almost not diss.	Diss. slightly.
1	200	0.5	Diss. slightly.	Alm. diss.
1	500	0.2	Alm. dissolved.	Dissolved.

The solubilities shown in the above table are the result of the observation of the dissolving condition of soft masses of agar-agar.

(B) Change of agar solution by boiling under normal pressure.

When one gram of square-agar is put in 500 c.c. of water and boiled for one hour at 90°, the agar dissolves completely, as is apparent in the above table.

We observed what change there occurs when such a solution is heated for a long time.

So we boiled further the solution for varying times and observed the colour reaction through a reagent which was added to a fixed quantity.

The result is as follows:

TABLE II.

Colour reaction of agar solution boiled with water.

Time of boiling (hours)	Fehling's test	Bial's orcin[1] test	Resorcin[2] test
1	—	—	—
3	—	—	—
5	—	—	+
8	—	—	+

Agar, it is understood, does not decompose through prolonged boiling with water at about 90°. By 5–8 hours' boiling we can observe a slight resorcin reaction. All solutions became jelly-like when they were cooled.

(C) Hydrolysis of agar-agar by superheating.

A certain quantity of square-agar was placed in Erlenmeyer's flask and heated with water in an autoclave. At about one hour after beginning heating it reached a temperature of 130°. After heating for the required time the burner was turned off and the solution left one hour for cooling. The cooled solution was poured into a measuring flask, filled to the mark with water and filtered, warming slightly. The test was made upon the clear solution.

The result is as in the following table.

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1) Biochem. Z., **3** 323.

2) B., **20** 181.

TABLE III.

Hydrolysis of agar-agar solution by superheating.

Quant. of agar (g)	Quant. of water (c.c.)	Time heated (hour)	Cond. of soln. after cooling	Viscosity[1] (second)	IKI test[2]	Acidity[3] (c.c.)	SO_4'' test[4]	Fehling's test	Resorcin test
0.4	200	1	Liquid	26.60	Yellow	0.98	Not clear	Slight	Slight
,,	,,	2	,,	20.50	Brownish-yellow	1.18	,,	,,	+
,,	,,	3	,,	20.00	,,	3.78	,,	,,	+++
,,	,,	5	,,	20.00	,,	4.80	,,	,,	++++
8.0	200	1	Liquid	26.18	Brownish-yellow	—	Not clear	Slight	++
,,	,,	2	,,	20.16	,,	—	,,	+	+++
,,	,,	3	,,	—	,,	—	,,	+++	++++
10.0	200	1	Jelly	—	Violet	—	+	+++	+++
,,	,,	2	Liquid but pptd. colloidal subst.	26.29	Faintly violet	—	+	++++	++++
,,	,,	3	Liquid	—	Yellow	—	+	++++	++++
,,	,,	4	,,	—	,,	—	+	+++++	+++++

1) 10 c.c. were taken and measured by Ostwald's Viscosimeter in water at 60° (The velocity of the flow of distilled water was 20″⅕).

2) Two drops of IKI-solution were added in 5 c.c.

3) 50 c.c. were titrated with N/100 NaOH. Phenolphthalein was used as indicator.

4) The decomposed solution of agar was left for one night. The upper clear solution was taken and tested with $BaCl_2$.

(D) Rate of hydrolysis of agar-solution by superheating.

1) Experiment on square-agar.

To 10 g of agar 200 c.c. of water were added and the solution heated at 130° in an autoclave for required time.

After cooling and filtering it was made up to 250 c.c. with water, and humas substance (I), viscosity (II), total reducing sugar (III), Aldose (IV), H_2SO_4 (V) and Hydrogen ion concentration (VI) were determined. The treatment was as follows:

I) Humas substance—The solution was taken out of the autoclave, filtered, washed with a small quantity of water, and dried at 115°.

II) Viscosity—The solution separated from the humas substance was brought up to 250 c.c. with 10 c.c. of this solution. Flowing time at 60° was measursd (T_1) and the ratio obtained for that of distilled water at the same temperature ($T_0 = 20'' 1/5$).

III) Reducing sugar—The reducing power was determined by Bertrand's method and calculated as galactose.

IV) Aldose—Ten c.c. of the solution was taken, 25 c.c. of N/10 IKI sol. and 25 c.c. of N/10 NaOH sol. were added. After having stopped stirring for 12 minutes the solution was neutralized with 25 c.c. of N/10 H_2SO_4 and titrated with N/10 $Na_2S_2O_3$.

V) H_2SO_4—Fifty c.c. was taken and treated with $BaCl_2$ sol. by the usual method.

VI) pH—A chinhydron electrode was used, 10 c.c. of the solution being taken.

The result is shown in TABLE IV.

TABLE IV.

Rate of hydrolysis of sq. agar.

Time heated (hours)	(I) Humas sub. for 10 g of agar g	(II) Viscosity T_1/T_0	(III) Total reducing sugar to 100 g of agar g	(IV) Quant. of Aldose to 100 g of agar g	(V) H_2SO_4 for 50 c.c. of sample mg	(VI) pH
2	—	2.514	5	12	2.046	4.64
3	—	1.118	23	30	3.023	3.49
4	0.1482	1.109	23	21	5.629	3.29
5	0.1500	1.109	28	33	15.011	3.08
6	0.2244	1.030	50	39	30.651	2.80
7	0.3596	1.020	55	42	45.031	2.52
8	0.4640	1.009	55	43	62.158	2.04

The above results are illustrated in Fig. I.

2) *Experiment on other kinds of agar.*

For comparison, the same experiment was executed with Saghalien agar (S) and Merck's agar (M).

Reducing sugar, viscosity and sulphuric acid were measured as described above. The result is as in the following TABLE V. The peculiarities are seen clearly in Figs. 2–4.

TABLE V.

Rate of hydrolysis by superheating of various kinds of agar.

Time heated (hours)	Viscosity			For 100 g of agar (%)					
				Reducing sugar			H_2SO_4		
	sq.	M.	S.	sq.	M.	S.	sq.	M.	S.
2	2.51	—	—	5	—	—	0.10	—	—
3	1.12	1.83	4.04	23	12	5	0.15	0.22	0.03
4	1.11	1.25	1.70	23	19	13	0.26	0.37	0.07
5	1.11	1.20	1.40	28	30	28	0.75	0.59	0.08
6	1.03	1.15	1.18	50	44	28	1.53	0.67	0.13
7	1.02	1.07	1.12	55	51	29	2.25	0.85	0.18
8	1.03	1.00	1.09	55	51	42	3.11	0.96	0.24

Fig. 1.

Hot-water-hydrolysis of sq. agar.

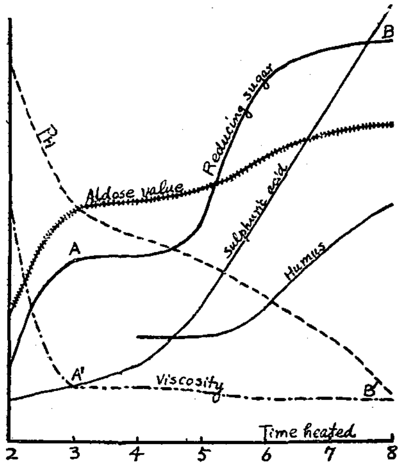


Fig. 2.

Viscosity.

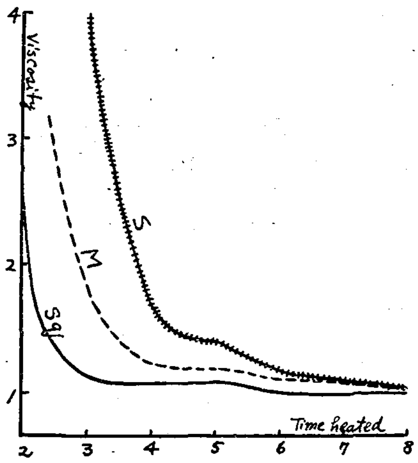


Fig. 3.

Reducing sugar.

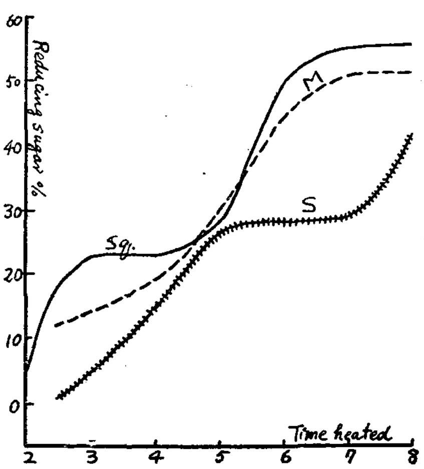
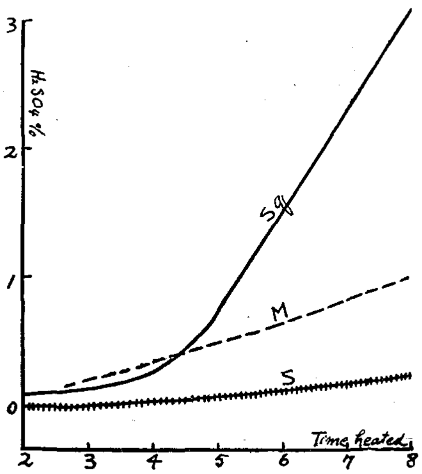


Fig. 4.

Sulphuric acid.



(E) Observations on the hydrolysis of agar.

It will be observed that when agar-agar is hydrolysed by heating under pressure the solution ceases to be coagulable after cooling, and its viscosity decreases remarkably. The longer it is heated the more hydrolysis advances. As seen in TABLE IV and Fig. 1 the free sulphuric acid and hydrogen ion concentration gradually increase. At the same time, an increase of reducing sugar occurs, attaining two maximum points in their courses.

If the first maximum point is denoted by *A* and the second by *B*, *A* corresponds to 3 hours after heating and *B* to 8 hours. Investigating the curve of viscosity, it is noticed that there are two minimum points. If the first minimum point is denoted by *A′* and the second by *B′*, *A′* and *B′* correspond respectively well with *A* and *B* regarding the heating time.

Now, if the maximum and minimum points are traced on the curve of reducing sugar and viscosity, the heating time of *A* and *A′* correspond almost at about 3, 4, 5, in the order of square, Merck's and Saghalien agar respectively. Though the relation of *B* and *B′* is not so clear as in the above case, it may nevertheless be held to provide an approximate agreement. It is to be noticed that the cause of the remarkable change of viscosity illustrated in TABLE III (10 g of agar was heated with 200 c.c. water) depends upon the appearance of a large quantity of jelly-like substance after about 2 hours' heating, by which time the solution becomes very viscid, though after 3 hours it becomes transparent without showing any jelly-like property.

The remarkable decrease of viscosity means the disappearance of such jelly-like substance. Further, in TABLE IV, we notice that the aldose value is higher than the reducing value during the first 5 hours. Curves appear similar to those seen in Fig. I, the maximum points being both equal. These points will be explained later.

II. Upon two kinds of hydrato* resulting from hot-water-hydrolysis.

(A) Separation of hydrato.

One kilogram of commercial square-agar was heated with 20 litres of water at 130° for 2 hours in an autoclave. It was filtered and

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*hydrato means partial hydrolytic product.

cooled, and the resultant white jelly-like precipitate was separated by centrifuge. The precipitate was then dissolved in a large quantity of warm water. After repeated treatment of cooling, precipitation and filtration, it was dehydrated by alcohol and dried in a desiccator. A light white powder was obtained. The yield was 250–260 g ... it is designated as " hydrato-kanten-δ."

To the filtrate from δ, 4–5 volumes of 95% alcohol were added. The resultant precipitate was separated and redissolved in water again precipitated as above, dehydrated finally by absolute alcohol and dried in a desiccator. A yellowish-brown powder was obtained. Its yield was 260-270 g ... it is designated as " hydrato-kanten-λ."

(B) Hydrato-kanten-δ.

This is a white tasteless amorphous powder, showing no constant melting-point, almost ash-free and insoluble in alcohol or ether. In water at room temperature it is hardly soluble but in hot water dissolves fairly rapidly and separates a sticky white precipitate on cooling. The precipitate separates easily from dilute solutions, but in concentrated solutions remains long in a glutinous condition. With iodine it changes to a reddish-brown colour, does not reduce Fehling's solution, and hydrolyses easily when treated with a hot mineral acid. The hydrolysed solution reduces Fehling's solution even at a low temperature. Seliwanoff's resorcin reaction is strong, but it fails to produce pentose and methylpentose reactions. Oxydation by nitric acid yields much mucic and oxalic acid. Uronic acid is absent. It appears to be a simple polysaccharide.

Minerals

Moisture	5.05%
Ash	0.8 % (Ca, 0.4%; Mg, 0.06%; SO_4, 0.38%)
SO_4	resulted by hydrolysis 0.82% (in δ)

Pentose and Methylpentose

The presence of pentose and methylpentose in the hydrolysed solution was tested by OSHIMA and KONDO's method, with a negative result. Further, 1.0906 g of δ was taken and distilled with HCl of 1.06 sp. gr. as usual.

To 400 c.c. of distillate, phloroglucin was added and left for 15 hours. The solution changed to a bluish dark colour. The precipitate was only 0.0002 g in weight. The presence of pentose and methylpentose was not probable.

Uronic acid

Naphthoresorcin reaction was negative. The result was as follows:

A {Ether layer—Yellow.
   Aqueous layer—Pale reddish brown.
B {Benzol layer—Yellow.
   Aqueous layer—Reddish brown.

By LEFÉVRE and TOLLENS's(33) method 0.622 g (Moisture 10.76%) sample was taken and the liberated CO_2 determined with DICKSON, OTTERSON and LINK's(34) equipment.

After 6 hours' heating 0.0308 g of CO_2 was measured, that is, 0.495% which is 0.59% for moisture free substance. The presence of uronic acid can be excluded.(35)

Rate of hydrolysis by dilute H_2SO_4.

One per cent solution of *δ* in sulphuric acid of varying concentration was made and heated at 80° for varying times. It was next neutralised with $CaCO_3$ and its total reducing power and aldose value were measured. The result was as follows:

TABLE VI.

Rate of hydrolysis of δ by H_2SO_4 of varying concentration.

Concentration of H_2SO_4 / Decomposition time (hours)	Reducing Value*				Aldose Value	
	0.1%	0.5%	2.5%	5%	2.5%	5%
1	17%	42% (a)	59%	53%	66%	64%
2	27	49	60	54	70	78
3	33	56 (b)	61	57	78	80
5	39	61	63	57	83	80
8	52	66	64	57	—	80

(a) and (b) reduce Fehlings solution in a cold place as follows:

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*Quantity as galactose for 100 g δ.

(a)=13%, that is 31%, for total reducing sugar (42%).
(b)=20%, that is 36%, for total reducing sugar (56%).

The maximum of total reducing sugar is 66% (70% for dry matter) and that of aldose is 83% (78% for dry matter). There is a difference of 19% visible between them.

The reducting value is highest with 8 hours' heating in 0.5% H_2SO_4; a decreasing tendency is seen in a more concentrated acid solution. Complete hydrolysis of δ may be performed in a 2–3% solution by heating for 3–5 hours at 80°.

Oxydation by HNO_3.

60 c.c. of HNO_3 of sp. gr. 1.15 were added to the sample and heated in a water-bath until evaporated to ⅓ of the original volume.

After 24 hours, 10 c.c. water was added; the resultant crystals were collected and washed with 70% alcohol.

Sample 1.4298 g (moisture 16.76%)
Mucic acid 0.3110 g
As galactose 0.3910 g × 1.33 = 0.52003 g
that is 39.37% (43.69% for mositure free δ).

In testing the crystal the naphthoresorcin reaction showed a distinct purple red colour at the benzol layer, but in the further purified sample, (The crude crystal is dissolved in the mixture solution in which $(NH_4)_2CO_3$ 1, conc. ammonia 1 and water 10 c.c. is mixed. A little HNO_3 is added and the mucic acid is reprecipitated), the above reaction disappeared. The melting point was then 205–207° which practically coincides with that of mucic acid.

According to TOLLENS[1], LIPMAN[2], KILIANI and SCHEIBLER[3], KÖHLER[4] and others, the melting point of mucic acid is 206–208° by heating slowly, and 212–215° by heating rapidly.

The nitric acid solution, the filtrate from the mucic acid was concentrated further to about 5 c.c. and put aside for 12 hours. Large colourless crystals appeared which were soluble in water and alcohol but not in glacial acetic acid. They were therefore washed with glacial

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1) B., **18** 26.
2) B., **20** 1004.
3) B., **22** 518.
4) N.Z., **24** 292.

acetic acid and dried over NaOH. They amounted to 0.3388 g, that is, 28.85% for moisture-free δ. In testing qualitatively with $AgNO_3$, it produces a white precipitate, soluble in HNO_3, NH_4OH. With lead acetate a white precipitate resulted, soluble in HNO_3. With $CaCl_2$ solution a white precipitate was also given which is insoluble in glacial acetic acid but soluble in HCl. With MnO_2 and H_2SO_4 it produced CO_2. These reactions agree well with that of oxalic acid.

The melting point of the crystal was 101°, but an anhydrous sample crystallised from alcohol melted at 189°. The elemental analysis was executed with the following results:—

Sample (Anhydride)...... 2.831 mg
Found CO_2 2.764 mg H_2O 0.745 mg
C = 26.62% H=2.94% O=70.44%
Calculated for $C_2H_2O_4$ C=26.67% H=2.22% O=71.11%
Sample (Ammonium salt)......4.590 mg; 4.77 mg
Found CO_2 3.294 mg; 3.403 mg H_2O 2.638 mg; 2.770 mg
C=19.57%; 19.45% H=6.43%; 6.49% N=22.65%
Ave. C=19.50% H=6.46% N=22.65% O=51.39%
Calculated for $C_2H_8O_4N_2$ C=19.35% H=6.45% N=22.58% O=51.62%

The obtained crystal can be nothing else than oxalic acid.

The appearance of oxalic acid by the oxydation of some sugar or organic acid with nitric acid has been known for a long time. MORIN⁽⁶⁾ reported that in the case of the oxydation of gelose with conc. HNO_3 oxalic acid is produced besides mucic acid. We took 1 g each of glucose, mannose and galactose treated under the same conditions as in the above experiment. But no crystals of oxalic acid were found. There remains for investigation the nature of the substance from which the acid is derived.

(C) Hydrato-Kanten-λ.

This is a yellowish-brown powder, and a fresh sample dissolves in water and dilute alcohol, making an opalescent brown solution. It is insoluble in ether and strong alcohol; and does not reduce Fehling's solution. It shows no reaction with iodine and is with great difficulty hydrolysed with dilute mineral acid compared with δ. But the hydrolysed solution reduces Fehling's solution in a cold place, showing also weak reactions for ketose, pentose and uronic acid, but none for methylpentose.

A point remarkably different from δ is that λ contains a large quantity of ash, a large part of which is calcium and sulphuric acid. The quantity of sulphate in ash is almost half that in a hydrolysed solution of λ.

In the electro-dialysis of the aqueous solution of λ only a little SO_4'' appears at the positive pole at the beginning of dialysis, but at the negative pole there is found a large amount of Ca and a small amount of Mg, Na and K. The dialysed solution then turns acid. From these facts it may be inferred that the greatest part of λ is present as the metal salt of an acid carbohydrate probably allied to "agar acid" such as separated by HOFFMAN and GORTNER. When the λ preparation preserved long in a desiccator, produces a substance insoluble in water amounting to 5–6%. This material contains a large quantity of ash (mostly SiO_2) and 10% uronic acid. From this result it is obvious that λ is not a simple substance.

Mineral component
  Moisture 17.75%
  Ash 5.16% (7.27% for dry matter)
  In 100 g ash Ca 28.33% SO_4 57.66%
  Mg 7.46% SiO_2 4.09 Na, K a little.

$$\text{In } \lambda \begin{cases} SO_4{}^{*} \text{ in hydrolysed solution......6.26\%} \\ SO_4 \text{ in ignited ash. ....2.98\%} \end{cases}$$

10 g λ was dissolved in 200 c.c. H_2O and dialysed electrically under 110 volts, 20–50 mA. for 20 hours.

Mineral ions appeared in both poles as follows:

Positive pole .... SO_4 in small quantity.
Negative pole............ Ca in large quantity and Mg, Na, K in small quantity.

The solution after dialysis was distinctly acid.

The rate of hydrolysis by dilute H_2SO_4.

One per cent solution of λ in H_2SO_4 of varying concentrations were heated at 80° for varying times as in the case of δ. It was next neutralised with $CaCO_3$ and its total reducing power and aldose value were determined. The result is shown in TABLE VII.

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*According to Haas and Russel-Wells's (Biochem. J., **17** 696 (1923)). Method: 1 g sample was heated with 40 c.c. conc. H_2SO_4 and 60 c.c. water, 6 hours on a sand bath.

TABLE VII.

Rate of hydrolysis of λ by dilute H_2SO_4.

Time hydrolysed (hours) / H_2SO_4 concentration	Reducing value.*				Aldose value.			
	1%	2%	3%	5%	1%	2%	3%	5%
1	33%	32%	35%	34%	28%	36%	39%	40%
2	33	34	37	40	34	38	42	45
3	32	37	41	41	39	43	47	47
5	36	41	44	49	39	47	51	56
8	38	46	52	51	44	52	57	58
10	40	47	**55**	**53**	48	53	59	**63**
12	—	—	52	49	—	—	**64**	60
14	—	—	54	47	—	—	63	60
17	—	—	53	46	—	—	—	58
20	—	—	50	45	—	—	62	61

The maximum reducing value is 55% (57% for dry matter) against 64% (78% for dry matter) of aldose value. A difference of just 9% is found between them. The concentration of H_2SO_4 and the time taken to acquire the maximum value of reduction or aldose are not comparable with δ. It required 10–12 hours in 3% H_2SO_4 in this case.

A high concentration of the acid, we see, increases the hydrolysis to some extent, as in the case of the above mentioned δ.

Oxydation by HNO_3.

As in the case of δ, mucic acid and oxalic acid were produced.

	(a)	(b)
Sample	1.9647 g	1.9806 g
Mucic acid	0.4983 g	0.4933 g
As galactose	33.73%	33.12%

Average 40.65 for dry matter.

	(a)	(b)
Oxalic acid	0.3451 g	0.3947 g
	19.93%	18.75%

Average 22.79% for dry matter.

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*A quantity as galactose in 100 g.

The crystals of mucic acid melted at 205° the crude crystals showed naphthoresorcin reaction as in the case of δ.

Pentose, methylpentose and uronic acid.

The distillate which was obtained from a 2 g sample according to the method of OSHIMA and KONDO was tested for orcin reaction. Five c.c. of HCl and a small amount of orcin solution were added to 5 c.c. of distillate and the coloration was observed with the following result:

a) Distillate—Yellow, changing to green.

b) Redistillate—Yellow, changing to blue after a few minutes. (a) indicates the presence of both furfurol and oxymethyl furfurol and (b) shows the presence of furfurol. Further, this redistillate gave the characteristic absorption spectrum of pentose.

As to the methylpentose, no characteristic reaction was obtained by the methods of ROSENTHALER(36) or OSHIMA and KONDO(30).

1.3900 g gave 0.0295 g of phloroglucid which corresponds to 2.8%, that is, 3.4% of its dry matter.

To test for uronic acid, 0.3717 g (H_2O 17.75%) was decomposed for 6 hours by LEFÈVRE and TOLLENS's method. The liberated CO_2 weighed 0.002332 g, that is, 0.76% of its dry matter. It is 3.40% as uronic acid lactone. In this experiment, as heating continues the remarkable effervescence of HCl solution and the separation of a resinous substance were observed. Such changes were not noticed in the case of δ.

Insoluble matter produced from λ.

When λ preserved for long time (for 6 months) was dissolved in water, a large quantity of brown insoluble matter was left. It dissolves gradually when treated with cold water for a long time, more rapidly with warm water.

By washing with water and alcohol it appeared as a white powder. The yield was 0.5–0.6 g, from 10 g, of crude material.

The substance contained much SiO_2 and a small amount of Ca and SO_4. Naphthoresorcin reaction was positive. The analysis gave the following results:

Ash:—Sample 788.0 mg ash 21.26 mg...... 26.99%
SiO_2 in soluble in HCl = 14.16 mg... 17.97%
CO_2:—Sample 59.90 mg

CO_2 0.96 mg (4 hours' heating) 1.88%
uronic acid lactone 7.52% that is 10.25% in ash free substance.

(D) Remarks.

Generally, agar-agar is considered to be a kind of hemicellulose filling up the cellular tissue and corresponds to pectin in its physiological function. Recently, ANDERSON(35) classified the hemicellulose from a chemical standpoint as follows:—

(a) Acid hemicellulose—Pectin, plant gums, Agar, etc.

(b) Polysaccharide hemicellulose — True hexosan and pentosan (Plant mucilages), etc.

Further, he stated that the fact that hemicelluloses are dissolved out of plant material by alkali might suggest that either they contain an acid group and are combined by it to some constituent (possibly the cellulose) of the plant material or that they are held in the plant by a glucosidic union. In either case alkali might break up the union and set free the hemicellulose. He drew attention to the fact that although all acid hemicelluloses so far studied have been found to contain a uronic acid, the fact that agar contains an acid not belonging to the uronic acid group would suggest that not all acid hemicelluloses contain uronic acids.

But so far as we are aware there have been, hitherto, few reports upon the constituent of the organic part of gelose. Gran, judging from the results of bacterial culture, presumed experimentally that the gelose must be composed from 2 kinds of carbohydrates: one producing a a violet colour with iodine and as nutriment by bacteria, and the other showing no colour reaction with the same reagent.

Samec and Isajevič conducted a colloidal research upon the sulphuric acid combination in agar, and detecting a similarity to the structure of starch, suggested the possible existence of some substances to be called "Agarose" and "Agaropectin" like "Amylose" and "Amylopection" present in starch.

Considering now the result of our experiment, three points are noticed in Chapter I, (E) (p. 108) as follows:

(1) By hot-water-hydrolysis the maximum points on the curves of reducing sugars agree well with the minimum points on their curves of viscosity regarding the heating time.

(2) When the viscosity of the hydrolised agar solution is greatly

reduced, a jelly-like substance appears in the *solution but afterwards* disappears, the solution turning opalescent.

(3) The aldose-value increases parallel to the reducing value until the first maximum point on the curves is reached and the aldose-value is markedly higher than the reducing value.

In the next experiment, as described in Chapter II, two kinds of hydrolytic product, " Hydrato-Kanten-δ " and " -λ " are separated by hot water-hydrolysis. δ is the jelly-like substance mentioned above (2), it being very easily decomposed and has an aldose-value higher than that of reducing sugar (a difference above 20%).

λ is not so easily hydrolysed and not such a simple polysaccharide as δ which is probably composed of pure carbohydrates. And the difference (about 9%) of the aldose and reducing values is smaller than that of δ.

λ contains a large quantity of ash, principally constituted from $CaSO_4$. The amount of SO_4 is equal to about half the amount in its hydrolysed solution. This fact leads one to suppose that a substance *having an ethereal sulphate may exist in* λ.

Considered from the relation between the three notes mentioned above and the nature of the two hydrolytic products, agar seems to be hydrolised principally into two components. The following assumption can be made:

$$R-R_1-SO_4-M \qquad \text{(M takes metals principally Ca)}$$

If R takes δ, the ethereal sulphate ($-R_1-SO_4-M$) may exist in λ and the union between them may be broken up by hot-water-hydrolysis. The nature of δ (R-), just described above in (2) and (3) and note (1) justifies the following facts: the first maximum curve composed of the reducing values represents a hydrolysable nature of R, and the second maximum curve is due to the compound in λ (which contains) $R'-SO_4-M$, which is contained in λ representing the nature of λ which is not easily hydrolysable.

Agar is then to be considered as a calcium salt of an acid ($R-R_1-SO_4H$) *which may* correspond to the so-called " Agar acid " reported by HOFFMAN and GORTNER. But it is to be noticed that the power of jelly formation depends upon the union between R and R_1, and not upon a splitting of the sulphate union such as reported by SAMAC and ISAJEVIČ.

III. Electro-dialysis of Hydrato-Kanten-λ.

(Isolation of the free acid $R'-SO_4H$)

When Hydrato-Kanten-λ is treated with water, it dissolves to a dark brown solution, a small amount of insoluble matter remaining. The clear filtered solution was poured into a Pauli's apparatus, and a moderate current passed through, using parchment paper as dialysing membrane. The dark solution fading to pale brown, no coloration of water was observed on either side.

A test was made of both these solutions which had been previously somewhat condensed.

Positive side—Though colourless at the beginning, the solution gradually changes to brown with evaporation; the reaction for SO_4 is positive; it reduces Fehling's solution; the resorcin-HCl reaction for ketose is positive; the reactions for pentose (phloroglucin, orcin reactions) and methylpentose (aceton-HCl reaction) were negative, naphthoresorcin-HCl reaction for uronic acid was as follows:

$$\begin{cases}\text{Ether layer (Reddish brown)}\\\text{Aqueous layer (yellowish green)}\end{cases}$$
$$\begin{cases}\text{Benzol layer (Pale pink—colourless)}\\\text{Aqueous layer (Yellowish green)}\end{cases}$$

Uronic acid is hardly detectable. Moreover, the alcohol solution of the precipitate produced by the reaction has a reddish brown colour and shows the ketose reaction.

Negative side—Colourless by evaporation; the reactions for $Ca^{\cdot\cdot}$ $Mg^{\cdot\cdot}$ $Na^{\cdot}$ and $K^{\cdot}$ were positive, and that for $Ca^{\cdot\cdot}$ most remarkable; it does not reduce Fehling's solution.

(A) Aqueous solution after electro-dialysis.

After electro-dialysis the solution is generally clear and yellowish-brown, but sometimes separates a white colloidal precipitate.

This precipitate seems to be related to insoluble matter in the λ fraction.

The λ solution which was turbid owing to insufficient filtration and the λ solution which was left for a long time and the precipitate produced once redissolved, both separate the precipitate in the course of dialysis.

The precipitate deposited on the bottom of the vessel (obtained by passing a current of 110 V and 50–100 m. Amp. for 25 hours using a 5% water solution), if it is once stirred does not deposit again easily, and is very difficult to separate a clear solution in any way.

But by adding 6–7 volumes of 99% alcohol, the turbid substance can be completely precipitated. It was separated, dried in a desiccator, and appeared as a white powder. Its yield was scanty and indefinite. It contained 2–3% ash, and had no uronic acid reaction. It is considered to be probably the carbohydrate mixed in λ.

Next will be described the nature of the clear dialysed solution without precipitate.

(1) General nature of dialysed solution.

10 grams of λ were dissolved in 200 c.c. of water, decanted and filtered after the precipitate was deposited. The filtrate was dialysed for 18 hours in 110 V, 50–100 m. Amp. The solution produced no more colloidal precipitates and showed a clear yellowish brown colour.

Reaction for the solution: The solution was strong by acid; it reduces FEHLING's solution slightly; the resorcin-HCl reaction for ketose was positive; the naphthoresorcin-HCl reaction for uronic acid was negative, the 95% alcohol solution of the precipitate which was produced in this case was reddish brown in colour, and the reaction for ketose was positive. Moreover, though pentose reaction was seen, methylpentose was not observed.

Acidity by titration:—5 c.c. of the solution required 0.85 c.c. of n/10 NaOH solution. The quantity of SO_4 corresponding to NaOH titre needed for 1 litre of the solution is 0.833 gram. It is calculated as SO_4 of—SO_4H form.

The quantity of SO_4 was investigated with 10 c.c. of the solution by HAAS's hydrolytic method. It was 1.060 g in 1 litre. It closely approaches acidity by titration.

pH value:—Measured by the chinhydron electrode. It showed 1.2–2.0.

Reducing power:—5 c.c. of the solution produced a small amount of copper oxide with FEHLING's solution.

Change on heating:—The reducing power of the solution heated to boiling point for 2–3 minutes was almost equal to that of the untreated solution, but prolonged heating increased the reduction.

Rotatory power:—20 c.c. of the solution was evaporated at 105–

110°, a dark residue being left. Its yield was 0.2839 g. Specific rotatory power was measured with a 10 cm tube.

$$[\alpha]_D = \frac{-0.33 \times 100}{1 \times 1.9195} = -23.2°$$

Ash:—The ash from the above residue showed the reaction of $Ca^{\cdot\cdot}$ $K^{\cdot}$ and SO_4''.

(2) Physical nature of dialysed solution.

After dialysis, the solution was of a colloidal nature. Neutralisation with alkali changes its colour and viscosity. The addition of salts after neutralisation causes further change of colour.

(a) Original sol. diluted with H_2O......Pale yellow, 60″ 1/5 (100)

(b) Neutralised with NaOH and made to the same quantity...... Dark brown, 55″1/5 (70)

(c) Neutralised with $Ba(OH)_2$ and made to the same quantity...... Brown yellow, 64″ (105)

(d) Neutralised with NaOH and made to the same quantity...... Pale or yellow, 71″.0 (116)

(e) Neutralised with $Ca(OH)_2$ and made to the same quantity...... Dark brown.

$$\left.\begin{array}{lll} \text{(a)} & +FeCl_3\ (1\%)\ 0.2\ \text{c.c.}\ \ldots\ldots\ldots\ldots & 63'' \\ \text{(b)} & +FeCl_3\ (1\%)\ 0.25\ \text{c.c.}\ \ldots\ldots\ldots\ldots & 62'' \\ & \text{,,}\quad\text{,,}\qquad 0.5\ \text{c.c.}\ \ldots\ldots\ldots\ldots & 62'' \end{array}\right\}\text{Relative viscosity.}$$

(3) Precipitation of the free acid or its salts.

When a small quantity of different kinds of salt were added to the dialysed solution, followed by 4–5 volumes of 99% alcohol, the free acid was precipitated easily.

The results are shown as follows:—

Salts	Colour of the solution	Addition of alcohol	Colour of precipitate dried
K_2CO_3	Brown.	Precipitate at once.	Reddish brown.
Na_2CO_3		Upper sol turbid.	
$(NH_4)_2CO_3$	Light brown.		
$MgSO_4$	Brown.		
$CuSO_4$	,,	Ppt. after one night.	
$MnSO_4$	,,	Ppt. at once.	Yellowish brown.

Salts	Colour of the solution	Addition of alcohol	Colour of precipitate dried
$CaCl_2$	Brown.	Ppt. at once.	Yellowish brown.
$BaCl_2$	,,	,,	
$HgCl_2$	White.	Ppt. after one night.	
$CdCl_2$	Brown.	Ppt. at once.	Yellowish brown.
$AlCl_3$	,,	,,	
$FeCl_2$	,,	,,	Gray.
$FeCl_3$	White, slightly.	Ppt. rapidly. (upper sol. clear.)	Turns red by sun light.
NH_4Cl	Yellowish white.	Ppt. at once.	White.
Brucin		No ppt.	......
Cinchonin		,,	......

Precipitation from the solution neutralised by titration with alkalies is as follows:

Neutralised sol. by NaOH	6–7 volumes of 99% alcohol added	Clear.
	Little NH_4Cl sol. and 5 vol. of alcohol	Ppt.
	Little $CaCl_2$ sol. and 5 vol. of alcohol	Ppt. rapidly.
Neutralised sol. by NH_4OH.	10 volumes of 99% alcohol added	Slightly Turbid.
	Little NH_4Cl sol. and 5 vol. of alcohol	Ppt. at once.
	Little Fe_2Cl_6 sol. and 5 vol. of alcohol	Ppt. at once.
	Little $CaCl_2$ sol. and 5 vol. of alcohol	Ppt. at once.
Neutralised sol. by $Ba(OH)_2$	5 volumes of 99% alcohol added	Ppt. at once.

By treating as above with small quantities of salts or by adding alcohol after neutralisation, the acid can be easily precipitated, but the separation of the acid, treating only by solvents, showed extreme difficulties.

It was impossible to form a precipitate by only one kind of solvent added in different proportions. By adding 10 volumes of 99% alcohol it turns turbid, but it was still unchanged a in cold place, even after 2 months.

Other solvents, methyl and butyl alcohols, ether, acetone, benzene and chloroform, all hand no effect. By investigating with mixed solvents in different proportions, the following two methods were found to give the best results and they produced pure white preparations.

Preparation (I).

10 volumes of 99% ethyl alcohol was added to the solution, concentrated to 4–5 c.c. at 40° under diminished pressure. Next the solution was poured into 300 c.c. of 99% alcohol, and an equal volume of ether added, followed by the further addition of two volumes of benzene. A large amount of flocculent precipitate appeared, leaving the upper solution clear. The precipitate was washed with alcohol and dried in a desiccator. It was obtained in the form of a white powder.

Preparation (II).

4 volumes of 99% alcohol and an equal volume of ether were added to the solution, followed by 25 volumes of benzene. The mixture was concentrated to about double the original volume at 40–50° and 3–4 volumes of ether were added, by which a large quantity of white precipitate was obtained. This was washed with alcohol and dried in a desiccator. It appeared as a white powder.

(B) Isolation of the free acid and its composition.

The results of the analyses made of the preparations obtained in different ways are described later. CO_2 was measured by the method of DICKSON, OTTERSON and LINK. SO_4 was measured by HAAS's hydrolytic method, galactose by mucic acid, and pentose determined by OSHIMA and KONDO's method.

Preparation 1. (Control, Hydrato-Kanten-λ)

Ash$=6.27\%$; $CO_2=0.76\%$; $SO_4=6.26\%$
Galactose$=40.65\%$; Pentose$=3.4\%$

Preparation 2.

10 g of sample was dissolved in 200 c.c. of water and the insoluble matter filtered. The filtrate was dialysed by a current of 110 V and 2.5 m. Amp. for 48 hours.

After dialysis the upper solution was clear and yellowish brown, and separated a small amount of white colloidal precipitate. The clear and turbid parts were separated through a syphon.

Clear part.

A small amount of NH_4Cl solution and 5 volumes of 99% alcohol were added. The precipitate produced was washed with alcohol and dried. It was in the form of a white powder and 2 grams in weight.

Ash 5.85% ($Ca=2.65\%$; $SO_4=3.0\%$, as it is 5.67% as $CaSO_4$, the greater part of the ash must be $CaSO_4$)

Turbid part.

A precipitate was made, adding 5 volumes of 99% alcohol to the solution. It amounted to 1.5 g. (The greater part of this seems to be Ca Salt mixed with a little colloidal precipitate)

Ash=3.05% ; $CO_2=0.62\%$

Naphthoresorcin-HCl reaction for uronic acid is negative.

Preparation 3.

5 g of λ was dissolved in 200 c.c. of H_2O. After the insoluble matter was excluded a current of 110 V. 10 m. Amp. was passed through for 24 hours.

Removing the clear upper solution by a syphon, a precipitate was made with NH_4Cl and alcohol as above. The yield was 2 g.

Ash=3.68% ; $CO_2=0.8\%$

The reactions of $Ca^{\cdot\cdot}$ SO_4'' $K^{\cdot}$ $Na^{\cdot}$ were positive.

Preparation 4.

15 grams of λ were dissolved in 200 c.c. of water and filtered. The filtrate was dialysed by 110 V. 50–100 m. Amp. for 30 hours.

The solution divided into a clear upper part and a lower turbid part. After a small amount of NH_4Cl was added to them, 99% alcohol was added up to 5 times of their volumes, by which 3 grams of precipitate from the clear solution were obtained and 1.5 g from the turbid solution. The analytical result of the substance obtained from the clear solution is as follows :—

$SO_4=8.36\%$; Galactose=39.47% ; Pentose=8.17%

Preparation 5.

10 g of λ was treated the same as before. (In this case the turbid part did not appear)

Ash=0.80%; $CO_2=0.56\%$; $SO_4=8.12\%$

$$[\alpha]_D=\frac{-0.28\times 100}{0.9503\times 1}=-29.40^\circ$$

The aqueous solution gives an acid reaction. In titrating the solution, 1.56 g of NaOH was necessary for 100 g of moisture-free preparation. Calculating as corresponding SO_4 which is in combination as $R{\cdot}SO_4{\cdot}Na$, it becomes 3.18 g (or %).

Next the N of NH_4 form was determined to be 0.81%. Calculating also as corresponding SO_4 which is in combination as $R{\cdot}SO_4{\cdot}NH_4$ it becomes 5.55%.

SO_4 by titrating with n/10 NaOH ....=3.18%
SO_4 supposed to be in combination with NH_4=5.55%
Total 8.73%

The above total is very close to the total quantity of SO_4=8.72%.

Preparation 6.

The clear solution which was obtained in the same way as above was neutralised, titrating with NH_4OH and adding a small amount of NH_4Cl, followed by the addition of 5 volumes of 99% alcohol, by which a precipitate resulted.

Ash=0.77%; SO_4=7.66%

Next the acidity was determined as in the above experiment.

The amount of NaOH for neutralising 100 grams of the preparation was 0.36 gram. The quantity of SO_4 corresponding to this is 0.86 gram (or %). On the other hand, nitrogen of the NH_4 form was 0.9%. Calculating the quantity of SO_4 combining with NH_4, it is 6.17%.

SO_4 by titrating with $^1/_{10}$ n NaOH ....=0.86%
SO_4 supposed to be in combination with NH_4 =6.17%
Total 7.03%

Though a small quantity of SO_4 is present in ash, it shows very close figures for the total SO_4=7.66%. It is practically an identical result with the one recorded above. However, it is worthy of notice that notwithstanding the fact that it was precipitated after neutralisation with NH_4OH and treated with NH_4Cl, the preparation still showed slight acidity.

Preparation 7.

The dialysed solution obtained in almost the same way as above was neutralised, titrating with baryta solution, and precipitated by adding 5 volumes of 99% alcohol. After desiccation the preparation was hydrolysed with HCl and the precipitated $BaSO_4$ was determined. From

80.04 mg (dry matter) sample, 0.27 mg $BaSO_4$ was produced, that is, 0.11 mg (0.138%) as SO_4.

Next, to the filtrate of the hydrolysed solution, $BaCl_2$ was added and $BaSO_4$ measured as above. It was 16.83 mg $BaSO_4$, 6.925 mg (8.66%) as SO_4. The quantity of SO_4 which is produced by hydrolysis seems comparatively much greater in this case than in the case of the above sample, notwithstanding that they were neutralised previously.

Preparation 8.

10 grams of λ were dissolved in 200 c.c. of water. After filtration a current of 110 V. 50–100 m. Amp. was passed through for 35 hours. The preparation was made by alcohol after treating with NH_4Cl solution. The yield was 1 gram.

Ash=trace (Reaction of $K^{\cdot}$ and SO_4'' was recognized slightly)

$SO_4=6.90\%$

$$[\alpha]_D=\frac{-0.53\times 100}{1.7062\times 1}=-31.0^\circ$$

The quantity of NaOH required for 100 grams of the preparation = 1.05 gram; corresponding above $SO_4=2.52$ gram (or %). $N=1.27\%$; respective $SO_4=4.36\%$; total=6.88%.

The quantity of SO_4 calculated from the titrating acidity and nitrogen amount of the NH_4 form is 6.88%. It is fairly near to the total $SO_4=6.90\%$.

The yield of the preparation was comparatively small, but it was a pure white powder.

Preparation 9.

10 g λ was dissolved in 200 c.c. of water and dialysed by 110 V. 50–100 m. Amp. for 30 hours. By the method described above in [A] (3) Preparation (II) it was separated as a free acid. Though the preparation is at first white, it turns brown after long preservation.

Ash=0.59%; $SO_4=6.62\%$

$$[\alpha]_D=\frac{-0.54\times 100}{1.75\times 1}=-30.8^\circ$$

Titrating acidity for 100 gram Sample: NaOH=3.13 gram; respective $SO_4=7.52\%$.

It is worthy of notice that the amount of the total SO_4 is somewhat lower than titrating acidity in this case.

When the preparation was dried by heat it turned from a gray colour to dark brown and showed the following properties:

$$[\alpha]_D = \frac{-0.07 \times 100}{1 \times 1} = -7^\circ$$

$SO_4 = 6.26\%$

Titrating acidity for 100 g sample:

$NaOH = 4.0$ g respective $SO_4 = 9.6$ g (or %).

That the amount of SO_4 calculated from titrating acidity is greater than the total SO_4 is the more remarkable when compared with the sample not dried. It is probable that in free acid the combination of SO_4, being unstable, partially separated sulphuric acid attacks secondary the carbohydrate by which doubles its titrating acidity.

Colour reaction for free acid:—

The colour reaction was investigated of the preparations 4, 5 and 7 which were precipitated by NH_4Cl and alcohol, and of preparation 9 which was precipitated only through a solvent.

Reduction:—Free acid by heating with Fehling's solution: a newly prepared sample reduces the solution slightly but the preservation or heating of the sample increases its reducing power. The ammonium-salt of the acid (preparations 4, 5 and 7) had no reducing power.

Reaction for ketose:—Resorcin-HCl reaction was observed in all samples.

Reaction for pentose:—A Furfurol distillation was made by Oshima and Kondo's method and the reaction was observed of the primary (I) and secondary (II) distillates.

Phlorglucin-HCl	I.	Yellow—dark red.
	II.	Yellow—dark blue.
Resorcin-HCl	I.	Faintly red.
	II.	Faintly blue.
Orcin-HCl	I.	Reddish purple.
	II.	Bluish purple.
Anilin acetate	I.	Slight red.
	II.	Red.

Pentose reactions were observed all in II solutions.

Reaction for Methylpentose:—Aceton-HCl reaction was not observed.

Reaction for uronic acid:—Naphthoresorcin-HCl reaction was not seen in any of these preparations.

Ether layer (yellow)—aqueous layer (fluorescent, green)
Benzene layer (colourless)—aqueous layer (fluorescent, green)

The method of separating the above preparations and their compositions will be scheduled as follows.

TABLE VIII.

Preparation and composition of free acid.

No. of Preparation	Method						Ash %	CO_2 %		SO_4 %	Titration acidity for 100 g sample	Remarks
	Concentration of the dialysed solution		Intensity of electric current			Treatment						
	(g)	Water (c.c.)	Volt	m. Amp.	Time							
1							6.27	0.76	—	6.26	Acid	Galactose=40.65% Pentose= 3.40%
2	10	200	110	2.5	48	NH_4Cl+Alc.	5.85	1.16	—	—	—	Ca = 2.65% SO_4= 3.02%
3	5	200	110	10	24	NH_4Cl+Alc.	3.68	0.80	—	—	—	
4	15	200	110	50–100	30	NH_4Cl+Alc.	—	—	—	8.36	—	Galactose=39.47% Pentose= 8.17%
5	10	200	110	50–100	30	NH_7Cl+Alc.	0.80	0.56	−29.4°	8.72	NaOH=1.56 g SO_4=3.18 g	NH_4 ... N= 0.81% SO_4= 5.55%
6	10	200	110	50–100	30	Neutralisation with NH_4OH. NH_4Cl+Alc.	0.77	—	—	7.66	NaOH=0.36 g SO_4=0.86 g	Amm. N= 0.90% SO_4= 6.17%
7	10	200	110	50–100	30	Neutralisation with Ba $(OH)_2$. +Alc.	—	—	—	8.66	—	SO_4 combined with Ba......... 0.138%
8	10	200	110	50–100	35	NH_4Cl+Alic.	Trace	—	−31.0°	6.90	NaOH=1.05 g SO_4=2.52 g	Amm. N=1.27% SO_4=4.36%
9	10	200	110	50–100	30	Mixed solvent	0.59	—	−30.8°	6.62	NaOH=3.13 g SO_4=7.52 g	

Colour reaction .... No reducing power; Ketose, hexose, pentose reactions positive methyl pentose and uronic acid reactions negative.

(C) **The isolation and some properties of free acid.**

A dilute solution of hydrato-Kanten-λ containing an ethereal sulphate group was subjected to dialysis, by which various preparations of free acid were obtained. As is seen in TABLE VIII, according to the strength of electric current and length of dialysed time, the content of ash decreased proportionally while the increase of free acid was observed on the other hand. SO_4 attains the maximum point (8.72%) but in further dialysis decreases again. The liberation of CO_2 reduces within the error of the experiment, specific rotatory power reaching the maximum value $-31°$.

It was noticed that the free acid was very unstable. A fresh sample such as prep. 9 which showed $[\alpha]_D -30.8$ was a white powder turning brownish during preservation for some time. The content of SO_4 calculated from the titration acidity ($-SO_4H$) increased somewhat compared with the acidity calculated from the hydrolysis ($BaSO_4$), which explains that the preservation causes splitting of the ethereal combination into H_2SO_4 form. Such a trend was noticed in the drying of the preparations. Thus, though the free acid was very unstable it turned stable when ammonia or metals were combined with it.

Further, the important nature observed of the acid will be detailed: in the preps. 1–5 and 8, when small quantities of NH_4Cl solution were added to the solutions, and followed by alcohol, the acids were precipitated easily. These were very stable but showed acidity. For example, in prep. 5, the amount of sulphate through titration was 3.18% ($-SO_4H$), and the amount of sulphate corresponding to ammonia-nitrogen was 5.55%, their sum being 8.73%. Its amount agrees well with 8.72% as determined by hydrolysis. The same result was also obtained with prep. 8.

Prep. 5.	Total SO_4 by hydrolysis	8.72%	
	SO_4 as $-SO_4 \cdot Na$	3.18%	(36% for total SO_4)
	SO_4 as $-SO_4 \cdot NH_4$	5.55%	(64% for total SO_4)
		8.73%	
Prep. 8.	Total SO_4 by hydrolysis	6.90%	
	SO_4 as $-SO_4 \cdot Na$	2.52%	(36% for total SO_4)
	SO_4 as $-SO_4 \cdot NH_4$	4.36%	(64% for total SO_4)
		6.88%	

In the above preparations, we see, sulphate combined with NH_4 was 64%, the residual 36% being in free state. Prep. 6 was precipitated by adding a small quantity of NH_4Cl and alcohol after neutralisation with NH_4OH. Prep. 7 was precipitated by adding alcohol only after neutralisation with $Ba(OH)_2$. Both preparations showed acidity also in spite of previous neutralisation.

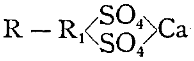
Prep. 6.	Total SO_4 by hydrolysis......	7.66%	
	SO_4 as $-SO_4 \cdot Na$..............	0.86%	(12% for total SO_4)
	SO_4 as $-SO_4 \cdot NH_4$	6.17%	(88% for total SO_4)
		7.03%	
Prep. 7.	Total SO_4 by hydrolysis......	8.66%	
	SO_4 as $-SO_4 \cdot Na$..............	——	
	SO_4 as $-SO_4 \cdot Ba \cdot SO_4-$	0.14%	

In prep. 6, though the amount of sulphate corresponding to ammonia nitrogen was increased as compared with the above preparation, its content was 88% for the total SO_4, but the residual 12% of the sulphate was in a free state. In prep. 7 a very small amount of barium was required for the neutralisation; the largest part of sulphate is thought to be in a free state in this case.

An important fact to be noticed was that after neutralisation with a small excess of NH_4OH the precipitation did not easily occur, while after the same neutralisation followed by a small excess of NH_4Cl the precipitation occurred immediately. These facts suggest to us that in these preparations OH' reacted as a peptizator and Cl' as an accelerator for the precipitation, a phenomenon usually occurring in colloidal substances. The acid shows consequently the properties of both ordinary acid and colloid simultaneously.

The constitution of the free acid may be considered as $R_1-O.SO_2 \cdot OH$ which differs naturally from the agar-acid reported by HOFFMAN and GORTNER. It was, as already described, separated from a fraction of the hot-water hydrolysis of agar setting free by the method of electrodialysis. It consisted of about 8% of sulphuric acid in ethereal linking, 39.47% galactose, 8.17% of pentose, a hexose other than galactose, and a substance giving a ketose reaction. It shows rotatory power, $-31°$; contains no uronic acid or methylpentose.

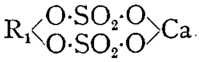
We propose to call this "Kanten-acid" to distinguish it from "Agar-acid." Agar may probably be shown by the following formula.



$$R - R_1 \begin{smallmatrix} \diagup SO_4 \diagdown \\ \diagdown SO_4 \diagup \end{smallmatrix} Ca \quad \text{or} \quad R - R_1 - (SO_4)_n - M \quad [\text{M takes metals}]$$

Summary.

We have investigated the hydrolysis of agar by heating this substance in aqueous solution under pressure at 130°, and measuring the accompanying loss of viscosity. We have been able to show that under the above conditions two products are formed; one of these appears to be a simple polysaccharide R, while the other contains an ethereal sulphate of the type



$$R_1 \begin{smallmatrix} \diagup O \cdot SO_2 \cdot O \diagdown \\ \diagdown O \cdot SO_2 \cdot O \diagup \end{smallmatrix} Ca \quad \text{or} \quad R_1(O \cdot SO_2 \cdot O)_n M \quad (\text{M takes metals})$$

A free acid has been isolated from the latter by the method of electro-dialysis. It is unstable with heat and preservation, and shows rotatory power, −31°; composed from galactose 39.47%, pentose 8.17%, about 8% of sulphuric acid in ethereal linking, a hexose other than galactose and a substance giving a ketose reaction. It contains no uronic acid or methylpentose.

We propose to call this acid "Kanten-acid" to distinguish it from the "Agar-acid" reported by HOFFMAN and GORTNER.

From these facts the following formula is suggested for Gelose,

$$R \cdot R_1 \cdot (O \cdot SO_2 \cdot O)_n M$$

The loss of gelatinising power of this substance would thus appear to result from the fission of the union between the two carbohydrate residues; this view is opposed to the suggestion of SAMEC and ISAJEVIČ, that the gelatinising property of Gelose is dependent upon the ethereal sulphate group.

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