A COMPARATIVE STUDY OF AGARS FROM VARIOUS GEOGRAPHICAL SOURCES

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Although small amounts of agar were produced in other countries, prior to the outbreak of war in 1939 almost the whole of the world's supply of this material came from Japan. During the period of the war, however, production of agar was developed in many countries or the British Empire, including New Zealand, South Africa, Australia, Canada and India, as well as England; also in some foreign countries, the United States of America, Denmark, China, Russia, Spain and Italy among others. Agars of New Zealand, South African, Australian, British and Danish origin are commercially available in this country at the present time, and it was, therefore, thought that it would be of some value to make a comparison of these varieties of agar with Japanese strip agar, normally in use before the war and now again on the market. The points to which attention has been particularly directed are:—(1) The character and strength of the gel produced, (2) The melting- and setting-points of the gel, (3) Ash values and the microscopical characters of the ash.

The agars have also been examined for their reaction to the British Pharmacopœia tests for identity and purity.

Sources and Materials

New Zealand Agar. Agar is prepared in New Zealand from *Pterocladia lucida* and *P. capillacea*[1,2]. The production has reached 100 tons per annum[3]. Four commercial samples have been examined (Nos. 5, 6, 7 and 18). The agar occurs as a coarse greyish-white powder.

Australian Agar. This is derived from *Gracilaria confervoides*[4]. Its manufacture has been described by Wood[5]. Of the two specimens of this variety examined, No. 11 consisted of coarse brown flakes and No. 12 of a greyish-brown powder.

South African Agar. The chief sources of agar prepared in South Africa are *Gelidium cartilagineum, Gracilaria confervoides* and *Suhria vittata*[6]. Five commercial samples have been examined (Nos. 8, 9, 10, 17 and 19). Each consisted of a coarse, light-brown powder.

British Agar. This is prepared from *Gigartina stellata* and *Chrondrus crispus.* The sources, production and properties of British agar have been described, in considerable detail, by Newton *et al.*[7] in a recently published monograph. The manufacture of British agar has also been described and illustrated in an anonymous communication[8]. The two specimens examined (Nos. 13 and 14) were more finely powdered than the other varieties and yellowish in colour.

Danish Agar. Danish agar has been presented in two forms, one consisting of thin strips, very much crinkled and twisted and almost white in colour, and the other of a fine white powder. Two samples of strip (Nos. 15 and 20) and one of powder (No. 16) have been examined. The botanical source of this agar is not known.

Japanese Agar. 24 different batches of Japanese strip agar (Kobe No. 1) (Nos. 1 to 4 and 21 to 40) have been tested to obtain quantitative data for comparison with those of the other varieties. All of these have been obtained during the last three years with the exception of No. 2, which was imported in 1937. A report of the British Intelligence Objectives Sub-Committee, issued in 1946[9], describes the conditions under which agar is manufactured in Japan at the present time. The report names 34 different species of seaweed which are used for the purpose: of these *Gelidium amansii* and *G. pacificum* are the most important. A detailed description of the collection of the seaweed and of the manufacture, testing and grading of the agar is given and also figures for the quantities produced and exported.

Properties of the Gel

Gel Strength. In the work on British agar[7], the gel strength was measured by a modification of the method suggested by Campbell[10], which consists of determining the force required to turn a vane through a predetermined angle when immersed in the jelly. Chakraborty[11] immersed one pan of a balance in the jelly at a known level and determined the weight required to pull the pan out. Another method for determining the gel strength of agar, suggested by Lockwood and Hayes[12], depends on measuring the change in height of a column of jelly when removed from its containing cylinder. The apparatus is called a " ridgelimeter."

In the present work, the gel strength was measured by the Bloom gelometer. This instrument is regularly in use for the determination of the jelly strength of gelatin, and forms the subject of a British Standard Specification[13]. It has also been described by the National Association of Glue Manufacturers of the United States of America[14]. In adapting this method for use on agar, the principal modifications necessary are in the method of preparation and strength of the jelly employed. It was found that 1 per cent. was normally a suitable strength, and the method of procedure was as follows: Heat 500 ml. of water to boiling point, add 5 g. of agar, in moderately coarse powder, and boil gently for 5 minutes, with constant stirring. Cool somewhat, adjust the total weight of the solution to 500 g. by the addition of water and mix thoroughly. Allow to cool to about 60°C. and pour into the standard bottles. Three bottles should be filled with each solution under test. Leave the bottles until the contents have cooled to room temperature, then stopper and place them in the chill bath at a temperature of 10° ± 0·1°C. for not less than 16 and not more than 18 hours. The jelly strength is then determined in triplicate, exactly as described in the standard method and the average value taken.

The gel strengths of the specimens under examination, determined by the above method, are recorded in Table I. The results on sample No. 2 confirm the observation of Chakraborty[11] that Japanese agar loses its setting power, to some extent, after long storage. The melting- and setting-temperatures of this sample also seem to have been affected by age.

TABLE I

	Country of Origin	Gel Strength 1 per cent. g.	Setting Temperature 2 per cent. °C.	Melting Temperature 2 per cent. °C.
Average of 23 samples ...	Japanese	260 to 310	33 to 34	89 to 93
Sample 2	,, 1937	177	35	82
,, 5	New Zealand	625	35·5	92
,, 6	,,	618	36	90
,, 7	,,	610	36	91
,, 18	,,	620	35	92
,, 11	Australian	56	34	76
,, 12	,,	85	34·5	77
,, 8	South African	280	36	86
,, 9	,,	306	36·5	86
,, 10	,,	243	36·5	88
,, 17	,,	287	36·5	88
,, 19	,,	250	36	87
,, 13	British	55	40	56
,, 14	,,	100	40	57
,, 15	Danish	135	44	64
,, 16	,,	135	44	65
,, 20	,,	130	43	64

Some determinations were also made, at strengths other than 1 per cent., with the object of finding what concentration of the various agars would be required to produce a jelly of strength equal to one made with a given amount of Japanese agar. The results are stated in Table II.

TABLE II

CONCENTRATION OF AGAR REQUIRED TO PRODUCE A GEL EQUAL IN STRENGTH TO THAT PRODUCED BY 1 PER CENT. OF JAPANESE AGAR

New Zealand	0·7 per cent.
Australian	2·0 ,,
South African	1·0 ,,
British	1·5 ,,
Danish	1·5 ,,

Melting- and Setting-Temperatures. These were determined on 2 per cent. gels, since this is the concentration most usually used in bacteriological work. The solution was prepared by boiling the agar in water for 5 minutes, as described above, and adjusting to the required weight. From this solution 10 ml. was poured into a test tube, a thermometer inserted and the whole allowed to cool slowly, the temperature at which the gel solidified being taken. It was found that this temperature was quite sharply defined. The tube was kept in a thermostat, at 15°C., overnight and the next day was placed in a water-bath and gradually heated and the temperature at which the gel melted was recorded. The

results will be found in Table I. Melting- and setting-temperatures of agar have also been recorded by Chakraborty[11] and by Newton *et al*[7].

Sensory Characters. The visible characteristics of the 1 per cent. gels, prepared as described above, were as follows. *Japanese.* Slightly brownish, somewhat opalescent, a trace of insoluble matter present. *New Zealand.* Colourless, somewhat opalescent, a slight trace of insoluble matter present. *Australian.* Distinctly brownish, very opalescent, some insoluble matter. *South African.* Brownish, opalescent, a trace of insoluble matter. *British.* Very pale yellow, clear, no insoluble matter. *Danish.* Colourless, slightly opalescent, a slight trace of insoluble matter.

Ash and Microscopical Characters of the Ash

Ash and Loss. Table III shows the total and acid-insoluble ash contents of the various specimens, determined by the methods of the British Pharmacopœia. The losses sustained, by most of the samples, on drying to constant weight at 100°C. are also shown in this table. The official limits and the average values for 24 samples of Japanese strip agar are included for comparison. These results show that, other considerations apart, British and Danish agars are ruled out, so far as the official standards are concerned, by their high ash values.

TABLE III

	Country of Origin	Total ash per cent.	Acid-insoluble ash per cent.	Loss at 100°C. per cent.
	B.P. limits	5·0	1·0	18·0
Average of 24 samples ...	Japanese	2·3 to 3·6	0·02 to 0·30	12·0 to 20·0
Sample 5	New Zealand	1·20	0·20	12·2
" 6	"	1·07	0·18	—
" 7	"	0·92	0·06	11·9
" 18	"	1·05	0·15	16·1
" 11	Australian	5·90	0·56	14·8
" 12	"	3·24	0·02	16·1
" 8	South African	2·80	0·20	14·6
" 9	"	2·70	0·18	8·2
" 10	"	2·30	0·10	—
" 17	"	3·00	0·10	8·1
" 19	"	2·50	0·13	15·9
" 13	British	35·1	0·40	8·4
" 14	"	37·2	0·31	10·4
" 15	Danish	18·3	0·80	13·2
" 16	"	16·4	0·09	16·8
" 20	"	16·5	0·13	—

Microscopical Characters of the Ash. The part of the ash insoluble in hydrochloric acid was examined microscopically and a search made, in particular, for diatoms and sponge spicules. All samples of a common national origin showed similar microscopical characters, which are summarised below. The diatoms of Japanese agar are well known. King[15] has listed and illustrated characteristic species.

New Zealand. A few small diatoms, mainly species of *Melosira*, and sponge spicules. In addition, large masses of silica and much amorphous débris are present (Fig. 1).

Australian. Mainly amorphous debris, a small amount of silica and a few sponge spicules and diatoms, also *Melosira* sp. (Fig. 2).

South African. Diatoms, whole and broken, of a species of *Coscinodiscus* with a hexagonal pattern on the surface are fairly numerous, also present are a few silico-flagellates, fragments of silica and amorphous débris. Sponge spicules are absent (Fig. 3).

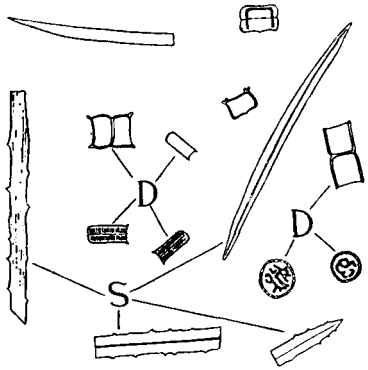

FIG. 1. Diatoms and sponge spicules from New Zealand agar. D, Diatoms, *Melosira sp.* S, spicules. × 330.

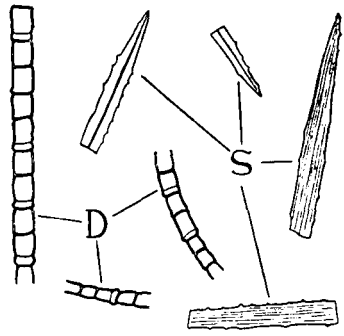

FIG. 2. Diatoms and sponge spicules from Australian agar. D, Diatoms, *Melosira sp.* S, spicules. × 330.

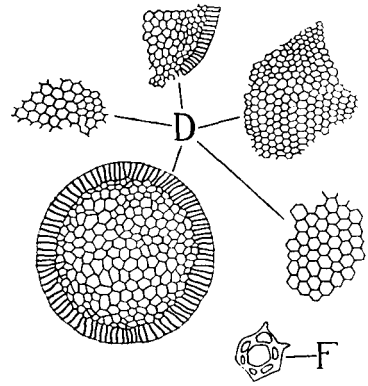

FIG. 3. Diatoms from South African agar. D, Diatoms, *Coscinodiscus sp.* F, A silico-flagellate. × 330.

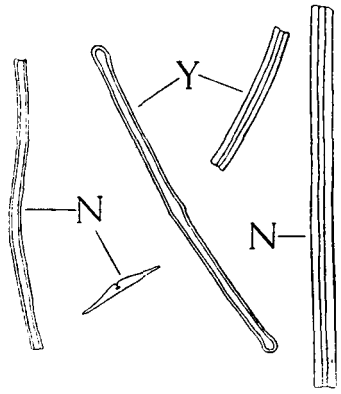

FIG. 4. Diatoms from Danish agar. N, *Nitzschia sp.* Y, *Synedra sp.* × 330.

British. Consists almost entirely of finely-divided amorphous matter, fragments of silica are very rare and diatoms and sponge spicules absent.

Danish. Mainly silica; some amorphous debris and a few diatoms, including species of *Nitzschia* and *Synedra*, no sponge spicules (Fig. 4).

PHARMACOPŒIAL TESTS FOR IDENTIFICATION

Ruthenium Red. All varieties are stained pink by solution of ruthenium red.

Potassium Hydroxide. All varieties give a yellow colour on warming with a 5 per cent. solution of potassium hydroxide but, with British agar, this colour is paler and longer in developing than with the other types.

Hydrolysis Test. All varieties of agar, after hydrolysis with hydrochloric acid, will reduce Fehling's solution. The barium chloride test for sulphate, however, when carried out in accordance with the instructions given in the British Pharmacopœia, gives no reaction with New Zealand, Australian and South African or even with Japanese agar. British and Danish agars do give a precipitate in this test.

Iodine Test. Powdered Japanese, New Zealand, South African and Australian agars give a deep crimson colour with a dilute solution of iodine. British and Danish agars give only a brown colour. It was found that 0·5 ml. of 0·05 N iodine with 0·1 g. of powder gave a more distinctive colour than 1 ml. of 0·05 N iodine recommended by the B.P.

Tannic Acid Test for Gelatin. This test will give satisfactory results with all varieties of agar, but when carrying out the test it is essential that the temperature of the mixed agar and tannic acid solutions be between 80° and 90°C. Within this range, pure agar gives no precipitate; the presence of 10 per cent. of gelatin will give rise to a distinct opalescence, while 20 per cent. produces a milky white turbidity. If, however, the temperature falls much below 80°C., an exactly similar turbidity is obtained from agar alone, if the agar be of Japanese, New Zealand, South African or Australian origin. British and Danish agars give no precipitate with tannic acid solution even in the cold.

Picric Acid Test for Gelatin. The United States Pharmacopœia gives a test for gelatin using picric acid. A 1 per cent. solution of the agar is prepared by boiling with water; this solution is cooled to about 50°C. and to 5 ml. is added an equal volume of a 1 per cent. solution of picric acid. None of the varieties of agar gave any precipitate when tested in this way. However, 10 per cent. of gelatin in the agar will give a marked turbidity and 5 per cent. is just detectable. This test is superior to the British Pharmacopœia test using tannic acid, mentioned above, because it is more sensitive and, even more, because there is no risk of getting false positives; no turbidity being produced by agar alone even on cooling to normal room temperature.

Conclusions

New Zealand, South African and Australian agars are of the same type as Japanese agar. New Zealand agar is generally superior to Japanese: South African is about equal to Japanese: Australian is much inferior in gel strength and colour, and contains more ash and insoluble matter. So-called British and Danish agars, on the other hand, apart from their inferiority in gel strength and in melting- and setting-temperatures, are of quite different character from the Japanese material and

are to be regarded as substitutes for, rather than as varieties of, agar. It would be preferable that these materials should be sold under some name other than "agar."

SUMMARY

1. New Zealand, South African, Australian, British and Danish agars have been examined. These agars are compared with Japanese agar in respect of the strength of the gels which they produce and also the melting- and setting-points of the gels.

2. The ashes, acid-insoluble ashes and losses on drying of the 5 varieties of agar are recorded and the microscopical characters of the ashes have been described and illustrated.

3. The agars have been tested by the British Pharmacopœia tests for identification of agar and also by the United States Pharmacopœia test for gelatin in agar.

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DISCUSSION

THE CHAIRMAN said that the results of this work were disappointing as far as British agar was concerned. Fortunately agars from Commonwealth sources were as good as the Japanese, but it was obvious that something giving 35 per cent. of ash and not giving the reactions of agar ought not to be called agar.

DR. T. E. WALLIS (London) said that one of the most important points arising from the paper was the quantity of ash in the different varieties. He thought that the ash in the Japanese, New Zealand and South African agars probably represented the ash in the seaweeds from which they were made, but with the British agar he understood that a considerable proportion of calcium salts was added in the course of preparation in order to make the material obtained from the algae gel when it was used. Perhaps this was also the case with the Danish variety. The picric acid test was a far better test for the presence of gelatin than

that now in the B.P. Some of the differences between the different agars depended upon the particular composition of the mucilaginous matter which was obtained from them. That obtained from the Japanese seaweeds was said to contain the sulphuric ester of galacturonic acid, and that was the cause of the positive reaction in the sulphate test. He could not explain why the author had failed to get a positive result in the test for sulphate after hydrolysis. Perhaps the process of preparation of Japanese agars had been altered recently. Did the author regard the diatoms as really good differential diagnostic characters for the different kinds of agar?

DR. J. M. ROWSON (London) said that he could confirm the author's findings with regard to the failure of the gelatin test. Students repeatedly reported to him the presence of gelatin in genuine agar because they did not get the temperature right. The tannic acid test required careful control to give a satisfactory indication, and the picric acid test was better. Like Dr. Wallis, he had been surprised to find that the author did not get a reaction with the sulphate test on Japanese agar. Also, he wondered whether the author had made any chemical investigation of the ash in British agar. Had the author plotted the log concentration graph for the concentrations to give equivalent gels as that would be interesting from the physico-chemical point of view? He also wondered whether the author had investigated the viscosities of dilute agar solutions and compared them with the gel strengths. This might form a further standard for agar, and, since U-tube viscometers were in the B.P. appendix, it might be more convenient to establish a viscosity strength than a gel strength, if the two were comparable.

DR. J. W. FAIRBAIRN (London) asked if there was any correlation between the botanical source of the agars and the gel strength. The Japanese and South African agars were similar in strength and both contained *Gelidium* species. The New Zealand agar, which was outstanding, was characterised by *Pterocladia*, of which there was a little in the Japanese agar. It was possible that the most important factor for any country wanting to cultivate seaweed was to select those species which yield a gel of very high strength. In evaluating gel strength the author had tried two methods. The results in each case were quite different, and even the order of gel strength varied a little. It seemed very important to get a method which would give the same results every time. He was sorry that it did not seem possible, from the paper, to distinguish with certainty the various commercial agars. Could the author guarantee, if he were given a powdered agar, to identify its geographical source?

MR. T. D. WHITTET (London) said that he had investigated a number of chemical tests to distinguish between these agars and had discovered a number of useful reactions, but, as yet, the work was uncompleted. He had also examined Norwegian agar (which seemed very similar to the British and Danish), a Californian sample (which was almost identical with Japanese) and Indian agar (which was more like South African).

He was able to get a positive reaction for sulphates with all the agars, and a very dense one indeed with the British agar. In the tannic acid test he had had the same results as the author except with Australian agar, which gave a slight precipitate even with the hot solution. This became very dense on cooling, and there was still a slight turbidity even on boiling the solution. He had included sodium alginate in his tests, and the only difference between this and British agar was the precipitate with calcium salts which occurred only with the former.

MR. G. SYKES (Nottingham) agreed that British and Danish agars probably did not justify the name of "agar" in the pharmacopœial sense. He wondered whether the author had considered the clarity of the agar solution in different solvents. British agar gave a clear solution in water but not in the presence of peptone. It was, therefore, no good for certain culture media. Instead of the Bloom gelometer for testing gel strength, had the author used the finger pressure test specified for gelatin in a British Standards Specification? As regards relative gel strengths he had found a 3 per cent. gel of British agar to have a weaker strength than a 2 per cent. one, and that the strength of gels in different solvents varied considerably before and after sterilisation.

DR. K. R. CAPPER (London) said that agar was sometimes regarded as being relatively inert in bacteriological media, but that was by no means true. The nature of the ash could have a great deal to do with the effect of agar on bacterial growth. With *B. subtilis*, for instance, the whole character and appearance of the growth on New Zealand agar was different from when Japanese agar was used. Certain ions, particularly cations, could have an effect on the degree of inhibition produced by some antibiotics, and by adding certain metallic salts to agar it was possible to obtain quite different results.

MR. A. R. G. CHAMINGS (Horsham) referred to the efforts and expense incurred during the war in the production of British agars, which, it appeared, unfortunately did not meet the B.P. requirements.

MR. H. DEANE (Sudbury) pointed out that large quantities of agar are used in the food industry.

PROFESSOR H. BERRY (London) said that he would like to know the nature of the water-soluble extractive. Before the war good quality Japanese agar had given little trouble, but since the war many difficulties had been due to variations in the quality of agar. It would be interesting to have some information on the quality of Japanese agar now on the market. One particular difficulty was with Brewer's medium, which should be perfectly clear. With post-war samples a gelatinous precipitate formed and was very troublesome. It would be interesting if the author could make arrangements, now that he had these specimens typed, to have bacteriological tests done on the various specimens. Was it possible to rely on samples of New Zealand agar as being of a constant type?

MR. R. L. STEPHENS (London) referred to the use of agar in emulsion of liquid paraffin with phenolphthalein B.P.C. It was a matter of considerable embarrassment to make this preparation with New Zealand agar and find that it was almost solid. The B.P.C. gave no instructions for adjusting the formula of that emulsion in the way in which the B.P. directed that the proportions of emulsifying agents might be changed according to the method of preparation. Had the author considered this aspect of agar?

DR. NORMAN EVERS (Ware) said that some agars had an effect on bacterial growth because they contained copper.

DR. W. P. KENNEDY (London) mentioned that material coming from India had been found to be heavily contaminated with copper, and it was discovered that this was due to the method of preparation, in which large copper pans were used. There was enough copper in some Indian agars to inhibit bacterial growth appreciably.

MR. G. R. A. SHORT (London) said that the objection to the use of British agar in confectionery would be its peculiar flavour, due probably to its high content of potassium chloride.

THE CHAIRMAN, referring to Professor Berry's suggestion for an investigation of the water-soluble matter of these agars, said it was to be noted that the unsatisfactory agars came from *Chondrus crispus*. This gave two extracts, one separable by hot water and the other by cold. They both contained sulphonated carbohydrates. The cold water extract had an inhibiting effect on the gelling of the hot water extract, but if one separated it one got a very viscous preparation derived from the hot water extract only. That might perhaps give a line to be followed in the investigation of the differences between the water-extracted matter of these different agars.

MR. J. L. FORSDIKE, in his reply, said that he had not investigated the ash of British agar, but it was stated by the manufacturers that it was principally potassium chloride, which was added to produce the gel. The preparation of agar in Japan had been described in the report of the British Intelligence Objectives Sub-Committee, 1946, No. JAP/PR/814. All the samples examined contained the same diatoms. They had tried the sulphate test on a hundred or more samples of Japanese agar and had never found it to work satisfactorily. They had not made concentration graphs, and they had not determined the viscosity. The reason for choosing the Bloom gelometer was that it was used for determining the gel strength of gelatin and was the subject of a British Standard Specification. It was, therefore, a well-established and quite invariable instrument. As to botanical source and gel strength, there was little doubt that the type of seaweed used to produce the agar was the principal factor in determining the gel strength, though no doubt the method of manufacture had something to do with it. In Japan 34 different species of seaweed were used to make agar, and that would cover most of the species in use in other parts of the world. He did

not think that the cultivation of seaweed for the production of agar had ever been attempted, and it would be rather impracticable. The usual practice was simply to collect the seaweeds which happened to grow in the locality where agar was to be produced. He did not quite understand Dr. Fairbairn's remarks about the gel strengths obtained by the two different methods; all the gel strengths were determined by the same method, although with gels of different strengths.

With regard to the differentiation of the various types, in those samples which he had examined he had found the same kind of diatoms and spicules present in the ash of every sample of a particular geographical variety, and therefore he thought it would be possible to identify Australian, New Zealand or South African agar by microscopical examination of the acid-insoluble ash. He had not examined either Californian or Indian agars. Only two samples of Australian agar had been examined and neither of them gave any precipitate in the gelatin test. He could not say whether the presence of peptone or any other substance would affect the gel as he had not tried making gels containing other substances and he had not examined the effect of sterilisation on gel strengths. He would try to determine what the water-soluble extractive was and also investigate the presence of copper. Whether the quality of Japanese agar was exactly the same as that obtained before the war, he did not know, but he was unaware of any obvious differences. He would make an attempt to have some bacteriological tests done on the samples of agar as had been suggested. If New Zealand agar was used in a formula intended for the Japanese variety, one would get very much stronger gelling.