proposed also to try other disinfectants, or combinations of disinfectants; and, as suggested by Colonel Reece, to compare the effects of the spray upon patients lying on their backs on the floor with the results obtained when they are sitting or standing; and also to see whether multiple inhalations are more successful than a single daily inhalation for the present purpose.

It should be added that during some recent experiments with a powerful experimental spray the use of 2 per cent. of chloramine produced a certain amount of bronchial irritation in two medical volunteers who underwent this treatment for catarrh. Although in no sense serious—both declared their catarrh benefited by the treatment—this effect is undesirable. No such bronchial irritation was observed when 1 per cent. of zinc sulphate was sprayed by the same apparatus. The result would appear to suggest that for disinfection of the nasopharynx of meningococcus carriers the finest possible spray is not essential, since the droplets of disinfectant, if very finely divided, penetrate further along the respiratory tract than is necessary, and reach the bronchi in some quantity. With a weaker solution of chloramine such irritant effect would doubtless be avoided, even in persons suffering, as these were, from a more or less acute form of catarrh at the time when they entered the inhaling room.

CONCLUSIONS.

These observations have led us to believe that, in the case of carriers of the meningococcus, prognosis with regard to the duration of their future period of carrying varies largely with the degree to which their nasopharyngeal secretion is infected by the meningococcus.

In our experience, the majority of carriers whose nasopharynx yields only a few colonies of the meningococcus clear up quickly. In the case of these persons good results follow local treatment either by direct application of a 1 per cent. solution of chloramine, or by subjecting them to inhalation of a steam spray charged with zinc sulphate.

On the other hand, carriers whose nasopharyngeal secretion yields a copious growth, or a practically pure culture of the meningococcus, are often far more difficult to cure. The results on these persons with zinc salts were disappointing, but they served to emphasize the success of those obtained with chloramine, which, though still somewhat few in number, are more than encouraging, and would appear to indicate that a large proportion of these chronic carriers can be definitely freed of the meningococcus by this form of treatment in a comparatively short space of time.

We would add that while these observations on meningococcus carriers were in progress, several cases of chronic nasal or post-nasal catarrh have been submitted to disinfectant treatment by the automatic steam spray. As in all of these persons improvement has resulted, we offer the suggestion that this method is worthy of trial on a large scale for the treatment of such cases.

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AN EXPERIMENTAL STUDY OF THE CULTURAL REQUIREMENTS OF THE MENINGOCOCCUS.

TOGETHER WITH A DESCRIPTION OF AN EASILY PREPARED
MEDIUM FOR THAT MICRO-ORGANISM.

BY

LIEUT.-COL. M. H. GORDON, AND MAJOR T. G. M. HINE,
R.A.M.C., R.A.M.C.

With a Note by
MARTIN FLACK, CAPTAIN R.A.M.C.

(From the Central Laboratory for Cerebro-spinal Fever,
R.A.M. College, London.)

ONE of the functions of the Central Laboratory established at the Royal Army Medical College for dealing with outbreaks of cerebro-spinal fever is to supply district laboratories with medium for cultivation of the meningococcus. For this reason the following investigation was the first of

a series of researches carried out in the Central Laboratory during the outbreak of 1915.

Ordinary agar medium, which consists of about 2 per cent. of agar dissolved in meat broth with the addition of 1 per cent. of Witte's peptone and $\frac{1}{2}$ per cent. of salt, is generally recognized to require the addition of protein in some form before it becomes a satisfactory medium for the meningococcus. Animal protein in the shape of ascites fluid or blood gives good results when added to ordinary agar medium, but neither of these materials was employed for routine purposes in the present instance because ascites fluid was not procurable in large enough quantity and blood was found to have two disadvantages. First, it made the medium opaque, thus interfering with the differentiation of the meningococcus colonies, and secondly, in spite of all efforts to prevent it, the blood which we were able to obtain from the slaughterhouse was found to be contaminated. There remained horse serum, which we were able to get in quantity, but at considerable expense, and without a guarantee of its sterility.

Satisfactory results having been obtained previously with nasgar, and this medium having the great advantage that it can be sterilized by steam without losing its capacity of growing the meningococcus, it was decided to send out nasgar in the first place for routine purposes, using serum instead of ascites fluid for its manufacture.

Two difficulties, however, had to be met in making nasgar. In the first place, the supply of nutrose failed, necessitating recourse to a substance asserted to be identical with nutrose chemically—namely, a mixture of sodium caseinogenate with glycerophosphates. Secondly, the supply of Witte's peptone ceased. We therefore made nasgar with serum instead of ascites fluid, with artificial nutrose, and with Armour's standard peptone in place of Witte's. As regards reaction, the medium was rendered + 1.0 to phenolphthalein.

Nasgar made in this way was carefully tested and found to grow the meningococcus quite well in primary cultures from the cerebro-spinal fluid and also in subcultures. When swabs from the nasopharynx of contacts were plated out on it, a proportion of them grew colonies of cocci indistinguishable from the meningococcus. The medium also had the great advantage that it gave good differentiation of colonies of the meningococcus from those of other Gram-negative cocci of the nasopharynx, such as *M. flavus* and *M. catarrhalis*.

While a start was made with nasgar, an investigation was begun for the purpose of defining the conditions of growth of the meningococcus in artificial culture more thoroughly, with the special view of obtaining, if possible, a superior medium for routine purposes.

The chief requirements that a satisfactory routine solid medium for the meningococcus should meet may be summarized as follows:

1. It must grow the meningococcus with constancy in primary culture, as well as in subculture.
2. It must be transparent, so that colonies of the meningococcus developing upon it can readily be distinguished from those of other Gram-negative cocci.
3. It must be simple and easy to make, sterilize, and handle, and as far as possible free from materials of foreign origin.
4. When the supply of a large number of laboratories is in question, as in the present instance, it must be cheap.
5. The medium should be capable of maintaining the meningococcus alive for a considerable time, thus obviating the necessity of frequent subculture.

The research which has been made for the purpose of obtaining an improved medium has been carried out in three stages. In the first two experiments ordinary agar consisting of agar 2½, Witte's peptone 1, salt $\frac{1}{2}$, and meat broth to 100, was used as base.

In the succeeding experiments, where the value of individual substances for growing the meningococcus was under investigation, water agar, consisting of agar 2½, salt $\frac{1}{2}$, and water 100, was used as menstruum.

As a result of these observations a medium was arrived at that appeared to be full of promise. The third stage of the investigation, therefore, was to test the capacity of this medium to satisfy requirements in practice.

In the experiments now to be described, the sign — signifies that the meningococcus failed to grow; the sign (+) signifies a distinct but feeble growth; + a moderate growth; and ++ a good growth of this micro-organism.

PEPTONE BROTH AGAR SERIES.

EXPERIMENT I.—Fresh human blood when added to ordinary agar encourages the growth of the meningococcus. Is this power lost when the blood is heated?

A meningococcus recently isolated from the cerebro-spinal fluid was sown in equal amounts on slopes of the following:

1. Ordinary agar (agar 2½, Witte peptone 1, salt 0.5, meat broth to 100).
2. Ordinary agar + 1 per cent. of fresh blood.
3. Ordinary agar + 1 per cent. of fresh blood heated to 55° C. for ten minutes.
4. Ordinary agar + 1 per cent. of fresh blood heated to 70° C. for ten minutes.
5. Ordinary agar + 1 per cent. of fresh blood boiled for ten minutes.

Owing to heat coagulation of the albumin 4 and 5 were very opaque.

Result.—The meningococcus grew on all the tubes, but more quickly and copiously on 2, 3, 4, 5 than on 1. There was no difference in the degree of growth on 2, 3, 4, and 5 respectively.

Inference.—Heating does not destroy the augmenting effect of blood on the growth of the meningococcus on ordinary agar.

EXPERIMENT II.—What are the relative values of various forms of protein for augmenting growth of the meningococcus on ordinary agar?

The same menstruum was used as before.

0.1 gram of the various nitrogenous substances of test were placed in a series of test tubes, and 1 c.cm. of water added to each; 9 c.cm. of melted agar was then run into each tube. The tubes were steamed on two successive days, shaken and sloped.

A parallel series was also put up in the same way, but with the addition of 1 per cent. of glucose.

A meningococcus recently isolated from the cerebro-spinal fluid of a case was then sown in equal amounts on all the tubes. The results were as follows:

Growth of the Meningococcus in Forty-eight Hours at 37° C.

No.	Substance.	Alone.	With Glucose 1 per Cent.
1	Alkali albumin	(+)	(+)
2	Serum albumin	++	++
3	Globulin	—	+
4	Haemoglobin	++	++
5	Fibrinogen	++	++
6	Protalbumose	—	(+)
7	Deuteroalbumose	—	(+)
8	Dysalbumose	—	—
9	Nuclein	—	—
10	Legumin	++	++
11	Control agar alone	—	—

It is seen that growth was excellent with serum albumin, haemoglobin, fibrinogen and legumin, and that the addition of glucose appeared to make little or no difference to the value of these substances for encouraging growth of the meningococcus.

The percentage composition of serum albumin, serum globulin, and legumin respectively is seen from the following figures taken from Hammarsten's *Textbook of Physiological Chemistry*, translated by Mandel, 1914:

Percentage Composition.

	Serum Albumin.	Serum Globulin.	Legumin.
Glycocoll	0.0	3.5	0.38
Alanin	2.7	2.2	2.08
Valin	—	—	1.0
Leucin	20.0	18.7	8.0
Isoleucin	—	—	—
Serin	0.6	—	0.53
Aspartic acid	3.1	2.5	5.3
Glutamic acid	7.7	8.5	13.8
Cystin	2.5	1.51	—
Phenyl-alanin	3.1	3.8	3.75
Tyrosin	2.1	2.5	1.55
Prolin	1.04	2.8	3.22
Oxyprolin	—	—	—
Tryptophan	—	—	—
Histidin	—	—	2.42
Arginin	—	—	10.12
Lysin	—	—	4.29
Ammonia	—	—	1.49

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It would appear that there is a broad similarity between them as regards components, so far as these have been determined.

As a practical medium was being sought for, and legumin has the great advantages that it is cheaply and easily prepared by extracting peafLOUR with salt solution, and that the crude solution of legumin thus obtained can be sterilized by heat without precipitating the legumin, an attempt was made to ascertain more closely the value of this vegetable albumin for the present purpose.

At the same time opportunity was taken of examining the actual values of various constituents usually included in media for cultivating the meningococcus.

WATER AGAR SERIES.

EXPERIMENT III.—What are the relative values of peptone (Armour's), peafLOUR extract, and meat broth respectively for growing the meningococcus?

An extract of peafLOUR was prepared by placing 10 grams of peafLOUR in a flask together with 10 grams of salt and 100 c.cm. of distilled water. The flask was then well shaken, steamed, and its contents filtered. The filtrate, which was sterilized by steaming it on two successive days, was the peafLOUR extract used in this and the following experiments. A 5 per cent. solution of Armour's peptone was made in the same way. The present experiment was carried out in duplicate.

In the first place 2½ per cent. of agar dissolved in distilled water was used as menstruum, and in the second 2½ per cent. of agar dissolved in meat broth. The meat broth was made in the usual way by boiling 500 grams of finely-minced beef in 1 litre of distilled water and filtering. Except where the peafLOUR extract was added the agar in both cases received the addition of ½ per cent. of salt. This experiment and the following ones were made in Petri dishes. One cubic centimetre of the solution of peafLOUR, peptone, etc., was first placed in the dish. Nine cubic centimetres of the melted agar, or in the case where trial was made of two added substances, 8 c.cm. were poured in, the contents well mixed, and allowed to set.

Three recently isolated meningococci were then each inoculated upon a different segment of the solid medium.

Result Forty-eight Hours at 37° C.

No.	Menstruum.	Addition.	Meningo-coccus 1.	Meningo-coccus 2.	Meningo-coccus 3.
1	Water agar		—	—	—
2	" "	Armour's peptone	—	—	—
3	" "	PeafLOUR extract	(+)	(+)	(+)
4	" "	Peptone and peafLOUR extract	—	—	—
5	Broth agar			+	+
6	" "	Armour's peptone		+	+
7	" "	PeafLOUR extract	++	++	++
8	" "	Peptone and peafLOUR extract	++	++	++

Inferences.—(1) The sample of peptone here used was of no value for growing the meningococcus. In Experiment 4 it seemed to inhibit. (2) PeafLOUR extract has some nourishing value for the meningococcus. (3) Meat broth also has this property. (4) Growth of the meningococcus was best when both peafLOUR extract and meat broth were present in the medium.

In the preceding experiment the meat broth was in far higher concentration than the peafLOUR extract in the agar. In the next experiment, therefore, equal quantities of meat broth and peafLOUR extract respectively were added to the water agar.

Result Forty-eight Hours at 37° C.

No.	Menstruum.	Addition.	Growth of Meningo-coccus.
1	Water agar		—
2	" "	Broth 10 per cent.	—
3	" "	PeafLOUR extract 10 per cent.	(+)
4	" "	Both.	(+)

When in equal concentration, therefore, in the agar, the peafLOUR extract gave better growth than the meat broth. As before, growth was best when both were present. With 10 per cent. of both in the agar, a moderate growth of the meningococcus took place.

EXPERIMENT IV.—Comparison of the values of peptone (Armour's), peptone (Witte), peafLOUR extract, nutrose, and serum (horse), in water agar and in meat broth agar respectively.

As in the case of Experiment III the present test was made in duplicate. Five per cent. solutions of each of the peptones and of the nutrose substitute were used. The peafLOUR extract was prepared as before. One cubic centimetre amounts of these fluids were mixed in plates with 9 c.cm. of melted agar. After setting, each plate was inoculated with three meningococci.

Result Forty-eight Hours at 37° C.

No.	Menstruum.	Addition.	Meningo-coccus 1.	Meningo-coccus 2.	Meningo-coccus 3.
1	Water agar		—	—	—
2	" "	Peptone (A.)	—	—	—
3	" "	Peptone (W.)	—	—	—
4	" "	Nutrose	—	—	—
5	" "	PeafLOUR extract	—	—	—
6	" "	Serum	—	—	—
7	Broth agar		(+)	—	+
8	" "	Peptone (A.)	—	(+)	+
9	" "	Peptone (W.)	++	++	++
10	" "	Nutrose	++	++	++
11	" "	PeafLOUR extract	++	++	++
12	" "	Serum	++	++	++

None of the five substances tested separately in the first half of this experiment sufficed by itself for growth of the meningococcus. On the other hand, when meat broth was used to dissolve the agar, a certain amount of growth occurred without further addition. This growth was greatly enhanced by the addition of each of the substances of test except Armour's peptone.

EXPERIMENT V.—Comparison of the effect of adding to water agar, meat broth, trypsinized meat broth (Douglas), nutrose, peafLOUR extract, serum, and ascites fluid respectively.

The meat broth, trypsinized meat broth, serum, and ascites fluid were each added to the extent of 10 per cent. of the water agar. The amount of nutrose and peafLOUR extract added was as before.

Result Forty-eight Hours at 37° C.

No.	Menstruum.	Addition.	Meningo-coccus 1.	Meningo-coccus 2.
1	Water agar.		—	—
2	" "	Meat broth	—	(+)
3	" "	Trypsinized meat broth	—	+
4	" "	PeafLOUR extract	(+)	(+)
5	" "	Nutrose	—	—
6	" "	Serum	—	—
7	" "	Ascites fluid	(+)	(+)

Both meningococci grew slightly but distinctly when peafLOUR extract or ascites fluid were added to the agar. Growth of meningococcus 2 was better on trypsinized broth than on ordinary broth.

EXPERIMENT VI.—Comparison of the effect of adding serum, ascites fluid, peafLOUR extract, and nutrose to agar containing meat broth and trypsinized broth respectively.

A bottle of water agar was melted and divided into two equal parts. To one portion 30 per cent. of meat broth was added, and to the other the same amount of trypsinized broth. Plates were then prepared of these two media with the addition of the various substances mentioned above. The serum and ascites fluid were added in the

proportion of 10 per cent.; the peafLOUR extract and the nutrose were in the same concentration as before.

Result Forty-eight Hours at 37° C.

No.	Menstruum.	Addition 1.	Addition 2.	Meningo-coccus 1.	Meningo-coccus 2.
1	Water agar		Broth	—	—
			Trypsinized broth	(+)	(+)
2	Water agar	Ascites fluid	Broth	+	+
			Trypsinized broth	++	++
3	Water agar	Serum	Broth	—	—
			Trypsinized broth	—	—
4	Water agar	PeafLOUR extract	Broth	(+)	(+)
			Trypsinized broth	++	++
5	Water agar	Nutrose	Broth	+	+
			Trypsinized broth	++	++

Both meningococci grew on agar containing trypsinized broth but not on that containing ordinary broth; and in the remaining three experiments in which growth took place it was always better on the trypsinized broth than on the other. For some reason not ascertained, but possibly from surviving antiseptic action of the sample of chloroform which had been added to it as a preservative, the serum used in this experiment inhibited growth.

It is clear, however, from this experiment that, as had been indicated in the preceding one, trypsinized broth is more favourable to the growth of the meningococcus than ordinary broth. Further, the addition of peafLOUR extract, nutrose, or ascites fluid to trypsinized broth increases the growth of the meningococcus in the way that these substances do when added to ordinary broth.

EXPERIMENT VII.

The following experiment was made for the purpose of confirming the previous ones, and also to ascertain if another sample of serum (bullock's) containing haemoglobin in solution from lysed corpuscles offered any advantage for culture of the meningococcus. Each addition was made to the extent of 10 per cent. of the medium.

Result Forty-eight Hours at 37° C.

No.	Menstruum.	Addition 1.	Addition 2.	Meningo-coccus 1.	Meningo-coccus 2.
1	Water agar	Trypsinized broth		+	+
2	" "	PeafLOUR extract		—	+
3	" "	H. B. serum		—	—
4	" "	" "	Trypsinized broth	+	+
5	" "	" "	PeafLOUR extract	+	(+)
6	" "	" "	Both above	++	++
7	" "	Trypsinized broth	PeafLOUR extract	++	++

As before, the addition of serum by itself to water agar failed to induce growth, and the best results were obtained with peafLOUR extract (experiments 6 and 7). Growth was so good when both these substances were added that the further addition of serum seemed to make no difference to it.

EXPERIMENT VIII.

The object of this experiment was to ascertain the effect of adding glucose.

Water agar was used as before. To it were added in eight successive plates, respectively, 10 per cent. of peafLOUR extract, serum, trypsin broth, peafLOUR extract and serum, peafLOUR extract and trypsin broth, serum and trypsin broth, and finally peafLOUR extract, serum and trypsin broth all three at once. In a duplicate series the experiment

was repeated with the addition in each case of 1 per cent. glucose to the medium.

No.	MEDIUM.					RESULT.	
	Menstruum.	Addition 1.	Addition 2.	Addition 3.	Addition 4.	Meningococcus 1.	Meningococcus 2.
1	Water agar					-	-
					Glucose	-	-
2	Water agar	Peafour extract				-	-
					Glucose	-	-
3	Water agar	Serum				-	-
					Glucose	-	-
4	Water agar	T. broth				-	-
					Glucose	-	-
5	Water agar	Peafour extract	Serum			(+)	(+)
					Glucose	(+)	(+)
6	Water agar	Peafour extract	T. broth			(+)	(+)
					Glucose	(+)	(+)
7	Water agar	Serum	T. broth			+	+
					Glucose	++	++
8	Water agar	Serum	Peafour extract	T. broth		++	++
					Glucose	++	++

Result.—It is seen that glucose only produced a marked effect in the second half of subexperiment 7. As trypsin broth itself contains a considerable quantity of sugar, no doubt when the agar contains 100 per cent. of broth instead of 10 per cent., as in this experiment, the addition of glucose is superfluous.

EXPERIMENT IX.

This experiment was made to ascertain the value of Savory and Moore's fluid extract of meat as a substitute for trypsin broth. The preparation was tried because it contains, as Mr. Mackenzie Wallis pointed out to us, a comparatively large amount of true peptones.

Water agar was used as before, and to 70 c.cm. of the melted agar 30 c.cm. of a 10 per cent. solution of Savory and Moore's preparation was added. To this was then added in a succession of plates, glucose, serum, peafour extract, finally glucose, peafour extract and serum all three together.

Result Forty-eight Hours at 37° C.

No.	Menstruum.	Addition 1.	Addition 2.	Addition 3.	Meningococcus 1.	Meningococcus 2.
1	S. and M. extract in water agar				-	-
2	"			Glucose	-	-
3	"	Serum			(+)	(+)
4	"	Serum		Glucose	+	+
5	"	Peafour extract			-	-
6	"	Peafour extract		Glucose	Trace	Trace
7	"	Peafour extract	Serum		++	++
8	"	Peafour extract	Serum	Glucose	++	++

It would seem that, although the preparation in question contained a considerable quantity of peptones, it had no striking value for the present purpose. Savory and Moore's meat extract, however, has some value for increasing growth of the meningococcus, for a control experiment made at the same time as subexperiments 7 and 8, without addition of this meat extract, showed less abundant growth than 7 and 8.

EXPERIMENT X.

This experiment was made to ascertain the point at which concentration of salt in this medium inhibits growth of the meningococcus. The medium used was agar

2½ per cent. dissolved in trypsin broth and containing 5 per cent. of peafour extract, the "trypagar" introduced as the result of the preceding experiments. This medium is rendered neutral to phenolphthalein.

Salt was distributed in plates of this medium in increasing amounts, and examples of four different types of meningococci isolated from the cerebro-spinal fluids of cases in the present outbreak were then inoculated on each plate. The results were as follows:

Result Forty-eight Hours at 37° C.

No.	Percentage of Salt in the Medium.	Meningococci.			
		Type 1.	Type 2.	Type 3.	Type 4.
1	1.0	+	+	+	+
2	1.5	(+)	(+)	(+)	(+)
3	2.0	(+)	-	(+)	-
4	3.0	Trace	Trace	(+)	(+)
5	4.0	-	-	-	-
6	5.0	-	-	-	-

Growth of all four meningococci became diminished when the salt exceeded 1 per cent., and at 4 per cent. it was arrested.

GENERAL RESULT.

As the outcome of these experiments it was decided to make a practical trial of the following medium:

Agar 2½ per cent. dissolved in the trypsinized broth of Douglas.

To this when it has reached the final stage is added 5 per cent. of a sterilized extract of peafour prepared as follows: Peafour 10 grams, salt 10 grams, distilled water 100 c.cm. Steam for twenty minutes and filter. Sterilize the filtrate by steaming on two successive days.

By reason of some observations which will be referred to later, this medium, trypsin broth, pea agar, or "trypagar," is now made neutral to phenolphthalein.

Trial of Trypagar as a Routine Medium for the Meningococcus.

Since its introduction over two hundred meningococci originally isolated from the cerebro-spinal fluid of cases of meningitis have been subcultured on to trypagar, with the result that they have all been found to grow readily on it; moreover, plates of this medium have been in continual use for growing meningococci in bulk for the purpose of making standard suspensions of them for agglutination work.

Trypagar has also been found to grow the meningococcus in primary culture from the cerebro-spinal fluid in several cases where an opportunity of testing this point has occurred.

The chief object, however, for which a culture medium was required in the present connexion was for cultivating the meningococcus from the nasopharynx of contacts. The following observations were made for the purpose of deciding the suitability or otherwise of trypagar for this purpose:

TEST I.

Swabs from the nasopharynx of six contacts were each plated on the following media:

1. Nasgar.
2. Nasgar with the addition of 1 per cent. of blood.
3. Nasgar with the addition of 2 per cent. of blood.
4. Trypagar with the addition of 2 per cent. of serum.

The growth of meningococcus-like colonies on these plates was as follows:

1. On nasgar one case showed meningococcus-like colonies, and two others showed slightly suspicious colonies.
2. On nasgar enriched with blood the same case showed meningococcus-like colonies, and three others showed slightly suspicious colonies.
3. On nasgar enriched with serum the results were the same as with blood.
4. On trypagar enriched with serum five of the six cases showed colonies resembling those of the meningococcus.

On examination of subcultures of meningococcus-like organisms, all were rejected except two. One of these had come up on all the media; the other had developed only on the plate of trypagar enriched with serum.

TEST II.

Five days after the test just related these same six contacts were re-examined, and at the same time nine persons in their proximity were swabbed, making in all fifteen cases.

Each of the fifteen swabs from these men was cultivated on plates of the following media in turn:

1. Trypagar.
2. Trypagar enriched by 2 per cent. of serum.
3. Trypagar enriched by 1 per cent. of nutrose.
4. Trypagar enriched by 2 per cent. of serum and 1 per cent. of nutrose.

Meningococcus-like colonies appeared on nine of the plates of media 1 and 2, and in only two of the plates in 3 and 4. On testing these suspicious organisms only two of the cases were found positive. These were the same two cases that had been positive before, and in the present series they were positive on all four media.

TEST III.

After a further interval of five days these fifteen men were again swabbed.

Each swab was plated on three media.

1. Trypagar.
2. Trypagar enriched by 2 per cent. of serum.
3. Nasgar.

Meningococcus-like colonies appeared on medium 1 in two cases, on medium 2 in five cases, on medium 3 in three cases. On examination of their characters all of these Gram-negative cocci were rejected except one, and that had come up on all three media. The case from which it had been obtained had been positive in the previous two tests.

The second and third of these tests, therefore, showed that trypagar by itself, without enrichment, was successful for growing micrococci indistinguishable from the meningococcus from the nasopharynx. In this respect it appeared to give as good results as, if not better than, nasgar, and as trypagar is much easier and cheaper to make than nasgar it was adopted for routine purposes.

It has been mentioned that trypagar was successful for the object in view without enrichment. The addition of 2 per cent. of serum, or better, ascites fluid, seems to increase the size of the meningococcus colonies, and sometimes their vitality on the medium.

Since these tests were made we have on over a dozen occasions plated swabs from the nasopharynx of contacts or carriers on to trypagar plates at the same time with and without addition of ascites fluid. In all those cases in which meningococcus-like colonies appeared they came up on both plates.

Reaction of the Medium.

At an early stage of the present inquiry an experiment was made to see what reaction of the medium was best suited for culture of the meningococcus. Strains of this micro-organism were tested on plates of trypagar the reaction of which ranged from 0.0 to +1.6 to phenolphthalein. Growth of the strains occurred at +0.4 and continued up to +1.6; it appeared to be best at about +1.0, and accordingly this titre was adopted.

When opportunity offered later on, however, of testing contacts and carriers, the question of titre was reopened, and it was found that while there was as a rule growth of the meningococcus-like colonies in plates having a reaction of +1.0, they grew even better in plates the reaction of which was neutral to phenolphthalein. On two occasions also a swab was found to yield a positive result on the neutral plate when it was negative on the corresponding plate +1.0 to phenolphthalein. Some further experiments with delicate meningococci from the cerebro-spinal fluid having given a similar result, from this time onwards the trypagar was sent out neutral in reaction.

CONCLUSIONS.

The five requirements of a medium desirable for the present purpose have been previously stated. Trypagar prepared in the manner described has been found to fulfil four of them with satisfaction, and has, therefore, been adopted for routine use. Since May, 1915, over eight thousand bottles of this medium have been made and supplied to district laboratories at about half the cost of nasgar. With each bottle, sterile horse serum is sent out for addition in the case of primary cultures. Some comparative tests having indicated that a solution of haemoglobin gives even better enrichment, attempts are being made to apply this in place of the serum for routine purposes.

So far the fifth requirement is the main one that this medium fails to satisfy. Although growth of the meningococcus is excellent in trypagar, this micro-organism as a rule does not survive on it for over forty-eight hours.

The cause of this brief vitality of the meningococcus on a medium so well suited to its development in the first place, and the task of defining the value of the peafLOUR extract more closely from the chemical standpoint, are matters that have engaged the attention of Captain Martin Flack, to whom the research was handed over at this point.

FORMULA FOR TRYPGAR.

1. PeafLOUR Extract.

Take 100 grams of peafLOUR (ordinary Pearce Duff's), and add 1 litre of distilled water with 100 grams of salt. Mix and steam for half an hour, stirring occasionally. Allow to settle, and filter, then sterilize and label "Saline Pea Extract." This peafLOUR extract should preferably be freshly made for each batch of agar.

2. Trypsin Broth (Douglas).

Take some fresh bullocks' hearts, free from fat and vessels, mince the meat very finely, and weigh. To each ½ kilo add 1 litre of water, and make faintly alkaline to litmus with 20 per cent. KOH solution. Heat this slowly to 75°-80° C. for five minutes. Cool to 37° C., and add 1 per cent. of liquor trypsinæ co. (Allen and Hanburys), and keep it at 37° C. for two and a half to three hours. When trypsinizing is finished, test for peptone with copper sulphate and KOH as below, then render slightly acid with glacial acetic acid, and bring slowly to the boil for a quarter of an hour. Leave overnight in a cool place, and siphon off the clear liquid in the morning. Make faintly alkaline to litmus, and sterilize in an autoclave at 118° C. for one hour on each of two days (if not to be used at once).

To Make Trypagar.

Take a measured quantity of the trypsinized broth, add 2 per cent. of agar fibre (see below for preparation) and 0.125 gram of calcium chloride per litre. Autoclave at 118° C. for three-quarters of an hour to dissolve the agar. Mix together in an urn or saucepan; titrate with N/10 KOH while boiling, using phenolphthalein as the indicator, and add the necessary amount of normal KOH to give an absolutely neutral reaction. Cool to 60° C., add white of egg (2 to a litre) beaten up with the crushed shells, autoclave again at 118° C. for 75 minutes (or in the steamer for two hours). Filter, add to the filtrate 5 per cent. of the sterile pea extract, and sterilize in the ordinary way.

Preparation of Fibre Agar.

Weigh out the required quantity, cut up small with scissors, place in a large flask or enamel pail and wash twice quickly in water. Drain thoroughly; add water just to cover, and put in glacial acetic acid, 2.5 c.cm. per litre of water. Mix thoroughly and leave for a quarter of an hour. Pour off the liquid and wash thoroughly four or five times to make sure that all the acetic acid is washed out. Drain carefully and use as above.

Biuret Reaction for Peptone.

Take 5 c.cm. of broth, add 0.1 c.cm. of 5 per cent. solution of CuSO_4 ; mix, and then add 5 c.cm. normal NaOH. A true pink colour indicates that trypsinization is sufficient; a bluish-purple shade that it is incomplete.

Trypagar medium prepared as above is sent out from the Central Laboratory to district laboratories in sterilized bottles each containing 200 c.cm. A small phial of sterile serum is supplied for addition in the proportion of 1 of serum to 50 of medium in the case of primary cultures.

NOTE ON THE CONSTITUENT OF THE PEAFLOUR AUGMENTING GROWTH OF THE MENINGOCOCCUS ON THE TRYPGAR MEDIUM.

By CAPTAIN MARTIN FLACK, R.A.M.C.

(From the London District Laboratory for Cerebro-spinal Fever, Royal Army Medical College, S.W.)

In order to ascertain the nature of the constituent of peafLOUR which favours growth of the meningococcus when added to a broth agar medium, investigation was pursued along the following lines:

1. Analysis of the PeafLOUR.

Analysis of the peafLOUR showed that it contained legumin, starch, dextrins, salts, and extractives. No sugar was present. Analysis of the peafLOUR extract as added to the medium in the course of its preparation gave quantitatively the following results:

Solids	...	...	...	...	...	13.58 per cent.
Ash	...	...	...	...	...	10.11 "
Organic matter	...	...	...	...	...	3.47 "

The amount of organic nitrogen as determined by Kjeldahl's method was 0.07 per cent. This small amount of nitrogen precludes the possibility of the presence in the

extract of any large amount of a body of protein nature. The high percentage of ash is accounted for by the 10 per cent. saline solution used in preparing the extract.

2. Cultural Experiments.

Cultural experiments were made upon media prepared by adding various solutions obtained from the peafLOUR.

- To agar dissolved in water.
- To water agar to which trypsinized broth was added.
- To water agar to which was added caseinogen hydrolyzed for sixteen hours in an autoclave.

The following solutions were employed:

Solution A.—Prepared by boiling peafLOUR with 10 per cent. NaCl and filtering. Such a solution contains starch, dextrins, extractives, and salts.

Solution B.—Prepared by extracting the peafLOUR with 10 per cent. saline in the cold, boiling to get rid of coagulable protein, and filtering. This solution contains dextrins, extractives, and salts.

Solution C.—Prepared by precipitating the starch and dextrins by alcohol from the first-named solution and then removing as much alcohol as possible from the filtrate by boiling. Such a solution contains extractives and salts.

No phosphotungstic or phosphomolybdic acid was available to test the amount of precipitate which could be obtained from this filtrate with these reagents.

In addition to these solutions, there was prepared from the peafLOUR (Solution D) a solution of legumin. This was used, since an early experiment in the previous paper had shown that legumin exerts a favourable effect upon the growth of the meningococcus.

EXPERIMENT I.

The first experiment was made by adding the solutions singly to water agar and inoculating each plate with one loop from a culture of meningococcus in ascites broth. The result was as follows:

Medium.	Contents of Solution Added.	Growth.
Water agar + Solution A ...	Starch Dextrins Extractives Salts	—
Water agar + Solution B ...	Dextrins Extractives Salts	—
Water agar + Solution C ...	Extractives Salts	(+)
Water agar + Solution D ...	Legumin	—

Water agar 9 parts.
Solution 1 part.

— = No growth.
(+) = Slight growth.

EXPERIMENT II.

In the second experiment the solutions were added to trypsinized beef broth agar.

Medium	Growth.
Broth agar + A	++
" " + B	++
" " + C	++
" " + D	++
Broth agar only	++

Broth agar 9 parts.
Solution 1 part.

++ = Abundant growth.

EXPERIMENT III.

A similar experiment was tried, using caseinogen agar in the place of broth.

Medium.	Growth.
Caseinogen agar + A	+
" " + B	++
" " + C	++
" " + D	—
Caseinogen agar only	—

Water agar 18 parts.
Caseinogen 1 part.
Solution 1 part.

++ = Abundant growth.
+ = Moderate growth.
— = No growth.

It will be observed that with broth agar good growth was obtained upon all the plates. With caseinogen agar moderate growth was obtained with Solution A, good growth with Solutions B and C, and no growth with legumin solution only, or with caseinogen only. The results with the broth agar showed that the proportion of broth in the agar was too great to bring out differences.

Other tests, therefore, were made, using a smaller percentage of broth—namely, one of solution, and two of broth to seven of water agar. As before, each plate was inoculated with one loop of meningococcus culture in ascites broth. The results were as follows:

Medium.	Experiment IV.	Experiment V.
Agar + Broth + Solution A	+	+(+)
" + " + Solution B	++	++
" + " + Solution C	++	++
" + " + Solution D	—	—
Agar broth only	—	—

Broth 2 parts.
Solution 1 part.
Water agar 7 parts.

++ = Abundant growth.
+(+) = Good growth.
+ = Moderate growth.
— = No growth.

It will be seen that best growth occurred with Solutions B and C, although a favourable growth also occurred with Solution A. No growth occurred with legumin and broth, or with broth only.

From a consideration of the contents of the various solutions used in these experiments it is obvious that the agent favouring the growth of the meningococcus is one which must be grouped among the extractives. Such an extractive may provisionally be classified as being of the nature of a vitamin.

It appeared of interest to put this conclusion to the test. The germ of wheat was selected for the purpose, since this has been shown by the writer and Leonard Hill to be rich in vitamin.

Similar 10 per cent. NaCl solutions were prepared from raw wheat germ and from the patent Hovis cooked wheat germ respectively. Each of these extractives was then separately added to water agar, to water agar to which broth had been added, and to water agar with the addition of caseinogen. Each of the media thus prepared was inoculated with one loop of meningococcus culture.

In the experiments with water agar only no growth occurred; with caseinogen the results were inconclusive; but in the broth experiments a distinctly favourable influence was exercised upon the growth of the meningococcus. This vitamin, however, did not produce by any means so favourable an effect upon the growth of this micro organism as did the extractive from the peafLOUR.

It has been suggested that vitamins are of two kinds, (1) those which favour growth, and (2) those which favour vitality.

Since the meningococcus is but short-lived upon tryptic agar, it appeared of interest to compare the above extractives with regard to their respective influence on the longevity of the meningococcus.

On examination of the cultures made in the preceding experiments after the lapse of some days at 37° C., it was found that the meningococcus was dead upon all the plates except those containing the cooked or raw germ. The cultures on the plates in the first test lived sixteen and twenty days. In further tests it was again found that the cultures after some days at 37° C. were alive only upon plates containing extract of germ.

The extractive from the wheat grain therefore appears to have the property of prolonging the vitality of the meningococcus upon medium to which it has been added. In illustration of this, it was found that a specimen of meningococcus Type 1 lived for ten days, of Type 2 for seventeen days, and of Type 3 for twenty-eight days upon a slope of peafLOUR tryptic agar to which germ extract had been added.

Stab cultures of this medium (trypticized broth agar containing extracts of both peafLOUR and germ) have been in use by me for some time, and have proved equally as efficacious as starch stabs for stock cultures. Subcultures

have shown that occasionally the meningococcus may live on either starch or germ agar for as long as two months.

The extractive in the peafLOUR, therefore, would appear to be concerned rather with favouring the growth of meningococcus than with preserving its vitality.

That the extractive contained in the peafLOUR is probably of a vitaminic nature is shown also by the fact that the tryptic medium, after it has received the peafLOUR extract, deteriorates if autoclaved, and after submission to such treatment may absolutely fail to grow the meningococcus. The presence of vitaminic is difficult to prove chemically, but the above experiments clearly indicate that the essential constituent of the peafLOUR is a body of an extractive nature, not precipitated from solution by alcohol, and that it may provisionally be classified in the group of bodies known as "vitamines." The experiments in question suggest, further, that possibly a deficiency, to which attention has been drawn in the preceding paper, may be remedied by adding to the medium an extract of the germ of wheat.

EPIDEMIC CEREBRO-SPINAL MENINGITIS:

ITS BACTERIOLOGY AND PATHOLOGY.

BY

W. J. DENEHY, M.D., B.S.MELB.,

CLINICAL PATHOLOGIST TO THE ALFRED HOSPITAL, MELBOURNE.

THE extreme variability, the comparative uncertainty of our bacteriological findings in this disease, and the impossibility of making an absolute diagnosis on a clinical basis alone, especially in the milder types, have combined to influence me in publishing my conclusions from the examination of an enormous number of specimens in the laboratory from the 368 cases that have been admitted to the Alfred Hospital, Melbourne, during the past eleven months (to July 1st, 1916).

An endeavour is made in this article to give the laboratory findings in the Victorian epidemic, the cases dealt with being those occurring in the adult male population of the metropolitan district, together with a considerable number from country districts.

Of the 368 cases under review 207 occurred among soldiers in camps and the remainder among the civil population—the great majority within the first few months being from the military population, the civil population providing the greater number during the summer months. For the last three months the numbers are as follows: 31 soldiers, 50 civilians. These figures show how, with the increased number of foci as the epidemic proceeds, the civil population begins to suffer more severely.

First, in dealing with suspected cases, the examination of cerebro-spinal fluid is all-important. If the fluid is turbid the patient is admitted without delay, the fallacy of this proceeding being evidenced by the occasional presence of a pneumococcal meningitis or a septic meningitis, and, in a few cases, the turbidity has been due entirely to red blood corpuscles from accidental sources or from cases of cerebral haemorrhage.

Procedure.

In my experience the greatest cause of mistaken positive diagnosis has been in regarding a turbid or opalescent fluid, due to red blood corpuscles, as a cerebro-spinal fluid, turbid owing to the presence of polynuclear leucocytes or organisms, this fault lying with both hospital residents and private practitioners.

The microscopic examination of a wet specimen is the next procedure—(1) not centrifuged, (2) sedimented or centrifuged. The presence of polymorphonuclear leucocytes in appreciable numbers is taken as a positive diagnosis of an acute meningitis, not necessarily cerebro-spinal. Coccal forms can be seen even in the wet state. They are usually intracellular, contrary to the experience of Douglas¹ in early cases. Frequently also in the early cases mononuclears are quite as numerous as polynuclears. In only 3 per cent. of cases were mononuclears found to be the outstanding feature of the cerebro-spinal fluid, and in half of these polynuclears were very numerous also. The period when mononuclears were most in evidence certainly appeared to be in the early stage (that is, the early

part of septicaemic stage under Lundie's classification²) of the disease. This period immediately succeeds the stage of clear fluid with a positive potassium permanganate test, as described later.

The presence of fibrin is noted; the fibrin clot in a positive case usually settles in a few hours together with the majority of cells present. Excess of globulin is estimated by the simple ring test with saturated solution of ammonium sulphate. An enormous increase in globulin is a marked feature in the clear fluid after centrifuging. In all cases the potassium permanganate test is performed. Frequently one finds a clear fluid with no cell content, excepting, perhaps, a few mononuclears, no fibrin, and negative globulin test, and with no organisms, but giving an immediate potassium permanganate test (Lundie²).

In 51 different cases of this series the above result was obtained—that is, no abnormality was found except positive potassium permanganate test. The fallacy of this test is in a cerebro-spinal fluid containing red blood corpuscles; this invariably gives a positive reaction. Even if cerebro-spinal fluid is contaminated with red blood corpuscles, we still have an accurate means of judging the reaction to potassium permanganate.

If we centrifuge the cerebro-spinal fluid till we get a clear fluid free of haemoglobin and cells, we find in an early case of cerebro-spinal meningitis a positive potassium permanganate test, a negative test being obtained in non-meningitic cases.

I wish to add my recommendation of this test to that of Lundie and his co-workers.² Especially will it be of value to men in general practice, or in practice away from the advantages of laboratory diagnosis; for it appears to me that not only in cases where the fluid is turbid (with exceptions referred to) a diagnosis of some type of purulent meningitis can be made, but also a positive diagnosis of acute meningitis can be made with little risk of mistake in cases showing clear fluid if the potassium permanganate test is positive (avoiding the fallacy as above, as I suggest).

I do not wish to say that this test is specific in early cerebro-spinal meningitis, but after a large number of examinations of cerebro-spinal fluids from non-meningitic cases (128 examinations) I have come to this conclusion as to the value of the test, my only doubt being that a positive reaction may be obtained in septic non-meningitic cases.

These cases (with clear fluid giving positive potassium permanganate test) have at all times been admitted to hospital, and my experience is that, within twenty-four to forty-eight hours of admission, the cerebro-spinal fluid is found to be of the usual turbid type of cerebro-spinal meningitis.

Case 207.

Admitted October 10th, 1915. Died October 13th, 1915.
October 10th.—Cerebro-spinal fluid: No cells; no organisms. Fibrin —; globulin —; potassium permanganate + (immediately).
October 11th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Fibrin +; globulin +; potassium permanganate + (immediately). Intracellular and extracellular diplococci.
October 14th.—Autopsy: Brain shows pus along sulci over vertex and at base. Enormous distension of ventricles. General congestion of organs. Internal hydrocephalus.

Case 354.

Admitted June 14th, 1916. Relieved July 3rd, 1916.
June 15th.—Cerebro-spinal fluid: No cells; no organisms. Fibrin —; globulin —; potassium permanganate + (slow).
June 17th.—Cerebro-spinal fluid: Polymorphonuclear + + +. Mononuclear + +. Occasional red blood corpuscles. Fibrin +; globulin +; potassium permanganate + (immediately). Many intracellular and extracellular diplococci.
June 21st.—Swab: Staphylococci.

In the well-marked meningeal stage the potassium permanganate test, of course, is unnecessary from a diagnostic point of view; but, following Sophian,³ I attempted a quantitative test, as follows: Potassium permanganate (1 in 1,000) is poured from a measure into 1 c.cm. of cerebro-spinal fluid. The purple permanganate colour is usually immediately lost, the brown colour, due to reduction, replacing it. More potassium permanganate solution is added in small quantities until, on standing for three minutes, some purple colour remains. It was found that to the cerebro-spinal fluid of those most seriously ill seven to eleven times its volume could be added before the fluid ceased to reduce the potassium permanganate. Further work may determine a very definite prognostic value for this quantitative method.