A STUDY OF MENINGOCOCCI OCCURRING IN THE SPINAL FLUID AND OF SIMILAR ORGANISMS IN THE NASO-PHARYNX.¹

By W. M. SCOTT, M.D.

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INTRODUCTION.

THE work has been done in the Local Government Board's Pathological Laboratory, and during its course has had the benefit of much advice and criticism from Drs Eastwood and Griffith, the Board's pathologists.

The task proposed was threefold, (1) to determine the period during which cases of cerebro-spinal fever remain infective, *i.e.*, for how long during convalescence meningococci can be cultivated from the naso-pharynx; (2) to establish if possible the specific identity of meningococci isolated from the cerebro-spinal fluid with those got from the naso-pharynx of the same case; (3) while these pathological throats were being studied, and providing presumably pathogenic meningococci, to search among the flora of the normal, or at least "non-contact" naso-pharynx, for micro-organisms liable to be mistaken for pathogenic meningococci or actually indistinguishable from these. Some opportunity was also afforded for observing whether meningococci were present in the blood stream during the acute stage of cerebro-spinal fever.

The case material was provided by Dr Foord Caiger, of the Metropolitan Asylums Board South-Western Fever Hospital, to whom I wish to express my very great indebtedness for the large facilities he granted for visiting and examining the cerebro-spinal fever cases under his care.

Dr A. L. Baly, the Medical Superintendent of the Lambeth Infirmary, was kind enough to allow me to take cultures from the naso-pharynx of "non-contacts" attending his out-patient department.

I have pleasure also in thanking Dr Arkwright, of the Lister Institute, who gave me cultures of 25 strains of meningococci which he had isolated from the spinal fluid of cases of cerebro-spinal fever during the recent epidemic.

A. INVESTIGATION OF CEREBRO-SPINAL FEVER CASES.

Technique of Culture.

Naso-pharyngeal Cultures. The examination of the naso-pharynx in all cases was performed personally by abstracting a small portion of mucus from the posterior nares by means of a swab attached to the end of a bent rod, taking care to avoid contamination by the fauces or buccal mucosa. In the case of patients unable to sit up I found it difficult to avoid such contamination, and many of the "negative"

swabs recorded during the acute stages of the disease were due to overgrowth of the plates by contaminating organisms.

The mucus abstracted was deposited immediately on freshly poured plates: these were conveyed without delay to the laboratory (usually within one hour), and there the mucus was rubbed over the plate with a right-angled glass rod, this rod being then used for inoculating a second plate of the same medium.

Plates were examined after 24 hours' incubation at 37° C. and again after 48 hours. Suspected colonies were examined first microscopically; when typical micrococci were found a little of the colony was emulsified in sterile saline and used for inoculating tubes of solidified egg, ordinary nutrient agar, ascitic agar, and ascitic agar containing one of each of the following sugars—glucose, maltose, saccharose, and levulose. Strains corresponding culturally with the meningococcus were kept on egg in sealed tubes at 37° C. On this medium I found that they maintained their vitality for at least four months without sub-culture, though routine sub-culture was performed on egg at intervals of one or two months.

Cultures from meningeal fluid were isolated in similar fashion from plates inoculated with the deposit from the centrifuged fluid.

Media Employed for Primary Plates. Kutscher's serum-agar was always employed. It was prepared from fresh human placenta 500 grams to the litre, a boiled extract being made as with meat after mincing; to this nutrose 2 %, glucose 1 %, peptone (Chapoteaut) 1.5 %, and agar 2.5 % were added, the reaction being brought to + 8 (Eyre) after steaming; this was stored solid in bottles containing 75 c.c., which for use were melted and brought to 50° C.; 25 c.c. of sterile (filtered) ox serum were then added and the mixture poured at once. Comparison with similar agar lacking the placental extract showed that on primary cultivation the meningococcus-like organisms on Kutscher's medium grow more rapidly and produce larger colonies before growth stops.

The medium used for fermentation tests was litmus ascitic agar brought to + 5 (Eyre), the different sugars being added in sterile solution to make 1 % just before sloping.

Microscopical and Cultural Characters.

Microscopically meningococci are micrococci varying considerably in size in different strains in primary colonies and showing much variation in size in individuals of the same colony: giant forms are usually present.

The *arrangement* is typically diplococcal, usually with distinct flattening of adjacent poles: tetrads are common but not invariably present. The presence of chain formation or pronounced staphylococcal groups rules out a colony as certainly not meningococcal.

Staining in young cultures is distinctly uniform with the exception of the giant forms which often overstain. All individuals are definitely Gram-negative [carbol-gentian-violet $\frac{1}{2}$ minute, Gram's iodine $\frac{1}{2}$ minute, absolute alcohol $\frac{1}{2}$ minute].

Colonies on Kutscher's agar are highly characteristic. After 24 hours they appear perfectly circular, slightly convex, with a sharp margin, and measure from 1 to 2 mm. across. The colour is pearl-grey, and when viewed by transmitted light they may show slight iridescence: this latter appearance, however, is much more common with colonies from cerebro-spinal fluid than with throat colonies, a fact which may depend on the material inoculated and on the presence or absence of contaminating organisms. The most typical feature, and that on which most reliance was placed, is the fine granularity when viewed under a $\times 8$ lens on the stage of a dissecting microscope and illuminated strongly but obliquely by tilting the mirror so that no direct rays reach the lens.

The consistence is also characteristic: the growth adheres well to the platinum needle without being tenacious; it is not watery nor does the colony break into fragments on touching. The colony is moist and emulsifies readily in water.

After 48 hours the colonies may reach 3 to 4 mm. in diameter but remain perfectly circular: they are less transparent in the centre, but do not become coarsely granular. Some strains acquire a rather characteristic ringed appearance after 24 hours, due apparently to a thinner zone of the colony between the centre and the outer part; these were mostly found to fall into a group, the "Clayton" group, which was distinguishable, as will be seen later, by fermentation and agglutination tests. Some members of this group, however, did not show this ringed appearance. There is never any *pigment* in primary colonies, but sub-cultures on egg usually show a faint orange-pink colour in the growth collected on a loop. This may even reach a distinct orange or buff colour with certain undoubted strains. Lemon-yellow pigment always excludes a colony; some primary colonies are perfectly colourless, but on sub-culture develop this pigment and can then be excluded on this as well as on other grounds.

Growth on ordinary agar slopes at 37° C., inoculated with an emulsion from the primary colony, never occurred with any strain

which passed the further tests. A certain number of pigmented organisms, however, also failed to grow on ordinary agar.

Growth at 22° C., on tubes similarly inoculated, depends on the medium employed: on ascitic agar it rarely, if ever, occurs, but on Kutscher slopes, as also on egg, definite colonies appear quite frequently in two to four days; on the latter media growth is usually abundant if the inoculation is made with a considerable mass of the colony.

Fermentation Tests.

The characteristic behaviour is fermentation of glucose and maltose only, but it has not been thought justifiable to reject strains which, inoculated from the primary colony, fermented only one of these two substances. Such single-sugar fermenters on later sub-culture ferment both sugars as a rule, though one may be only slightly attacked. One cerebro-spinal fluid strain, however, fermented no sugar when first isolated, and later fermented glucose only. All the yellow-pigment formers which were isolated fermented levulose in addition to these two sugars, and most of them also saccharose.

Differences in degree of fermentative activity and comparison with agglutination. Of the cerebro-spinal fluid strains sub-cultured from primary colonies five fermented glucose more strongly than maltose, seven *vice versâ*, while two fermented them equally. Of the cerebro-spinal fluid strains isolated by Dr Arkwright, I found that eight fermented glucose more strongly than maltose, eleven *vice versâ* and six equally. Of the strains isolated from the naso-pharynx of convalescent patients nineteen fermented glucose more strongly than maltose, ten *vice versâ* and ten equally.

The glucose-preferring strains showed often but not always the "ringed" type of colonies; they were further separated by the *agglutination* tests which follow and form a fairly definite group, the "Clayton" group (*v. infra*). Most of the maltose-preferring strains and most of the equal-fermenters fell into the "Boscombe" agglutination-group. Exceptions occurred however: one glucose-fermenting strain fell into the Boscombe group, and several equal-fermenters were placed by agglutination in or closely related to the Clayton group. The question of grouping will be discussed after describing the agglutination reactions, but it may be stated that on the strength of observation of the relative activity with which these two sugars were fermented, I could generally predict with which serum the strain would agglutinate.

AGGLUTINATION TESTS.

Technique of Preparation of Sera.

Monovalent sera alone have been used, and rabbits have been successfully immunised with four *strains*—Boscombe, Clayton, Chandler and Smith. Boscombe and Clayton were strains found in almost pure culture in the *naso-pharynx* of cases of cerebro-spinal fever early in convalescence (1 week and 4 weeks respectively from commencement of the disease). They were chosen as representing the best marked examples in my possession of predominating fermentation of maltose and glucose respectively, the other sugar in each case being only slightly attacked. Chandler and Smith were selected later on the ground of feeble agglutination with Boscombe and none with Clayton serum. They were isolated from the *cerebro-spinal fluid*, Chandler by Dr Arkwright, Smith by myself. Both fermented maltose and glucose equally strongly.

In the *preparation* of the sera two methods have been used. For Boscombe and Clayton sera prolonged immunisation was employed, but the agglutinating titre was not raised much higher in the succeeding three months of treatment than it appeared ten days after the second injection. In the case of Chandler and Smith sera of equal potency to the above were obtained in a fortnight by giving two maximum intravenous doses at an interval of three days. Ten days later a satisfactory serum was obtained from four of the six animals; two died (one with each strain) 24 hours after the second dose.

The *antigens* in the case of Boscombe and Clayton were living cultures on solidified egg, the dose being raised from $\frac{1}{2}$ a culture to $\frac{2}{3}$, and finally to a whole culture; the injections were made intravenously at intervals of one week. The animals all lost weight during the first two months, but were regaining it during the later stages of immunisation. They were young rabbits of 1500 grams to 1800 grams. In the case of Chandler and Smith whole living cultures on Kutscher's slopes were injected intravenously on each occasion.

The titre of all these sera may be stated as complete agglutination of the homologous strain in a dilution of 1 in 600; well-marked agglutination with some remaining turbidity took place at 1 in 800; higher dilutions were not systematically employed.

Failures. One rabbit of three immunised with Clayton never produced a serum agglutinating completely at a higher dilution than

1 in 200. With two other strains, one from the throat and one from the lumbar fluid, all the rabbits employed (two and three respectively) failed to produce sera agglutinating completely higher than 1 in 200. These sera have not been used for systematic tests pending repetition of the attempt to produce with the strains sera of higher titre. The failure may depend on poor agglutinogenic properties in the *antigens*, but it must be taken into account that rabbits vary much in their response to immunisation.

Technique of agglutination tests.

The macroscopic method was used throughout and the mixtures were incubated for 24 hours at 55° C.

The micrococcal emulsions were made from glucose ascitic agar cultures of 24 hours' incubation, sown from 24 hours' egg cultures; the growth was washed off with saline, the emulsions thoroughly shaken, allowed to stand for 4 hours and then pipetted off so as to avoid coarse flakes of growth. Such emulsions remain in suspension for 2 to 4 days without producing visible deposit.

Newly-made emulsions, unheated, were used for all tests. All were brought to a standard opacity by comparison with a standard barium sulphate suspension corresponding in opacity to an emulsion containing 10 mg. moist growth per c.c.

The mixtures were put up in Durham's tubes calibrated so that equal portions were marked off, each containing about 0.3 c.c.; the diluted serum was first put in, then the emulsion run in quickly so as to insure mixture. A control of normal rabbits' serum diluted 1 in 100 was put up on each occasion with each emulsion.

Variations in agglutination.

Four to five agglutination tests have been made with each strain at intervals of several weeks during which the strains remained in cultivation.

The great majority of the strains kept were very stable throughout both as regards the dilution of a particular serum required to produce complete agglutination and as regards the specificity, *i.e.*, the presence or absence of agglutination with different sera. This stability was no doubt favoured by the fact that the stock was maintained on solidified egg at 37° C., and the sub-cultures employed for agglutination tests,

with three exceptions, had not more than three or four sub-cultures intervening between them and the original colony.

None of the strains isolated from the lumbar fluid varied to a greater degree than might depend on small errors in the technique of the agglutination test, with the exception that with one strain, A 2, a gradual increase in agglutinability took place; originally giving only slight clumping with 1-100 Boscombe serum, it increased to complete agglutination at 1-100 and definite clumping at 1-200.

On the other hand one of the South-Western Hospital naso-pharynx strains, T. 1a, became suddenly inagglutinable or rather incapable of complete agglutination since clumps appeared at as high a dilution as before, but turbidity persisted in the strongest serum. An emulsion prepared from a 24 hours' growth sown from an old stock culture, two "generations" instead of six from the original colony, agglutinated to the original titre. The same phenomenon appeared again later with this strain, but was not so well marked. Both A. 2 and T. 1a were strains sub-cultured more frequently than the others owing to the fact that they were being used for immunisation, and this may account for the variation.

Four strains developed "auto-agglutinating" properties. In one case by returning to an old stock the original characters were recovered; another strain died out in spite of all efforts to revive it. The two others lost the auto-agglutinating property on further sub-cultivation. All four were throat strains and the appearance of auto-agglutination coincided with evident diminution in the vigour of growth.

Agglutination of spinal strains.

Tables are subjoined recording the results of agglutination tests with 14 strains from the South-Western Fever Hospital, and 25 isolated by Dr Arkwright. It will be observed that with each serum the maximum dilution is given which produced complete agglutination, *i.e.*, perfectly clear supernatant fluid above the mass of agglutinated cocci; with each serum the homologous strain showed this at 1 in 600. With most strains well-marked clumping, but with some remaining turbidity of the liquid, took place at considerably higher dilutions, this being represented in reading results with the mark ++. Dilutions higher than 1-800 were not used for systematic tests as it was considered that the complete agglutination point was the most satisfactory one for comparison. The ++ point is of some value in comparing poor agglutinators where no complete agglutination occurred.

TABLE I.

*Agglutination Reactions of Cerebro-Spinal Fluid Strains
from South-Western Fever Hospital.*

Strain	Serum Boscombe		Serum Clayton		Serum Chandler		Serum Smith	
	Highest dilution with complete agglutination	Highest dilution with agglutination = ++	Highest dilution with complete agglutination	Highest dilution with agglutinating ++	Highest dilution with complete agglutination	Highest dilution with agglutinating ++	Highest dilution with complete agglutination	Highest dilution with agglutinating ++
C.S. 1	600	800	—	—	100	200	600	800
„ 2	600	800	—	—	100	200	600	800
„ 3	600	800	—	—	200	200	600	800
„ 4	400	800	—	—	200	400	200	400
„ 5	400	600	—	—	—	100	400	800
„ 6	400	800	—	—	—	200	200	800
„ 7	600	800	—	50	200	400	600	800
„ 8	100	400	—	50	100	200	600	800
„ 9	—	100	400	600	—	—	50	100
„ 10	—	—	800	800	—	—	—	100
„ 11	—	—	400	400	—	—	—	—
„ 12	—	—	400	600	—	—	—	—
„ 13	—	—	400	600	—	—	—	—
„ 14	—	—	200	400	—	—	—	—
Blood 1	—	—	400	400	—	—	—	—

The table shows that strains C.S. 1 to 7 agglutinate strongly with the sera Boscombe and Smith, moderately with serum Chandler and are negative with serum Clayton except for slight agglutination at 1-50 with C.S. 7.

Strain 1 is from the cerebro-spinal fluid of Boscombe, whose nasopharynx strain was used for preparing the serum of that name.

Strain 8 is Smith and agglutinates accordingly to the full titre with its own serum; it responds rather feebly to Boscombe and Chandler and is almost but not quite negative to Clayton.

Strain 9 agglutinates strongly with Clayton, weakly with Smith, is negative to Chandler and almost but not quite so to Boscombe. Strains 10 to 14 and the blood strain, which was got from the same patient as strain 11, respond strongly to Clayton and are negative to the other three sera with the exception of 10 which reacts very slightly to Smith.

So far, then, a rough but fairly satisfactory grouping into two sets is evident with these strains of cerebro-spinal origin; Boscombe, Chandler and Smith sera pick out eight of the fifteen strains and reject almost but not quite entirely the remaining seven which respond instead to the Clayton serum.

On applying this grouping, however, to a further set of cerebro-spinal strains, those supplied by Dr Arkwright of the Lister Institute, evidence appears of additional affinities which bring into relationship strains which, on the strength of agglutination with Boscombe and Clayton sera, appear to be sharply distinguishable.

TABLE II.

*Comparison with strains supplied by Dr Arkwright.
Agglutination Reactions of Dr Arkwright's Cerebro-Spinal Strains.*

Strain	Boscombe		Clayton		Chandler		Smith	
	Highest complete agglutination	Highest ++ agglutination	Highest complete	Highest ++	Highest complete	Highest ++	Highest complete	Highest ++
A. 1	400	800	—	—	200	400	200	400
A. 2	100	200	—	—	600	800	200	800
A. 3	600	800	—	—	—	200	800	800
A. 4	600	800	—	—	100	400	400	800
A. 5	600	800	—	—	100	200	400	800
A. 6	800	800	—	—	200	800	400	800
A. 7	200	800	—	—	100	200	800	800
A. 8	600	800	—	—	200	600	400	800
A. 9	400	800	—	—	100	200	400	800
A. 10	200	800	—	—	600	800	200	400
A. 11	600	800	—	—	100	200	400	800
A. 12	600	800	—	—	100	200	400	800
A. 13	200	400	—	—	100	400	400	800
A. 14	400	800	—	—	—	200	400	800
A. 15	600	800	—	100	100	200	400	800
A. 16	200	800	—	—	—	200	200	400
A. 17	400	800	—	—	100	200	800	800
A. 18	400	800	—	—	—	100	400	800
A. 19	—	—	600	800	—	—	—	—
A. 20	—	—	400	800	—	—	—	—
A. 21	—	—	600	800	100	200	—	—
A. 22	—	—	400	800	400	600	—	—
A. 23	—	100	200	400	200	400	—	—
A. 24	—	—	200	800	100	200	400	800
A. 25	—	—	400	800	100	200	400	800

Here again the Boscombe and Clayton sera make a fairly sharp division, there being eighteen responding definitely to Boscombe and not appreciably to Clayton serum, while with the remaining seven the response is exactly reversed.

But among those agglutinated by Boscombe serum, one, A. 2, agglutinates weakly, the complete reaction not extending above 1-100. This is Chandler, which provides the serum of the name, and this serum

picks out along with fourteen of the Boscombe type four of the Clayton group which it agglutinates on the average quite as strongly.

Further the Smith serum, while it agglutinates all the Boscombe strains fairly strongly, also agglutinates two which are definitely of the Clayton type.

There is evidence thus that it is easy to erect groups among a collection of meningococcal strains on the basis of agglutination with single-strain sera, and it is interesting to note that the fairly definite grouping brought out by Boscombe and Clayton sera coincides very closely with the grouping by means of the fermentation tests referred to above. On the other hand, members of these groups have affinities unrevealed by the particular pair of sera chosen for grouping. In the table above it is evident that the gap between the well-defined Boscombe and Clayton groups is bridged by the Chandler agglutinated strains and by those agglutinated by serum Smith.

It may eventually be possible to correlate by agglutination tests all the different strains of meningococci which occur in nature and to align them in a series. In such a correlated series the Boscombe and Clayton cultures would occupy opposite ends and be connected with each other by a great number of gradually differing but closely related strains. The central members of the alignment would differ equally widely from both Clayton and Boscombe, while the Boscombe and Clayton type characters would increasingly predominate in the strains towards one or other end.

*Agglutination Reactions of Meningococcus-like Organisms in
the Naso-pharynx of Convalescents.*

Table III expresses the behaviour as regards agglutination with the same four sera of strains of micrococci which produced characteristic colonies on plates inoculated from the naso-pharynx of convalescents and which microscopically and culturally were indistinguishable from the meningococcus. No strain which passed the morphological and cultural tests failed to give complete agglutination with one or more sera diluted 1-100.

Each arabic numeral represents an individual patient while the letters attached indicate that the strain was isolated from the same patient on different occasions, the dates of which from the time of admission to hospital are given in the second column. Further, an additional column not included in previous tables is devoted to recording

TABLE III.

*Naso-pharyngeal Strains from South-Western Fever Hospital.
Cases of Cerebro-spinal Fever and Contacts.*

No. of case	Period after admission at which strain was isolated	Boscombe serum			Clayton serum			Chandler serum			Smith serum		
		Highest complete	Highest +	Agglutination at 1-100	Highest complete	Highest +	Agglutination at 1-100	Highest complete	Highest +	Agglutination at 1-100	Highest complete	Highest +	Agglutination at 1-100
T. 1a	6 days	600	800	c	-	-	+	100	200	c	600	800	c
T. 1b	2 months	-	100	++	-	100	++	200	600	c	-	100	+++
T. 1c	10 weeks	-	100	++	100	200	c	200	400	c	100	200	c
T. 1d	3 months	-	100	++	-	200	+++	-	100	++	200	800	c
T. 2a	3 weeks	400	600	c	-	-	trace	-	-	+	-	100	++
T. 2b	5 weeks	400	600	c	-	-	+	-	-	+	-	200	+++
T. 2c	7 weeks	400	600	c	-	100	++	-	200	+++	400	800	c
T. 3a	10 days	200	400	c	-	-	-	100	...	c	600	800	c
T. 3b	3 weeks	400	600	c	-	-	-	...	...	...	...	...	...
T. 4a	6 days	600	800	c	-	-	-	-	100	++	400	800	c
T. 4b	2 weeks	-	-	+	400	800	c	...	...	...	...	...	...
T. 4c	4 weeks	-	-	+	400	800	c	...	...	...	...	...	...
T. 4d	7 weeks	-	-	-	600	800	c	-	100	++	-	200	+++
T. 4e	8 weeks	-	-	-	400	800	c	...	...	...	...	...	...
T. 5	5 days	600	800	c	-	-	-	-	-	+	100	400	c
T. 6a	9 days	400	600	c	-	100	++	-	200	+++	400	800	c
T. 6b	40 days	400	600	c	-	-	+	-	200	+++	400	800	c
T. 7	2 days	200	800	c	-	-	+	100	200	c	600	800	c
T. 8	1 day	-	-	trace	200	400	c	-	100	++	100	200	c
T. 9	Contact, 3 weeks	-	-	-	400	800	c	-	-	trace	-	-	trace
T. 10	3 weeks	-	100	++	200	600	c	-	100	+++	-	100	+++
T. 11a	6 weeks	-	-	trace	100	200	c	-	-	-	-	-	-
T. 11b	7 weeks	-	-	-	200	400	c	-	-	trace	-	-	+
T. 12a	4 days	-	-	+	600	800	c	-	100	++	-	200	+++
T. 12b	3 weeks	-	-	-	400	800	c	-	-	+	-	100	++
T. 13a	4 weeks	-	-	trace	600	800	c	-	-	-	-	-	-
T. 13b	5 weeks	-	-	-	600	800	c	-	-	-	-	-	-
T. 13c	6 weeks	-	-	+	600	800	c	-	-	+	-	-	+
T. 13d	7 weeks	-	-	-	600	800	c	-	-	+	-	-	+
T. 13e	8 weeks	-	-	+	600	800	c	-	-	-	-	-	+
T. 13f	9 weeks	-	-	-	600	800	c	-	-	-	-	-	+
T. 14	3 weeks	-	-	+	200	400	c	-	-	-	-	-	-
T. 15	10 days	-	-	-	100	200	c	-	-	-	-	-	-
T. 16	4 weeks	100	200	c	-	-	+	-	-	+	-	-	+
T. 17a	Contact, 2 weeks	-	-	+	200	400	c	-	-	+	-	-	+
T. 17b	Contact, 3 weeks	-	-	+	200	400	c	-	-	+	-	100	++
T. 18	Contact, 3 weeks	-	-	-	100	200	c	-	-	+	-	100	++
T. 19	Contact, 2 months	-	-	trace	400	600	c	-	-	-	-	100	++

with each serum its agglutinating effect when diluted 1 in 100. The sign “+++” indicates that agglutination was almost but not quite complete, faint turbidity persisting, while “+” indicates clumping present but not well-marked. The “++” mark indicates, as before, well-marked agglutination with a good deal of turbidity persisting.

It will be seen that 12 of the 38 strains agglutinate most strongly with Boscombe serum, while 23 strains agglutinate most strongly with the Clayton serum; two strains agglutinate most strongly with Chandler and one with Smith.

Of those agglutinating completely with Boscombe one does not do so higher than at 1-100, but all but two of the others reach 1-400, while ten of those agglutinated completely by Clayton serum do not exceed the level of 1-200. Four of these last were from contacts with no meningeal symptoms who presented doubtful if any clinical evidence of naso-pharynx infection. The possibility will be shown later, when discussing non-contacts, that some of these strains were not abnormal inhabitants of the naso-pharynx.

*Persistence of Meningococci in the naso-pharynx of cases
of Cerebro-spinal Fever.*

In 16 cases regular examination of the naso-pharynx was possible. In eight of these, patients 1, 2, 4, 10, 11, 12, 13, 16, meningococcus-like organisms were got up to the end of treatment in hospital and were still presumably present on discharge, in one case after over 3 months, after 9 weeks in another, and 7 weeks in 3 others; the periods at which meningococci were found are shown in Table III.

Thus in 50 % of cases complete convalescence occurred before the naso-pharynx was free; the maximum period of persistence of meningococci was not determined but evidently exceeded 3 months.

In five cases negative results were obtained on all (at least two) successive examinations after positive results had been established; the last positive result was on the fifth day in one case and at various periods up to five weeks in the others: *vide* Table III, patients 3, 5, 6, 7 and 15. In the three remaining, undoubted cases of disease, no meningococcus-like organism was found in the naso-pharynx either during the acute stage or at any of five weekly examinations thereafter.

Cases 8, 9 and 14 in Table III presented doubtful symptoms of cerebro-spinal fever, but were not established as cases of the disease, spinal puncture not having been performed.

Cases 17 and 18 were contacts of the disease, but presented indefinite symptoms only and were not tapped, while case 19 was an enteric fever patient nursed in the same ward as the cerebro-spinal fever cases.

In addition, 23 patients from the cerebro-spinal fever wards were swabbed once with negative results; 10 of these were in advanced convalescence from the disease when first seen and 13 were enteric fever patients. These were the first swabs I took, and the negative results may in part be ascribed to lack of experience in examining plates from the naso-pharynx; the smaller colonies which later experience showed to be meningococcal might have been overlooked.

Meningococci in the Blood Stream.

Three attempts at cultivation of meningococci from the blood were made in three acute cases; two of these failed in spite of having incubated 9 c.c. of blood in 200 c.c. of serum broth. From the third, the most acute and eventually a fatal case, cultures were easily got by direct inoculation of a few drops of blood on slopes of Kutscher's agar. The strain thus isolated agreed in all respects with that obtained from the spinal fluid of the same case. Microscopical examination of the blood in this and five other acute cases was entirely negative as regards the appearance of diplococci within or without cells.

Comparison of Strains cultivated from the Spinal Fluid and the Naso-pharynx of the same patient.

This was possible in seven cases; the spinal strains being, in Table I, strains C.S. 1, 2, 4, 5, 7, 8, and 10, while the naso-pharynx strains corresponding are, in order, T. 1 (a, b, c and d), T. 2 (a, b and c), T. 3 (a and b), T. 4 (a, b, c, d, and e), T. 6 (a and b), T. 15, and T. 12 (a and b).

It will be seen that in all but the pair C.S. 7 and T. 15 a strain was isolated from the naso-pharynx corresponding closely in specific agglutination with that got from the spinal fluid. In four cases no other specific type than that found in the spinal fluid was cultivated from the throat.

In two cases, however, the pairs C.S. 1 with T. 1 and C.S. 5 with T. 4, only the first positive swab furnished a strain agglutinating like the spinal strain.

T. 1 a, like C.S. 1, was a strongly agglutinated "Boscombe" strain; T. 1 b, c and d were very feebly agglutinated by "Boscombe" serum,

but the first two agglutinated fairly well with "Chandler" serum and the last with "Smith" serum.

T. 4 a, like C.S. 5, was similarly of the Boscombe type, but T. 4 b, c, d, and e were almost pure Clayton strains agglutinating almost to the full titre of this serum and feebly or not at all with all other sera.

The question is raised by these results whether modification may go on in the naso-pharynx so that one type changes into the other. It is noteworthy that the plates from which these strains were obtained showed almost pure cultures of colonies indistinguishable from those picked for isolation of the strains.

An alternative hypothesis is that the later swabs were furnishing cultures of another, perhaps a normal, inhabitant of the naso-pharynx which had been swamped by the infecting strain at the time of the first examination.

The strain T. 15 which is also different from its spinal strain C.S. 8 raises the same question, but in this case the homologous strain was not recovered from the naso-pharynx, only the variant.

B. INVESTIGATION OF NON-CONTACTS.

Meningococcus-like Organisms in the Naso-pharynx of Non-Contacts.

The observations just discussed lead up to the question whether organisms resembling meningococci may be normal inhabitants of the naso-pharynx.

Among the Lambeth Infirmary out-patients from June 1st to July 15th, 1915, 150 swabs were taken, 38 from males, 112 from females; 12 of these were repeat swabs, 11 being seconds and 1 third. Of a total of 138 individuals 19 (13½ %) were of 14 years or under, while 66 (47·8 %) were of 50 or over.

Twenty-six were normal individuals, the rest suffered from various ailments—chronic coughs, rheumatism, "bad legs," etc. No connection with cases of cerebro-spinal fever could be discovered with any.

Thirty-five swabs yielded colonies *culturally* and *microscopically* indistinguishable from the meningococcus. Of these, however, two were repeats reducing the percentage of individuals with such suspicious organisms to 24 %. Of these, moreover, three eventually developed abnormalities necessitating their exclusion, so that the final percentage

of "positives" on the strength of microscopical and cultural characters was 22 %.

The colonies were noted as being in "almost pure culture" in 2 cases, "numerous" in 15 cases, "few" in 8 cases, while in 10 cases a single suspicious colony was found on the plates.

Cultural Characters and Fermentation Tests.

Colonies selected were those showing sharply defined round outline, slightly raised towards centre, pearly grey in colour, translucent and with characteristic fine granularity when illuminated obliquely from below under $8\times$ lens.

With few exceptions on plates of 24 hours they were *smaller* and *slightly more opaque* than the meningococcus colonies isolated from the naso-pharynx of convalescent C.S.F. patients and in only two instances presented the bluish tint by transmitted light characteristic of colonies grown from the lumbar fluid. After growing for 48 hours the differences above noted were less marked, though the opacity was still rather greater in most cases.

A good many other colonies were sub-cultured as being doubtful, but not definitely distinguishable; these fermented other sugars (levulose and saccharose), grew on first sub-culture on ordinary nutrient agar, showed good growth on ascitic agar at 22° C. in two days, and were hence not further studied.

Of the 35 strains kept for further study, 2 were lost before all the tests were complete, while 2 began to develop traces of pigment on successive sub-cultures and were then found to ferment levulose in addition to glucose and maltose. A fifth without developing colour was found later to ferment levulose. The last three were tested along with the "normal" strains as regards agglutinating properties. They were entirely negative with all sera used.

Slopes of litmus ascitic agar containing the various sugars in 1 % strength were inoculated direct from suspected colonies. The results were as follows: of the 35 strains kept, 12 fermented glucose more strongly than maltose, 5 fermented maltose more than glucose, while 16 were apparently equal, and 2 fermented glucose only. Saccharose, levulose and galactose were not fermented by any. On repeating the fermentation tests after five to six months' sub-culture on egg the following results were got:—(1) 3 strains now fermented levulose, 2 by this time being known as pigment-producers (yellow), the third not

pigmented; (2) no strain which formerly preferred glucose to maltose now preferred maltose to glucose and *vice versâ*, i.e., there were no reversed activities; (3) none of the previously equal-fermenters now preferred glucose; (4) but 10 of these equal-fermenters now preferred maltose; (5) 3 of those formerly preferring glucose now fermented maltose equally well; (6) 2 of those preferring maltose but also fermenting glucose now fermented maltose only. There was evidently a general increase in the maltose fermenting power as compared with glucose. The medium employed at the second examination differed in the ascitic fluid being of a different sample, but was in reaction as nearly as possible the same, + 5.

Agglutination Reactions.

The thirty surviving strains were tested as regards agglutination with the same four sera as in the previous tests and the results are shown in Table IV (p. 481).

The symbol "+" signifies definite but slight agglutination; the symbol "++" means well-marked but incomplete agglutination, while "+++" means agglutination almost complete but with a trace of turbidity persisting.

It will be seen that 5 strains were completely agglutinated at 1-200 or over with "Clayton" serum; of these 1 was also complete at 1-100 with "Smith" serum, while the other 4 were negative with all other sera. Six strains were complete with Clayton serum at 1-100, and of these 4 were also complete at the same dilution with Smith serum, the other 2 with no other serum than Clayton.

One strain was complete at 1-200 with Boscombe and at 1-100 with Smith, while one was complete at 1-100 with Boscombe, and at 1-200 with Smith.

Three strains were complete at 1-200 and 12 at 1-100 with Smith.

Nine strains were not completely agglutinated with any serum, but showed some clumping at 1-100, 7 with Clayton serum, and 1 each with Boscombe and Chandler.

If these 9 are excluded as showing insufficient agglutination to justify their position as meningococci, there are 21 strains which could not readily be thrown out of the meningococcus category. Of these, 13-14 and 16-17 are pairs from the same throat at different examinations. There are, after deducting these, 19 individuals in whose nasopharynx organisms were found identical with or closely related to the

TABLE IV.

Titre of each serum with its own strain = complete agglutination at 1-600.

Strain	Boscombe			Clayton			Chandler			Smith		
	Highest dilution with complete agglutination	Highest dilution with agglutination = +	Agglutination at 1-100	Highest dilution with complete agglutination	Highest dilution with agglutination = +	Agglutination at 1-100	Highest dilution with complete agglutination	Highest dilution with agglutination = +	Agglutination at 1-100	Highest dilution with complete agglutination	Highest dilution with agglutination = +	Agglutination at 1-100
T.N. 1	-	-	trace	-	-	+	-	-	o	200	400	c
T.N. 2	200	400	c	-	100	++	-	-	o	100	200	c
T.N. 3	-	100	++	100	200	c	-	-	-	100	200	c
T.N. 4	-	-	o	-	-	+	-	-	o	-	-	o
T.N. 5	-	-	trace	100	200	c	-	-	o	-	-	+
T.N. 6	-	-	o	-	-	+	-	-	o	100	200	c
T.N. 7	-	-	+	100	200	c	-	-	o	100	200	c
T.N. 8	-	-	o	-	200	+++	-	-	o	-	-	+
T.N. 9	-	-	trace	-	100	++	-	100	++	200	300	c
T.N. 10	-	100	+++	-	-	o	-	-	o	-	-	+
T.N. 11	-	-	o	-	100	++	-	-	trace	100	200	c
T.N. 12	-	-	o	200	400	c	-	-	o	-	-	o
T.N. 13	100	200	c	-	100	++	-	-	o	200	400	c
T.N. 14	-	-	+	100	200	c	-	-	o	100	200	c
T.N. 15	-	-	o	200	400	c	-	-	o	100	200	c
T.N. 16	-	-	o	200	400	c	-	-	o	-	-	+
T.N. 17	-	-	o	400	800	c	-	-	o	-	-	+
T.N. 18	-	-	o	-	100	++	-	-	o	-	-	+
T.N. 19	-	-	o	-	-	+	-	-	o	-	-	+
T.N. 20	-	-	trace	200	400	c	-	-	o	-	-	trace
T.N. 21	-	-	o	-	-	+	-	-	+	100	200	c
T.N. 22	-	-	trace	-	100	+++	-	-	+	100	200	c
T.N. 23	-	-	trace	100	200	c	-	-	o	-	-	+
T.N. 24	-	-	o	-	100	+++	-	-	o	-	-	trace
T.N. 25	-	-	o	-	100	++	-	-	o	-	-	trace
T.N. 26	-	-	o	-	-	trace	-	100	+++	-	-	+
T.N. 27	-	-	trace	-	100	++	-	-	o	-	-	o
T.N. 28	-	-	o	-	100	++	-	-	o	100	200	c
T.N. 29	-	-	o	100	-	c	-	-	o	100	200	c
T.N. 30	-	-	trace	-	100	+++	-	-	o	100	200	c

meningococcus as shown by microscopical, cultural and serological tests; this is equivalent to 13.7 % of the throats examined at Lambeth.

Of the males examined (32), 15.6 % were positive, while 13.2 % of the females (106) were positive. Of those of 50 years and over 18.2 % were positive, while of those between 14 and 50 years 11.3 % were positive.

Only 1 "positive" was found among children under 14, of whom 19 were examined (5.3 %).

Non-contact school children.

Fifty-six children of ages from 5 to 13 attending a rural school were examined early in May. One plate only showed "suspicious" colonies, giving a strain whose morphological, cultural and fermentative characters were meningococcus-like. It gave a "trace" of agglutination with serum "Smith" at 1-100, but with no other serum.

Agglutinin-Absorption Experiments.

Further evidence of serological relationship of the above organisms to meningococci was sought by estimating their capacity of absorbing the specific agglutinins.

The growth was scraped from 48-hour glucose ascitic agar plates sown with the strains to be examined, weighed moist, mixed with Clayton serum diluted 1-50, so that each c.c. of the mixtures contained 40 mg. of growth. The mixtures were incubated 18 hours at 55° C., centrifuged, and the clear (or in some cases opalescent) fluid used as an agglutinating serum of 1-50 dilution. A control specimen of the diluted serum was similarly heated and centrifuged.

The results are recorded in Table V (p. 483).

The table shows that the non-contact throat strains T.N. 17 and T.N. 12, which are well agglutinated by Clayton serum, also absorb the agglutinin for the strain with which Clayton serum was prepared as well as the agglutinin for themselves, and this to almost the same extent as the homologous strain does with the same serum. Another strain, T.N. 4, which is agglutinated, as shown above, only to the smallest degree by Clayton serum, absorbs a just perceptible amount of agglutinin, while a non-agglutinating meningococcus strain, T. 1 (Boscombe), absorbed practically none.

Four other similar experiments were made with similar results which may be summarised thus: five known meningococcus strains and nine non-contact strains were treated with Clayton and with either Boscombe or Smith sera, *i.e.*, sera of another type; Clayton and Boscombe strains (which do not agglutinate at all with each other's sera) did not absorb agglutinin from each other's sera: agglutinability and absorptive capacity for agglutinin ran parallel: with the relatively weakly agglutinated strains increase in the quantity of the bacterial growth used for

TABLE V.

Absorption of Agglutinin from Serum Clayton.

Test Emulsions	Treatment of Serum	Agglutinations				
		1-100	1-200	1-400	1-600	1-800
T. 13 c (Clayton)	Control	c	c	c	c	+++
T.N. 17	showing	c	c	c	+++	+
T.N. 12	titre without	c	c	++	+	o
T.N. 4	exhaustion	+	o	o	o	o
T. 13 c (Clayton)	Serum Clayton	trace	o	o	o	o
T.N. 17	exhausted with	trace	o	o	o	o
T.N. 12	T. 13 c	trace	o	o	o	o
T.N. 4	(Clayton)	o	o	o	o	o
T. 13 c (Clayton)	Serum	+	o	o	o	o
T.N. 17	Clayton	trace	o	o	o	o
T.N. 12	exhausted with	o	o	o	o	o
T.N. 4	T.N. 17.	o	o	o	o	o
T. 13 c (Clayton)	Serum	+++	++	o	o	o
T.N. 17	Clayton	++	o	o	o	o
T.N. 12	exhausted with	o	o	o	o	o
T.N. 4	T.N. 12	o	o	o	o	o
T. 13 c (Clayton)	Serum	c	c	+++	++	++
T.N. 17	Clayton	c	c	++	+	o
T.N. 12	exhausted with	c	+++	+	+	trace
T.N. 4	T.N. 4	o	o	o	o	o
T. 13 c (Clayton)	Serum Clayton	c	c	c	c	++
T.N. 17	exhausted with	c	c	c	++	+
T.N. 12	T. 1	c	c	+	+	o
T.N. 4	(Boscombe)	+	o	o	o	o

absorption increased the amount of agglutinin removed. There was no adequate evidence of a "group agglutinin" in the sense of one which could be removed by absorption with a related strain leaving the more "specific" agglutinin intact.

Complement-Fixation Experiments with Strains from Cases of Cerebro-spinal Fever and from Non-contacts.

Two sets of these were done: in the first, with an *extract* from meningococcal growths as antigen, the specific complement absorption was slight in amount and not in agreement in every case with the agglutination reactions. For example, T. 1 absorbed a little complement in combination with the non-agglutinating Clayton serum and no more with its homologous serum. T.N. 17, however, was consistent, as it

absorbed 5 doses of complement in combination with Clayton serum and less than one with Boscombe serum.

In the second experiment the antigens used were *heated emulsions* [65° C.], and the results were parallel as regards specificity with the agglutination tests; .05 c.c. of the agglutinating sera with .2 c.c. of bacterial emulsion took up 4 to 5 doses of complement, while the non-agglutinating sera in similar amounts took up 1 to 2 doses only.

SUMMARY.

(1) The maximum period during which meningococci may be isolated from the naso-pharynx of convalescents exceeds three months.

(2) Meningococci were isolated from the naso-pharynx and proved identical with those isolated from the spinal fluid of the same patient in seven cases.

(3) In two of these cases the type of meningococcus found in the naso-pharynx at first resembled exactly that found in the spinal canal of the same patient, and later was persistently replaced by a meningococcus differing markedly in serological reactions from the spinal strain.

(4) Micro-organisms indistinguishable from meningococci by microscopical and cultural methods (including fermentation tests) were found in the naso-pharynx in 22 % of 138 individuals, non-contacts, from an urban population (Lambeth out-patients).

(5) With 63 % of these organisms the serological tests confirmed their identity with or close relationship to meningococci; they agglutinated specifically with anti-meningococcus serum and exhibited a tendency to fall into the same serological groups as the spinal strains.

(6) Their agglutinating properties were not, in general, so strongly marked with the sera used as those of the known pathogenic strains but they showed definite absorption of the specific agglutinin. They appeared to differ from the majority of the spinal strains not in the quality but in the quantitative intensity of their specific affinities: some spinal strains, however, resembled them in this.

(7) Thus, in 13.7 % of the 138 non-contacts micro-organisms were found in the naso-pharynx indistinguishable by any test from strains of meningococci known to have caused meningitis: these are regarded by the writer as meningococci and the individuals harbouring them as meningococcus-carriers.