SOME POINTS BEARING ON THE BACTERIOLOGY OF CEREBRO-SPINAL MENINGITIS.

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Fermentative activity of the Diplococcus intracellularis meningitidis (Weichselbaum).

In determining the fermentative powers of this organism both fluid and solid cultures were used. *The fluid medium* was made up according to the formula employed by Dr M. H. Gordon. It consisted of Lemco ·1 gram, peptone ·1 gram, sodium bicarbonate ·1 gram, 10 per cent. watery solution of litmus 10 c.c., water to 100 c.c. This medium was sterilized by heating under pressure and to it was then added the substance to be investigated in the proportion of 1 per cent. Subsequent sterilisation was effected by heating in the steamer for 10 minutes on three successive days. In the case of laevulose, arabinose, and xylose the flasks were heated over the Bunsen flame, as in the steamer the medium turned red.

To each 500 c.c. of the medium 50 c.c. of sterile ascitic fluid were added. The medium was then put into tubes which were incubated at 37° C. for three days to see that they were sterile. It was found that in this fluid the meningococcus grew luxuriantly, a pellicle forming on the surface of the bouillon.

Most of the strains of meningococci used were isolated from cases occurring in Belfast and were in cultivation for various lengths of time, some being isolated only a few days, others having been in the laboratory for over a year. In addition to the Belfast strains we had three cultures obtained from the Hygienic Institute, Hamburg. The cultures were examined daily and the colour of the medium was carefully compared with controls, the tubes being examined against a white background.

In no medium was any gas produced. Glucose, maltose, and dextrin were fermented with the production of acid, whilst no fermentation of any other substance was observed. With glucose and maltose in nearly all cases the medium became red and remained red, in a few cases bleaching preceded the reddening. With dextrin the medium became red, it was then bleached and finally, about the end of a fortnight, became blue again.

With the various other substances the medium remained blue; sometimes bleaching occurred but the blue colour returned when the tubes were left all night at room temperature.

After each of the substances in the following list is placed the number of strains of meningococci employed in determining the fermentability of the substance:—Glucose 60, Laevulose 43, Galactose 53, Maltose 59, Saccharose 47, Dextrin 31, Lactose 20, Inulin 23, Arabinose 20, Raffinose 19, Glycerine 25, Erythrite 17, Mannite 29, Dulcite 8, Rhamnose 15, Sorbite 14, Xylose 5, Adonite 21, Salicin 32, and Amygdalin 15.

The media were all prepared in the same manner and the same ascitic fluid was used in all cases, so that the conditions were uniform throughout the series.

We desire to call especial attention to the negative results we obtained with laevulose and galactose. We used galactose prepared by *Merck and by Kahlbaum.*

The results of these fermentation tests confirm those obtained by von Lingelsheim and by ourselves in previous experiments. Sheenan and Ritchie at Edinburgh have obtained similar results. On the other hand, Gordon, Buchanan, Rundle, Mottram and Williams, and Arkwright have obtained positive results as regards the fermentation of galactose and laevulose.

In solid media also we got no fermentation of laevulose and galactose.

Solid media. Nutrient agar was made containing 3 per cent. of Chapoteaut's peptone and to it was added 1 per cent. of the sugar and some litmus solution. Sterilisation was effected by steaming for 10 minutes on 3 successive days. To 2 parts of this medium, which had been melted and cooled to 50° C., 1 part of sterile ascitic fluid was added. The medium was then put into tubes which were placed in a slanting position.

After the agar had set firmly the tubes were incubated for 2 days at 37° C. and those that were contaminated were discarded.

The action of ten strains of meningococci was tested on media which had been thus prepared and which contained respectively glucose, laevulose, galactose and dextrin.

In the case of glucose permanent reddening of the medium in the neighbourhood of the condensation fluid occurred—with dextrin at the same place the medium first became red and then became blue again. In the case of laevulose and galactose the medium and condensation fluid remained blue.

Fermentative activity of Gram-negative cocci isolated from cases of sporadic cerebro-spinal meningitis.

The same media were used as in the previous experiments.

The results obtained with 10 Gram-negative cocci isolated from cases of sporadic cerebro-spinal meningitis (and which were proved by Houston and Rankin to differ from Weichselbaum's cocci in respect of their agglutinins and opsonins) are shown in the following table:—

Number of strains	Glucose	Laevulose	Galactose	Maltose	Saccharose
8 strains	+	–	–	+	–
1 strain	–	–	–	+	–
1 strain	–	–	–	–	–

\+ indicates production of acid. – indicates no change in reaction.

It is evident from this Table that 8 of these cocci are identical with Weichselbaum's coccus as regards fermentative power; of the remaining two, one differs from the true meningococcus in not fermenting glucose though able to ferment maltose, the other had no fermentative activity, though in morphology, feeble vitality, and appearance of its growth it closely resembled the meningococcus. In its absence of fermentative powers it resembles the *Micrococcus catarrhalis.*

From the above investigation it would appear that Weichselbaum's diplococcus has constant fermentative characters whilst there is some variability among the Gram-negative cocci occurring in sporadic cases of cerebro-spinal meningitis.

Characters of certain other Gram-negative cocci occurring in cerebro-spinal fluid.

In addition to Weichselbaum's and Still's cocci there are sometimes met with in cerebro-spinal fluid Gram-negative cocci which belong to

an entirely different class. The members of this class grow well on ordinary media both at 20° C. and at 37° C., survive for several weeks without transplantation and finally on the Drigalski-Conradi medium give an abundant growth. In morphology also they differ from the meningococci. They show no tendency to tetrad formation but rather to formation of short chains consisting of four or six individuals, though the diplococcal is the commonest arrangement. Moreover the organisms stain well, there being no evidence of autolysis. In cultures on both solid and fluid media among the diplococci short bacillary forms and even unsegmented uniformly staining threads 20μ in length are frequently seen. It is quite certain that these bacillary forms and threads are not contaminations but represent variant forms of the organism.

We may here note that D'Este Emery found extremely pleomorphic diplococci in the cerebro-spinal fluid of three cases of posterior basic meningitis which, though in some respects differing from the ones here described, agreed with them in the assumption at times of the bacillary form.

We have met with this class of organism on four occasions. In one case it appeared to be the only organism present in the cerebro-spinal fluid, in two cases it was associated with Weichselbaum's diplococcus, in the fourth, a case of posterior basic meningitis, it was associated with a Gram-negative coccus which, though in most respects resembling the meningococcus, produced no acid in media containing glucose and maltose.

These micro-organisms had no action on glucose, laevulose, galactose, maltose, lactose and saccharose.

Their growth on ascitic agar was somewhat more opaque than that of the meningococcus.

On the Drigalski-Conradi medium we find that Weichselbaum's and Still's meningococci and Pfeiffer's *Micrococcus catarrhalis* (culture obtained from Král) exhibit no growth.

Cerebro-spinal meningitis due to Gram-positive cocci.

On seven occasions we have obtained cultures of Gram-positive diplococci from the cerebro-spinal fluid. One of these organisms had the characteristic lanceolate shape of the pneumococcus and possessed a capsule; all the others were found to be non-capsulated when the sediment from the cerebro-spinal fluid was examined. From the fermentative activity of the other six when grown in Gordon's nine media it appeared

that four of them were probably members of the *Streptococcus faecalis* group. Another one was probably a member of the *Streptococcus salivarius* group. The 7th had a thick moist growth on agar quite different from that of streptococci and somewhat like that of the meningococcus but more opaque. Its vitality was good as it survived several weeks without subculture. Morphologically it showed large Gram-positive diplococci resembling in size and shape the giant forms occurring in cultures of the meningococcus.

A full account of these organisms has been given in a previous communication (1907) in which we also point out that the post-mortem findings in these cases may be identical with those met with in cases of cerebro-spinal fever.

On one occasion we found the *Bacillus anthracis* responsible for a haemorrhagic lepto-meningitis.

From the fibrino-purulent cerebro-spinal fluid of another case we isolated the *Bacillus typhosus* in pure culture.

From a third case we obtained a culture of the *Bacillus enteritidis* (Gaertner). The proof that in these latter two cases we were dealing with the typhoid bacillus and with Gaertner's bacillus was established by a consideration of the cultural, fermentative and morphological characters of the bacilli, as well as by agglutination and saturation experiments.

Agglutination of meningococci.

On 47 occasions the agglutinative effect of the blood serum of cerebro-spinal fever patients was investigated. The blood was taken from patients in all stages of the disease as well as from those who were convalescent.

An emulsion in normal salt solution was made from a 12—24 hours' culture of the meningococcus. With this emulsion 1 in 10, 1 in 20, 1 in 50 and 1 in 100 dilutions of the blood serum were made in Wright's capillary pipettes. Finally the pipettes were sealed and incubated for 2 hours at 37° C. At the end of this time they were examined for clumping both by the naked eye and the microscope.

According to the degree of the agglutination we have applied the terms small, medium sized and large to the clumps.

On three occasions clumping visible to the naked eye occurred instantaneously on making the 1 in 10 dilution.

Control tests were carried out with serum taken from normal individuals or from typhoid fever patients; the results were that with

a 1 in 5 dilution of the serum medium sized clumps formed, with a 1 in 10 dilution either no clumps or very small ones.

With a 1 in 10 dilution	45	sera	gave	large clumps.
,, ,, ,,	1	serum	,,	medium sized clumps.
,, ,, ,,	1	,,	,,	small ,, ,,
With a 1 in 20 dilution	19	sera	,,	large clumps.
,, ,, ,,	6	,,	,,	medium clumps.
,, ,, ,,	10	,,	,,	small ,,
,, ,, ,,	12	,,	,,	no clumps.
With a 1 in 50 dilution	4	,,	,,	large clumps.
,, ,, ,,	7	,,	,,	medium clumps.
,, ,, ,,	5	,,	,,	small ,,
,, ,, ,,	31	,,	,,	no clumps.
With a 1 in 100 dilution	0	,,	,,	large clumps.
,, ,, ,,	2	,,	,,	medium clumps.
,, ,, ,,	4	,,	,,	small ,,
,, ,, ,,	41	,,	,,	no clumps.

Different degree of agglutinability of old and young cultures of the meningococcus.

We have found that meningococci that have been in cultivation for a long time are much more readily agglutinated than recently isolated cocci not only by immune serum but also by normal serum. Roughly stated, old avirulent cultures of the meningococcus are twice as easily agglutinated as virulent cultures, when the dilutions herein mentioned are used.

For example a sample of Flexner and Jobling's serum which agglutinated a recently isolated culture of the meningococcus in a dilution of 1 in 300, caused an equal degree of agglutination with an old avirulent culture in a dilution of 1 in 600. Similarly the serum of a patient which agglutinated an old culture in a 1 in 50 dilution caused a similar amount of agglutination in the case of a recently isolated culture in a 1 in 20 dilution only.

Difference between old and young cultures of the meningococcus with reference to the opsonic index.

Houston and Rankin have shown that old cultures of the meningococcus are readily phagocytosed when acted on by normal serum, whereas with young cultures in the same conditions phagocytosis is very feeble.

An experiment which we made seemed to indicate that old cultures are able to absorb from a serum both normal and immune opsonins whilst young cultures combine only with immune opsonin.

Serum taken from a cerebro-spinal fever patient was divided into two equal portions, one portion was saturated with several loopfuls of growth taken from a young culture of the meningococcus on Chapasgar[1], the other was saturated with an equal quantity of growth from an old culture. The sera were left for two hours at 30° C. and then for two hours at room temperature. They were then centrifugalised and the opsonising effect of the clear supernatant serum was determined on an old and young culture of the meningococcus. The serum that had been saturated by the old culture became completely devoid of opsonising action both on old and young cultures; the serum on the other hand that had been saturated with the young culture still caused marked phagocytosis of an old culture. The above deduction, drawn from a single experiment, we regard as merely indicative of what is to be expected on further investigation.

Agglutinating action of the blood serum of cases of cerebro-spinal fever on bacilli of the typhoid, colon and alkaligenes groups.

In a previous communication (1908) we have pointed out that the blood serum of cerebro-spinal fever patients occasionally agglutinates the typhoid and colon bacillus and that it almost constantly agglutinates an organism closely related to *Bacillus faecalis alkaligenes* which was isolated by us from Belfast tap water and to which we gave the name *Bacillus Grosvenor*. Later observations have confirmed the frequency of this phenomenon, but we have found that it is not invariably present, some cases having failed to show the reaction though examined on several occasions.

Since the paper above referred to was published we have used as additional controls the blood serum of nine patients suffering from typhus fever, three cases of meningitis (not meningococcal), and one of pneumonia, and in no case was there agglutination in a dilution of 1 in 50. With no blood except that taken from cases of cerebro-spinal fever have we got agglutination of the Grosvenor bacillus in higher dilutions than 1 in 100 although 168 specimens have been examined.

[1] This medium consists of:

3 % agar (made with Chapoteaut's peptone)	2 parts.
Raw ascitic fluid	1 part.

In 16 cases of cerebro-spinal fever the blood serum gave marked clumping within an hour to dilutions of 1 in 1000, and further we found that one of the 16 agglutinated in 1 in 1400, two in 1 in 1500, four in 1 in 1600, and four in 1 in 2000 dilutions respectively.

We have shown that it is possible to remove the agglutinins from the serum by saturation with the Grosvenor bacillus, whilst saturation with the *Meningococcus*, *Bacillus typhosus*, *B. coli communis* or *B. faecalis alkaligenes* (Král) fails to do so.

The agglutinins for the Grosvenor bacillus and for the meningococcus are quite distinct. Heating the serum for 10 minutes at 65° C. completely destroys the agglutinins for the Grosvenor bacillus.

By means of precipitation with various strengths of ammonium sulphate solution it was found that these agglutinins were associated with the globulin component of the serum and moreover that it was that portion known as the "pseudo-globulin" fraction which contained them in greatest amount.

As we have never succeeded in cultivating *Bacillus Grosvenor* from the bodies of any of the patients we can advance no explanations of the phenomenon and content ourselves with merely stating the facts.

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