AN INVESTIGATION INTO THE ETIOLOGY OF PUERPERAL FEVER.*

BY

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WHILE certain cases of puerperal sepsis are due to staphylococci, *B. coli*, or other bacteria, the majority are attributable to streptococci. Of these the greater number are caused by haemolytic streptococci (chiefly *Strep. pyogenes*), but the residue of cases, due to non-haemolytic streptococci (*viridans* type) are sufficiently numerous to deserve consideration.

Infection of the uterus, as of other parts of the body, may be either exogenous or endogenous. There can be no doubt that a true exogenous infection does occur, as is shown by the recorded instances of doctors and midwives acting as carriers of infection. In many cases, however, the possibility of this mode of infection seems to be slight, and it was with the object of throwing light on what may, for the moment, be called spontaneous puerperal sepsis that the investigation here recorded was undertaken. Briefly stated, we sought to discover whether, in the case of normal labours, streptococci were present in the vagina before delivery and in the vagina and uterus after delivery and of what type. All the cases examined were intern patients in the Rotunda Hospital, Dublin, and, as is to be expected, show a higher percentage of abnormalities than would a normal series. In only one, however, was there any evidence of the existence of any septic condition in the body before delivery.

Swabs were taken from the posterior fornix of the vagina either on admission or soon after. In the majority of cases labour had commenced when the specimens were collected. In a small number of cases where, for some reason, a swab was not then taken, one was secured after the rupture of the membranes. This was also done occasionally in addition to the first swabbing. In all cases swabs of the posterior fornix and of the interior of the uterus were taken during the puerperium, usually between the second and the seventh day after delivery.

Method of Swabbing.

The patient was placed in the left lateral or Sims's position. The vulva was opened and a Sims's speculum passed in to draw back the perineum, thus fully exposing the cervix. The vaginal swabs were then taken from the posterior fornix. For the uterine swabs the external os and just inside was wiped completely clear of discharge. The cervix was then steadied with a clip and was again wiped out with sterile cotton passed well inside and the culture swab passed directly up into the uterine cavity. The swabs were obtained between noon and 2 p.m., so as to be sent to the laboratory for culture at 2 p.m. The first eighteen in the series were taken by Dr. Wyndham Williams and the remainder by Dr. C. Coghill, who were attending post-graduate courses. To both these gentlemen we are deeply indebted for their co-operation and for their interest in the investigation.

Since the object of the investigation was to determine whether streptococci were present or not, the laboratory technique was different from that used in dealing with swabs from patients clinically suspected of sepsis, in which cases the diagnosis depends largely on whether streptococci are present in significant numbers, the presence of one or two colonies being usually of no importance. Every effort was made to supply any streptococci present with their optimum nutrient material and to prevent any deaths of the bacteria from drying or delay. Cultures were made within, at most, a few hours of the collection of the swabs. We wish to lay stress on this point: our technique was devised to find whether even a single streptococcus was present on the swab examined. Had the swabs been cultured on solid media, as are swabs sent for diagnostic purposes, the result would have been, in the majority of cases, either no growth or a growth of only a few colonies,

and very many of the streptococci actually isolated would have been missed. We satisfied ourselves on this point by carrying out duplicate cultures on solid and fluid media on the first dozen or so swabs. The method we finally relied upon gave infinitely better results than were obtained by the use of solid medium.

Method of Cultivation.

The basis of the fluid medium used for primary culture was trypsin broth (pH 7.4) to which was added 20 per cent. of sterile hydrocele fluid and 10 per cent. of a sterile 10 per cent. solution of glucose. The swabs were thoroughly washed out in this medium, which was incubated at 37° C. until the following day, when it was examined. The medium is almost a selective one for streptococci of all varieties, and renders possible their isolation from material in which these organisms are scanty and other bacteria numerous. Streptococci may produce a turbidity with only slight deposit, but more usually form a dense deposit, leaving the upper part of the fluid clear. This deposit was examined microscopically, and if streptococci were seen the result was noted as positive. At the same time the average number of cocci in each chain was recorded. A loopful of the deposit was plated on human blood agar, and the plates incubated for twenty-four hours. Colonies believed to be of streptococci were then subcultured into glucose hydrocele broth, which was incubated until the following day. In many cases two or more colonies were picked where these presented differences visible with a hand lens. The glucose hydrocele broth cultures were examined for purity and to determine the morphology of the streptococci, and subcultured into carbohydrate media for purposes of classification. Blood agar plates were also made which, when examined after twenty-four and again after forty-eight hours, supplied the first basis of classification of the streptococci into the two main groups, haemolytic and non-haemolytic. At the same time notes of the size and morphology of the colonies were made as a control of the other basis of classification.

The classification of streptococci cannot at present be regarded as satisfactory, and since in this investigation some simple basis appeared desirable it was decided to follow Holman's method¹—splitting up the two main groups of haemolytic and non-haemolytic streptococci by means of the fermentative action of the isolated bacteria on three carbohydrates—lactose, mannite, and salicin. The basis of the medium was equal parts of trypsin broth and 1 per cent. peptone water. Trypsin broth was used, as better growth was secured by its presence than when peptone water was alone employed. It was not used exclusively owing to the possible action of streptococci on the glycerin which is present in it in small amount. It is doubtful if this precaution is really essential, as, in many controls made, no evidence of such action in sufficient amount to introduce any fallacy was observed; 1 per cent. of the carbohydrate was added and also 1 per cent. of Andrade's indicator. The tubes containing this medium were sterilized by the fractional method, and, after steaming, were enriched by the addition of 20 per cent. of sterile hydrocele fluid. They were always incubated for forty-eight hours before use to determine if any had become contaminated. After the three tubes were inoculated with each strain isolated, they were incubated and examined daily for five days. Usually the reactions were sharp and clearly read after twenty-four or, at most, forty-eight hours. Only very rarely were subsequent changes noted.

It is not contended that Holman's classification is perfect. Probably the number of types thus found is too small, but this appears to be preferable to a classification based on fermentative action on a large number of carbohydrates, which results in a multiplication of types. In all cases the morphology of the cocci and types of growth were compared with the results of carbohydrate fermentation, and only in a very few cases was any possible discrepancy observed.

Results.

In all, 158 swabs were examined, and streptococci were found to be present in 101. In 3 cases two types of streptococci were found in the one swab. It is probable that if the number of colonies picked had been increased this would have occurred more frequently. In 13 cases streptococci were seen in the original broth culture, but were not typed. The reason, in 5 cases, was that it was impossible to isolate them owing to the presence of other organisms (*B. proteus*, etc.), while eight accidents in the laboratory (breaking of plates or tubes, too hasty discarding of plates, etc.) were responsible for the other omissions to type the organisms.

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An analysis of the results must now be attempted. For this purpose "probables," as explained above, are treated as if the organism had been correctly typed. The error so introduced, if any, is small.

TABLE I.—Gross Results Obtained.

Number of patients	50
Number of swabs	158
Streptococci absent	57
Streptococci present	101
Streptococci found	104
<i>Strep. faecalis</i>	43
<i>Strep. mitis</i>	42
<i>Strep. equinus</i>	10
<i>Strep. salivarius</i>	7
<i>Strep. equi</i>	1
<i>Strep. infrequens</i>	1

Of these streptococci only two, *Strep. equi* and *Strep. infrequens*, were haemolytic. *Strep. equi* was only moderately haemolytic, while *Strep. infrequens* was very actively so. The rarity of the haemolytic streptococci and the fact that neither of those found was identical with the *Strep. pyogenes*, the commonest cause of puerperal sepsis, tend to confirm our preconceived opinion that this organism is usually, if not always, introduced into the vagina from without in those cases in which puerperal sepsis from this cause appears, and that it is incapable of existing there as a saprophyte which becomes pathogenic on reaching the damaged uterine tissues. It must not, however, be forgotten that we only examined fifty cases, and that further material might cause us to change our opinion on this point.

All the other streptococci isolated were of the non-haemolytic or *viridans* type. The most numerous were the *Strep. faecalis* and the *Strep. mitis*, which were about equal in numbers, *Strep. equinus* and *Strep. salivarius* being less frequently found.

TABLE II.—Showing the Occurrence of the Various Organisms in the Vagina Before Delivery and in the Vagina and Uterus After Delivery.

	Before Delivery.		After Delivery.	
	Vagina.		Vagina.	Uterus.
Number of swabs	58	...	50	50
Streptococci absent	26	...	8	23
Streptococci present	32	...	42	27
<i>Strep. faecalis</i>	16	...	16	10
<i>Strep. mitis</i>	10	...	20	13
<i>Strep. equinus</i>	5	...	3	2
<i>Strep. salivarius</i>	3	...	2	2
<i>Strep. equi</i>	—	...	1	—
<i>Strep. infrequens</i>	—	...	1	—

We may first contrast the bacterial condition of the vagina before and after delivery. The figures seem to show, beyond doubt, that streptococci are more frequently present after than before the birth of the infant. In only 4 of the 50 cases were streptococci absent at all examinations. In 3 streptococci were present in the vagina before delivery, but were not found subsequently, while in 15 they were absent before delivery, but were found in that situation after the birth of the infant. The flushing of the vagina by the waters had evidently no more than a transient effect, if any, which was outweighed by the more suitable environment in which the streptococci found themselves after delivery, owing probably to the food material supplied by the lochia.

From the uterus itself we get our most surprising result. In 27 out of the 50 cases, streptococci were present in the swabs taken from the interior of the uterus with great care during the puerperium. It would appear that invasion of the uterus by vaginal streptococci does occur after delivery, but every invasion does not, of necessity, mean an infection. In four of our cases morbidity was shown by the temperature of the patient. One of these can be eliminated owing to the existence of pyelitis, leaving three for consideration. In two of these streptococci (*Strep. faecalis*) were isolated from the uterus, while the uterine swab from the other contained no streptococci. It is noteworthy that of the 27 instances of uterine invasion, the same organism was present in the uterus as in the vagina in 25; in 1 different

organisms were present, and in 1 streptococci were found in the uterus but not in the vagina.

The constancy of the vaginal and uterine flora at the three or more examinations made is worthy of some remark. Out of the 50 cases, in only 4 were all examinations for streptococci negative. In 37, including one case where a double invasion was found, either the same streptococcus or none was found at all examinations. In 9 there was a change of type. It is probable that a more extensive sampling of colonies would have revealed double invasions in many of these cases.

We do not wish to obscure by further analyses of our results what we consider our most important finding—namely, uterine invasions by streptococci in 54 per cent. of cases. While we were satisfied that our technique was good, we felt that we must face one obvious and fair criticism. To pass a swab into the uterus it is necessary to pass through the cervix in intimate contact with the vagina, admittedly containing streptococci. This passage permits the possibility of the picking up, by the swab, of streptococci not actually in the uterus. That this criticism is sound would appear by considering the first 25 and the second 25 of our cases separately. In the first 25 cases 17 uterine invasions were recorded, and only 10 in the second. Two gynaecologists were engaged in the collection of swabs in the first half of the series, both of whom had to learn by experience the best technique. In the second half all the swabs were taken by one individual who had already mastered the technique.

In order to meet this criticism a further investigation, which was confined to the puerperal uterus, was undertaken. The technique, which was as follows, was entirely carried out personally by one of us (G. FG.). The patients were placed in a gynaecological chair, a posterior speculum was gradually passed into the posterior fornix, and the vagina wiped quite clear of lochia. The anterior lip of the cervix was then held and the cervical canal wiped clear. A 3/8 in. glass tube which had about 1 in. of cotton plug at one end and overlapping the edges of the tube, was prepared by boiling, and was then passed direct from the sterilizer for at least 2 in. into the uterine cavity. The culture swab was then passed up the glass tube and dislodged the cotton plug as it entered the uterine cavity. In all cases a flow of lochia came from the uterus into the glass tube, and in most cases flowed freely through the tube. The culture swab was then withdrawn and restored to its protecting test tube. It was thought that it would be helpful to attempt a numerical estimation of any streptococci found in these swabs, and therefore each swab, on receipt, was thoroughly washed in 5 c.cm. of glucose hydrocele broth. Of this 0.5 c.cm. was transferred to a second tube of the same medium and 0.1 c.cm. to a third. A positive finding in the third tube indicated at least 50 streptococci in the swab, in the second 10, and in the first 1. The methods used for the isolation and typing of the streptococci was similar to that previously stated. In this series 25 patients were examined, on either the fifth or sixth day after delivery.

TABLE III.—Swabs from Puerperal Uterus.

Patient.	Streptococci found.	No. in Swab.
18 patients	None	—
A. J.	<i>Strep. mitis</i>	10
A. M.	<i>Strep. mitis</i>	1
M. McL.	<i>Strep. faecalis</i>	50
C. D.	<i>Strep. mitis</i>	1
M. P.	<i>Strep. faecalis</i>	50
C. H.	<i>Strep. faecalis</i>	50
M. K.	<i>Strep. non-haemolyticus</i>	10

Streptococci were therefore found in 28 per cent. of the uteri, as contrasted with 54 per cent. in the first series. This would seem to point to faulty technique in the earlier investigation, but, even if this be allowed, 28 per cent. of positive results is still very considerable. The very sceptical may hesitate to accept a single streptococcus (more probably a single chain of streptococci) as significant, but even if these are discarded there remain 20 per cent. of puerperal uteri invaded by streptococci of the *viridans* type in women exhibiting no sign of morbidity as determinable by temperature, pulse, or local condition.

SUMMARY.

1. Streptococci were found in the vagina before and after delivery in 63 per cent. of the cases examined. They may therefore be regarded as among the normal flora of the part.

2. Haemolytic streptococci were only found twice in the examination of 108 vaginal swabs. *Strep. pyogenes* was never found.

3. The predominant non-haemolytic streptococci of the vagina were *Strep. faecalis* and *Strep. mitis*.

4. Non-haemolytic streptococci were present in the post-partum uterus in at least 20 per cent. of the cases examined.

We hesitate to draw any conclusions from our findings, but we suggest the following without wishing to dogmatize.

1. The commonest form of puerperal sepsis, that caused by haemolytic streptococci (and particularly *Strep. pyogenes*), is due to exogenous infection.

2. Non-haemolytic streptococci do occasionally cause puerperal sepsis. These organisms are present in the vagina of the majority of parturient women and are found fairly frequently in the uterus post partum. They are normal body saprophytes, but are opportunists as regards pathogenicity. (The *Strep. faecalis* may give rise to suppuration in wounds, and the *Strep. mitis* to septicaemia and subacute endocarditis.) Puerperal sepsis due to non-haemolytic streptococci is an endogenous infection. The cause is the streptococci of the vagina plus some unknown factor, which may be a local or general lowering of the patient's resistance or a rapidly enhanced virulence of the organism.

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BLOOD COAGULATION TIME IN THE INSANE.

BY

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WHEN discussing with my friend Dr. F. E. Taylor the occurrence of haematoma auris in insane persons, he mentioned that it had been found that in cases of spontaneous haemorrhage into the vitreous the coagulation of the patient's blood had been delayed, and suggested that haematoma auris might be due to the same cause. It was Dr. Taylor's suggestion that induced me to make the investigation here recorded.

I found that the blood coagulation time was markedly prolonged in cases of haematoma auris, and I then deemed it advisable to test the blood of cases of insanity in general. The only reference I can discover in the literature to the blood coagulation time of insane persons is a paper by E. J. Larsen and H. Lind, who in 1920 recorded a series of 50 insane patients. They found that in all these patients the time was within normal range. I have not been able to see the original paper and do not know what method they used.

I have not succeeded in finding any reference to the matter in any of the textbooks or other works to which I have been able to refer. The subject of blood coagulation time, but without reference to insane subjects, has occupied the attention of many observers.

The method of measuring blood coagulation time adopted by different observers not only varies extremely, but there is no uniformity in the end-reaction which is sought. Thus, E. H. Lepper,¹ adopting the method of Dale and Laidlaw, takes the onset of coagulation as the point to be determined. This method is obviously objectionable as coagulation would be hastened by the agitation employed.

J. Addis² views under the microscope the edge of a drop of blood suspended in oil and acted on by a current of oil streaming against the edge of the drop. When a continuous smooth flow of the corpuscles ceases he concludes that coagulation has occurred in the drop. The apparatus used is not simple, and is open to the objection that contact with oil hinders or prevents coagulation.

The method of Sir Almroth Wright³ is the simplest and most easily applicable. I adopted his method in a modified form in my experiments. I used capillary glass tubes 1 mm. in diameter and 6 cm. long; 2 cm. of the tube were filled with finger blood, the blood aspirated into the middle of the tube, and one end sealed in the flame. The unsealed ends

are then fixed in plasticine, and nipple, glass tubing, and plasticine are floated in a beaker of water kept at 37° C. A Hagedorn straight needle was used for pricking the finger, momentarily congested by a tape, and it is easy to fill simultaneously the four tubes used for each experiment by holding the tubes fan-shape between finger and thumb and inserting the approximated ends in the drop of blood.

I examined 117 cases, all being free from organic disease. In addition I examined 12 cases of normal sane persons. The average coagulation time in the 117 cases was 4.69 minutes. The average time in 12 normal sane persons was 3.75 minutes. This corresponds closely with the time given by other observers. J. H. Drysdale⁴ says that normal coagulation time at temperature of body is about three or four minutes. E. R. Stitt⁵ says that by Sabrazès's method finger blood coagulates in three to four minutes at room temperature.

Of the whole 117 cases, 63 gave coagulation times over the normal in sane patients. Of 10 cases of haematoma auris, 7 gave coagulation times above the normal. While these were the general results, it was noted that in 16 cases the time was six minutes, and 11 of these cases were mania. On the other hand, 14 cases gave a time under four minutes, and 10 of these were cases of dementia.

For some reason or another cases of haematoma auris are now very rare among the insane. In the private asylum where I am employed there are only 2 cases (recent), and at the East Sussex County Mental Hospital at Hellingly, with 1,350 patients, there were only 8 cases, and these were all old ones.

Tabulating the results we find—giving coagulation time over the normal: haematoma auris cases, 70 per cent.; insane cases, excluding haematoma, 52.3 per cent.; sane cases, 16.6 per cent. Coagulation time tends to decrease with increase of age of the patient.

Conclusions.

1. The proportion of insane patients showing an abnormal condition of the blood is sufficiently large to indicate a line of treatment.

2. The prolonged coagulation time may well account for malnutrition of the brain tissue, and for the cerebral haemorrhages which so often occur in the insane.

3. The method of determining the coagulation time, as I have described it, is so simple that it can be easily done at the bedside.

4. Further investigation is needed to ascertain the factor or factors which prolong the coagulation time.

Is delayed coagulation in insanes due to decalcification, or to deficiency of prothrombin, or to deficiency of thrombokinase, which activates prothrombin, or which, according to Howell, is of lipid nature and neutralizes antithrombin?

It is probably *not* due to toxic substances produced by infective agents, as these would liberate a certain amount of nucleo-protein in the blood, and so produce coagulation.⁷

Halliburton² says that if we reject the thrombin theory with all its superstructures we accept a physical and not a chemical change as the cause of coagulation.

Sir A. E. Wright⁸ points out that diminished coagulability of the blood is of very great practical importance, because it interferes with haemostasis, and also because it conduces to serous haemorrhage (in other words, to increased transudation).

It is to be noted that the observed blood coagulation time varies according to conditions of observation, temperature, etc., and the relative time between sane and insane is the important point. All my observations were made under exactly the same conditions. The absolute time will vary according to the method of observation.

My thanks are due to Dr. Taylor for the suggestion and much kindly advice, and to Dr. J. N. Greene Nolan of the East Sussex County Mental Hospital, Hellingly, where most of the work was done by kind permission of the medical superintendent. The rest of the work was done at Ticehurst House, Sussex, and I have to thank Dr. Colin McDowall for the facilities he gave me.

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