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UPON THE BACTERIOLOGY OF THE SUMMER DIARRHOEA OF INFANTS.

By H. DE R. MORGAN, M.A.OXON., M.R.C.S.ENG.,
L.R.C.P.LOND., D.P.H.CAMB.,

ERNEST HART MEMORIAL SCHOLAR.

(FROM THE BACTERIOLOGICAL DEPARTMENT, LISTER INSTITUTE
OF PREVENTIVE MEDICINE.)

INTRODUCTION.

THE diarrhoeal diseases of infancy, on account of their appalling mortality, demand the most careful and thorough investigation.

A large number of inquiries have been made in various directions with reference to the predisposing and etiological factors in these diseases, for example, the influence of seasonal and hygienic conditions, of natural and artificial feeding, of milk pasteurization, etc.

These inquiries have yielded valuable results, to which it is beyond the scope of this paper to refer. Whilst recognizing that in infantile diarrhoeas many possible factors have to be taken into consideration, there exists a particularly virulent type occurring in summer,which there is every reason to believe has an infectious origin. To this type of infantile diarrhoea bacteriological research has been especially directed. Escherich[1] in Germany and Booker[2] in the United States were among the earlier observers to study the bacteriological flora of the diseased intestine. They were, however, unable to obtain evidence as to the presence of pathogenic organisms of a specific type in the diarrhoeic stools.

A fresh impulse was given to research by Shiga's[3] studies of Japanese dysentery in 1898, and the isolation by him of the *B. dysenteriae.* Shiga's results were confirmed by Flexner[4] in Manila in 1900, and by Kruse[5] in Germany in 1901. Acute bacillary or epidemic dysentery is now regarded as due to the bacillus first isolated by Shiga, with which the type described by Kruse is identical. The Flexner type of *B. dysenteriae* differs in certain cultural characteristics, and in its agglutinative properties from the former. A number of intermediate forms allied either to the Shiga-Kruse or the Flexner type of organism have since been described.

Bacilli of the dysentery group are widely distributed, and have been detected in cases of acute endemic, epidemic, sporadic, and institutional dysentery.

Duval and Bassett[6] in 1902 concluded that the dysentery bacillus " is an important, if not the most important, cause of the summer diarrhoeas of children." In 1903, the Rockefeller Institute for Medical Research undertook, under Dr. Flexner's direction, an investigation on the local or general occurrence of the dysentery bacillus in the summer diarrhoeas of children in the United States. The reports on the bacteriology of the cases are published in *Studies from the Rockefeller Institute,* vols. i and ii. 1904. I may quote from. Dr. Flexner's summing up of the results.

The type of *B. dysenteriae* which preponderated in the children is the so-called "Flexner-Harris" organism. The "Shiga" type of the organism is exceptionally met with, and occasionally both types are found in association. . . . Types of *B. dysenteriae* of less well-established properties have also been encountered. . . . The central fact brought out by this collective investigation is the frequent occurrence in the diarrhoeal diseases of children of a specific micro-organism, which hitherto has been held to be of specific pathogenic action in human beings, and to be the cause of that form of dysentery among adults and also among children which is characterized by necrotic and pseudo-membranous lesions of the intestine and marked infectiousness.

More recent papers are those by Dunn,[7] Parks, Collins, and Goodwin.[8] Critical observations on the above results are made in the papers of Weaver and Tunnicliffe[9] and of K. Collins.[10] Charlton and Jehle[11] consider that summer diarrhoea in children may be caused by various forms of micro-organisms of a non-dysenteric type, more particularly by the *B. coli communis.* They note the occurrence of "vast numbers" of streptococci in the green stools. Streptococci in large numbers were also found by the Rockefeller observers associated with the *B. dysenteriae.*

It remains to be determined how far the summer diarrhoeas of other countries conform in type to those of the United States.

SCOPE OF RESEARCH.

Last summer (1905) I undertook the bacteriological examination of cases of acute infantile diarrhoea occurring in London. The results are sufficiently advanced to be made the subject of a preliminary report.

The clinical aspect of the disease here is different from that described in America. Blood in the stools is quite the exception, whereas in America it is apparently very frequent. There seems very little doubt, therefore, that we are not dealing with the same type of disease in the two countries, and that the causal agent may very well be different. This being the case, I determined to make a research into the bacteriology of as many cases of the disease in this country as I could collect during the time of its prevalence last summer. To this end it seemed advisable to collect all the aërobic bacteria of intestinal type present in the stools and intestines of these cases, excluding, however, all the lactose-fermenting and the gelatine-liquefying bacteria, in order to determine which types were the most prevalent. This was controlled by an examination of the stools of healthy children of similar age, so that one might exclude from the list of possible causes any bacteria found constantly present in normal intestines. I am indebted to the staff of the Victoria Hospital for Sick Children, Chelsea, and also to that of the Hospital for Sick Children, Great Ormond Street, for their aid and co-operation in this matter, the former sending me the stools from 9 cases of infantile summer diarrhoea, and the latter either the stools or the intestines, or both, from 49 cases of that disease, as well as blood samples from each patient when obtainable, for agglutination reactions.

Of the 58 cases examined, 28 were diagnosed clinically as "acute infective diarrhoea," the stools from these being of the typical liquid consistency, green colour, and without blood. The remaining 30 cases were diagnosed as "catarrhal diarrhoea," blood being found in the stools of 4 of them; some of these latter were liquid and green, and of very similar appearance to those from the former cases.

METHODS ADOPTED FOR THE ISOLATION OF THE BACTERIA.

A small portion of the material, either the faeces or scrapings from the mucous surface of the large or small intestine, was transferred to a tube of sterile peptone beef broth and an emulsion made; from this the bile-salt-neutral-red-lactose-agar-plates of MacConkey[12] were inoculated, and incubated for twenty-four hours at 37° C. On the following day all the colourless colonies (that is, non-lactose fermenters) were picked off and put into tubes of lactose broth. These tubes were then incubated for three days at 37° C., at the end of which time all those that had not produced acid and gas were retained and the rest discarded. The former were then used to inoculate gelatine tubes, and set aside for future investigation.

In the numerous instances where no colourless colonies were to be found on the agar plates, the same material, which had been kept frozen in the cold room, was used for reinoculating fresh agar plates, until colourless colonies had been obtained.

The advantages of the bile salt lactose agar were found to be very great in this research, as practically all except intestinal bacteria are excluded by the bile salt, and the lactose fermenters are readily separated out by means of the deep red colour produced by the fermentation of the lactose in the presence of neutral red; at the same time it was found advisable to apply the further test of growing the cultures from the colourless colonies on lactose broth for three days at 37° C., to exclude those bacteria that ferment lactose slowly.

The next step was to examine all the gelatine cultures that had not liquefied at the end of six weeks, and to grow them on the various media used for identification—namely, broths containing glucose, mannite, dulcite, lactose and cane sugar, and litmus milk; to examine

their morphology, motility, etc., and their capability of producing indol in peptone beef broth when incubated for five days. It was found when the gelatine liquefiers had been discarded, that the number of cultures to be examined amounted to 304, and each of these cultures was then grown on all the media above mentioned for identification.

Each bacillus isolated was, immediately after isolation, tested as to its agglutination properties with the blood of the patient from which it had been obtained, and the same blood was also tested against Shiga, Flexner, typhoid, Gaertner, paratyphoid A, and paratyphoid B organisms. The strength of the dilution of serum employed in each case was 1 in 30, and of the forty-four bloods tested only one gave an agglutination reaction with the bacillus isolated from its own patient, and two with one or more of the before-mentioned test bacilli. The result is unlike what was found in America, where the agglutination of the patient's blood with dysentery bacilli was so frequently demonstrated. This procedure involved great labour, and was most disappointing in its results.

It will be observed that No. 3 also resembles *B. typhosus* in that it produces acid on glucose, mannite, and sorbite ; in fact, it has some of the characters of both Flexner's bacillus and the typhoid bacillus, differing from the latter in its absences of motility, its alkaline reaction on milk, and its indol production. It was an interesting fact that No. 3 agglutinated equally well with either a Flexner or typhoid serum, as if it were a connecting link between the two bacilli. No. 4 agglutinates well with Flexner serum, but scarcely at all with typhoid serum.

That No. 3 was an infective agent in the case of one patient is possible, as it was agglutinated in a 1 in 30 dilution of the patient's blood serum, whilst the same serum agglutinated typhoid bacilli in a similar dilution.

No. 5 resembles *B. dysenteriae* of Shiga in its cultural reactions, but differs in its distinct motility and its absence of agglutination with either a Shiga or Kruse serum. Nos. 10 to 14 are organisms, found fairly commonly in drinking water, and for that reason seem to be of small importance.

TABLE I.—*Morphological and Cultural Tests applied to the various Bacteria Isolated.*

No.	No. of Cases in which Found.	Morphology.	Glucose.	Mannite.	Dulcite.	Lactose.	Cane Sugar.	Litmus Milk. 1 Day.	Litmus Milk. 3 Days.	Litmus Milk. 15 Days.	Litmus Whey. 1 Day.	Litmus Whey. 7 Days.	Indol.	Sorbite.
1	28	B. Motile	A. G.	—	—	—	—	0	0	Alk.	Alks.	Alks.	+ +	
2	3	B. Non-motile	A. G.	—	—	—	—	A.	A.	Alks.	A.	Alk.	—	
3	5	,,	A.	A.	—	—	—	A.	A.	0	A.	A.	+ +	A.
4	2	,,	A.	A.	—	—	—	A.	A.	0	A.	A.	—	—
5	4	B. Motile	A.	—	—	—	—	A.	0	Alk.	A.	Alk.	—	
6	1	,,	A. G.	A. G.	A. G.	—	—	A.	A. S.	Alk.	A.	Alk.	—	
7	2	,,	A. G.	A. G.	A. G.	—	—	A. S.	A.	Alk.	A.	Alk.	+	
8	1	B. Non-motile	A. G.	A. G.	A. G.	—	—	A.	0	A.	A.	A.	—	
9	1	,,	A. G.	A. G.	A. G.	—	—	A.	A.	Alk.	A.	Alk.	—	
10	4	,,	A. G.	A. G.	A. G.	—	A. G.	A.	A.	A. C.	A.	A.	+ +	
11	3	B. Motile	A. G.	A. G.	A. G.	—	A. G.	A.	Alk.	Alk.	A.	Alk.	+	
12	2	,,	A. G.	A. G.	—	—	A. G.	A.	A.	A. C.	A.	A.	—	
13	1	,,	A. G.	A. G.	—	—	A. G.	A.	A.	A. C.	A.	A.	+	
14	5	,,	A. G.	A. G.	—	—	—	A.	A.	Alks.	A.	Alks.	+ +	
15	2	Streptococci	A.	A.	A.	A.	A.	A. S.	A.	A.	A.	A.	—	
16	1	,,	A.	—	—	A.	A.	A.	A. C.	A. C.	A.	A.	—	
17	1	Coccus	A.	—	—	—	—	0	A.	A. C.	0	A.	—	
18	1	,,	A. G.	A. G.	—	—	—	A.	0	Alk.	A.	Alk.	+	

In order to compare the above table with the morphology and cultural reactions of some of the best known pathogenic intestinal bacteria. I add the following table compiled from my previous work. (Reference : Some Observations upon the Micro-organisms of Meat Poisoning, and their Allies, BRITISH MEDICAL JOURNAL, June 10th, 1905, p. 1259.)

Bacterium.	Morph	Glucose.	Mannite.	Dulcite.	Lactose.	Cane Sugar.	Litmus Milk. 1 Day.	Litmus Milk. 3 Days.	Litmus Milk. 15 Days.	Litmus Whey. 1 Day.	Litmus Whey. 7 Days.	Indol.	Sorbite.
B. dysentery, Flexner, Philippines	B. Non-motile	A.	A.	—	—	—	A.	Alks.	Alks.	A.	Alks.	+	
B. dysentery, Shiga	,,	A.	—	—	—	—	A.	Alks.	Alks.	A.	Alks.	—	A.
B. typhoid	B. Motile	A.	A.	—	—	—	A.	A.	A.	A.	A.	—	
B. enteritidis, Gaertner	,,	A. G.	A. G.	A. G.	—	—	A.	Alk.	Alk.	A.	Alk.	—	
B. paratyphoid B, Schottmüller	,,	A. G.	A. G.	A. G.	—	—	A.	Alk.	Alk.	A.	Alk.	+ Slight	
B. paratyphoid A, Schottmüller	,,	A. G.	A. G.	A. G.	—	—	A.	A.	A.	A.	A.	+	
B. hog cholera, McFadyean	,,	A. G.	—	—	—	—	A.	A.	A.	A.	A.	+ Slight	

A. = Acid.   G. = Gas.   C. = Clot.   S. = Slight.   — = No reaction.

Table I shows the results of the examination of the morphology and cultural characteristics of 67 organisms of the lactose non-fermenting class isolated from the intestines or faeces of patients suffering from infantile diarrhoea.

It will be seen on comparing the two tables that the cultural reactions of none of the bacteria found by me in infantile diarrhoea correspond with those of known pathogenic bacteria, with the exception of Nos. 6 and 7, which are similar as regards their reactions to *B. enteritidis* of Gaertner and *B. paratyphoid* B of Schottmüller respectively. That they are identical with these organisms is open to doubt, as they did not agglutinate when tested with specific serums even in such low dilutions as 1 in 20.

There is a partial resemblance between Nos. 3 and 4 and the Philippine dysentery bacillus of Flexner ; No. 3, however, differs in its reaction on sorbite, on which it produces acid, whereas Flexner's bacillus causes no reaction, and No. 4 does not produce indol, whereas Flexner's bacillus produces an abundant indol reaction.

No. 1, if one can argue from its prevalence, seems to be the most important organism which I have so far isolated, having been found in 28 cases out of the 58 examined, and in 17 of these it was the only lactose non-fermenting organism present. It resembles in some respects the bacillus of hog cholera of McFadyean, differing, however, in its alkaline reaction on litmus milk, its greater production of indol, and in its failure to produce acid and gas on arabinose, maltose, and dextrin. Bacillus No. 1 produces indol in glucose broth in the presence of an excess of glucose, differing in this respect—as Dr. A. Harden kindly demonstrated for me—from most of the other indol-producing bacteria. That it produces true indol and not skatol-carboxylic acid I proved by distillation.

To determine in how far all the 28 cultures of No. 1 were identical each was grown on 11 additional media, as also was the bacillus of hog cholera of McFadyean, which in some respects resembles them. The cultures of Nos. 3 and 4 were also compared in the same way with all obtainable cultures of dysentery of the Flexner type,

including two cultures isolated from infantile diarrhoea in America (Duval, Baltimore, and Duval, New York). Various strains of *B. dysenteriae* (Shiga and Kruse) were also tested in the same way, as also *B. typhosus.* To these were added three strains of dysentery bacilli isolated by Eyre from asylum dysentery, and two strains of bacilli isolated by MacConkey from monkey's faeces, one of which resembled *B. dysenteriae* (Flexner), and the other *B. dysenteriae* (Shiga).

In compiling Table II, two laboratory strains of *B. dysenteriae* (Flexner, Philippines) were used, and one from Kral; they were found to agree in all their reactions. In the same manner, three strains of *B. dysenteriae* (Shiga) were tested, and agreed culturally in every respect —they were obtained from Berlin, Kral, and the Institute. Two strains of *B. dysenteriae* (Kruse), one from Laar, and one from Kral were also found to be identical. It will be noticed that no cultural differences were found between *B. dysenteriae* (Shiga) and *B. dysenteriae* (Kruse), which many observers now regard as being identical.

The long list of media used proved very useful in distinguishing between bacilli Nos. 1, 3, and 4, and the other known bacteria which resemble them in some respects. It will be noted that No. 1 is quite different from the bacillus of hog cholera (McFadyean), and that Nos. 3 and 4, although of the dysentery type, are not identical with any known dysentery bacilli, nor with either of the Duval bacilli isolated from infantile diarrhoea in America.

No. 1, which will be subsequently shown to be of pathogenic importance, is a motile rod, slightly smaller than the typhoid bacillus, and, like the latter, multi-flagellated. As far as I can ascertain, this bacillus has not been recognized before. Dr. Houston very kindly examined his notes for me on the numerous bacteriological analyses of human faeces, sewage, and water, made by him, but was unable to find any record of a bacillus of this type, nor at the Lister Institute has this bacillus been isolated from the numerous samples of drinking water sent for analysis. This fact led me to make a bacteriological examination of the stools of twenty normal children under 2 years of age admitted to the Hospital for Sick Children, Great Ormond Street, for such operations as harelip or cleft palate.

The method of examination was identical with that employed by me in the cases of infantile diarrhoea, with the result that out of the 20 cases only one gave a colony at all resembling No. 1, and none gave colonies resembling Nos. 3 and 4. The culture resembling the No. 1 differed from those isolated from the cases of children's diarrhoea in its failure to produce alkalinity in litmus milk, in which it produced no change of reaction, and its production of a smaller amount of indol in peptone beef broth. This bacillus I subsequently found to be non-pathogenic for experimental animals, whereas No. 1 is distinctly pathogenic for young rabbits and rats when administered by the mouth.

The examination of the stools of normal infants established the fact that the non-lactose fermenting bacteria are very infrequent in the normal faeces, as, in spite of repeated plating on lactose agar, it was very difficult to obtain any colourless colonies. In addition to the single colony resembling the No. 1 beforementioned, I was only able to isolate one colony of a bacillus resembling No. 5, and a few colonies resembling Nos. 10 and 14.

AGGLUTINATION REACTIONS WITH THE BLOOD OF PATIENTS SUFFERING FROM INFANTILE DIARRHOEA.

Forty-four samples of blood were tested against all the bacilli isolated from the patients, against the dysentery bacilli of Flexner and Shiga, and the typhoid, Gaertner, paratyphoid A, and paratyphoid B organisms, in each case in a dilution of 1 in 30. Only one, however, gave a reaction with the bacillus isolated from its own patient, namely, bacillus No. 3, which was agglutinated by the patient's serum.

In the case of two of the patients, the serum agglutinated known bacilli, one in a case in which bacillus No. 1 was present in pure culture (lactose fermenters excepted), the serum agglutinating both typhoid and Gaertner bacilli, in a dilution of 1 in 60. The other case was that of the serum of a patient from whom bacillus No. 1 was isolated; this serum gave a reaction with the typhoid bacillus in a dilution of 1 in 30.

TABLE II.—*The various cultures are so arranged that those isolated by me from infantile diarrhoea can be easily compared with known bacteria which, in some respects, resemble them.*

Bacterium.	Motility.	Glucose.	Levulose.	Mannite.	Dulcite.	Maltose.	Dextrin.	Cane Sugar.	Lactose.	Galactose.	Inulin.	Amygdalin.	Salicin.	Arabinose.	Raffinose.	Sorbite.	Erythrite.	Indol.	Litmus Milk. 1 day.	Litmus Milk. 3 days.	Litmus Milk. 15 days.
No. 1	B. Motile	A. G. S.	A. G. S.			A. G.	A. G. S.			A. G. S.				A. G.				+ +Slt. +	O.	Alk.
B. hog cholera, McFadyean...	B. Non-motile	A. G.	A. G.							A. G.				A.				+	A.	A.
No. 3	,,	A.	A.			A.	A.			A.				A.			A.	+	A.	A.	O.
No. 4	,,	A.	A.			A.	A.			A.				A.				+	A. S.	O.	Alks.
B. dysentery, Flexner, Philippines	,,	A.	A.	A.		A.	A.			A.				A.			A. S.	+	A. S.	O.	Alks.
B. dysentery, Gray	,,	A.	A.	A.		A.	A.			A.				A.			A. S.	+	A. S.	O.	Alks.
B. dysentery, Strong	,,	A.	A.	A.		A.	A.			A.				A.			A. S.	+	A. S.	O.	Alks.
B. from infantile diarrhoea, Duval, Baltimore	,,	A.	A.	A.		A.	A.			A.				A.		A.	A. S.	+	A. S.	O.	Alks.
B. from infantile diarrhoea, Duval, New York	,,	A.	A.	A.		A.	A.			A.				A.			A. S.	+	A.	O.	Alks.
B. from monkey's faeces, MacConkey II.	B. Motile	A. S.	A. S.	A.		A. S.	A. S.			A.				A. S.			A. S.		A.	O.	Alks.
B. typhoid	B. Non-motile	A.	A.	A.		A.	A.			A.						A.	A.	+ trace	A.	A.	A.
B. dysentery, Shiga	,,	A.	A.	A.		A.	A.			A.				A. S.					A.	O.	Alks.
B. dysentery, Kruse	,,	A.	A.	A.		A.	A.			A.				A. S.			A.		A.	O.	Alks.
B. asylum dysentery, Eyre 7	,,	A.	A.	A.		A.	A.			A.							A.		A.	O.	Alks.
B. asylum dysentery, Eyre 8	,,	A.	A.	A.		A.	A.			A. S.				A. S.			A.		O.	O.	O.
B. asylum dysentery, Eyre 9	,,	A.	A.	A.		A.	A.			A.							A.		A.	O.	Alks.
B. from monkey's faeces, MacConkey I.	,,	A.	A.	A.		A.	A.			A.				A.	A.	A.	A.		A.	O.	Alks.

A. = Acid.   G. = Gas.   G. S. = Slight Gas.   Alk. = Alkalinity.   Alks. = Slight Alkalinity. | = No Reaction.

The time that had elapsed between the onset of the disease and the obtaining of the patient's blood was found to have varied from twenty-four hours to two months, practically all intermediate periods being represented. Of the three instances in which a reaction was obtained, one was with that of a blood taken at the end of twenty-four hours after the onset, one at the end of fifteen days, and the third at the end of two months.

The other agglutination reactions investigated were those relating to the bacilli found by me with known serums. In these experiments bacillus No. 3 was found to agglutinate equally well with both dysentery (Flexner) serum, and typhoid serum, thus accentuating its resemblance to these organisms. No. 4 was found to agglutinate well with dysentery (Flexner) serum, but only feebly and in low dilutions, with typhoid serum. No. 5, which but for its motility culturally resembles the B. dysenteriae (Shiga), failed to agglutinate with Shiga serum even in so low a dilution as 1 in 20. No. 7, which culturally resembles B. paratyphoid B of Schottmüller, failed to agglutinate with a paratyphoid B serum.

The various cultures of bacillus No. 1 obtained from different patients did not in every instance interagglutinate. Five rabbits were immunized with five different strains of this bacillus, and, although some did, there were many that failed to agglutinate with any of the five serums.

All the cultures, however, obtained from the same patient were found to agglutinate with a serum prepared by immunizing a rabbit with one of their number. The bacillus No. 1 in this respect forms no exception to other groups of bacteria, such as the colon and Gaertner groups, the members of which do not invariably interagglutinate.

THE PATHOGENICITY OF THE BACILLI ISOLATED FROM THE PATIENTS.

For this test, organisms Nos. 1, 3, and 4 were selected, as they appeared to be the most important.

Two young rats were fed with half an agar culture each of No. 3 mixed with their food; on the following day both rats were dead, and the bacillus was recovered from the spleen of one of them. There was no evidence of diarrhoea having been caused in either case. Two young rats were also fed with No. 4 in the same way; one rat died on the following day, and the other on the second day. The bacillus was not recovered from either of these rats, though it was looked for in the spleen and in the blood. There was no diarrhoea before death.

Eighteen successful feeding experiments were made with the No. 1 bacillus, twelve with young rats and six with young rabbits. Half an agar tube proved to be invariably fatal in the case of young rats, and an agar tube in the case of young rabbits. Death generally took place within twenty-four hours, and was preceded by violent diarrhoea. The bacilli were in each case recovered from the spleen in pure cultures, and in a few instances from the heart blood as well. That this was not a mere post-mortem invasion was proved in one instance by killing the sick animal, and taking out the spleen immediately. The No. 1 bacillus was recovered in pure culture in this instance from the spleen.

As a control experiment, two young rats were fed on half an agar tube each of a mixture of three different varieties of B. coli freshly isolated from human faeces. One young rat died at the end of four days, and the other was still alive at the end of a month, proving that the virulence of No. 1 bacillus is very much higher than that of B. coli.

The first experiments to investigate the pathogenicity of the No. 1 bacillus were by intraperitoneal and intravenous injection. These, however, entirely failed to have any effect on the animals experimented upon, even so large a dose as 1 c.cm. of a very thick emulsion from an agar culture injected intravenously into a small rabbit failing to produce any result, nor did ¼ c.cm. injected intraperitoneally into a mouse or 1 c.cm. into a guinea-pig have any effect. No. 1 bacillus, therefore, seems only to be pathogenic when introduced directly into the digestive tract.

The pathogenicity was next investigated of the bacillus which had been isolated from the stools of a normal infant, and which resembled the No. 1 bacillus in all its cultural reactions with the exception of its reaction in litmus milk. An agar tube culture of this was mixed with the food of two young rats, this being the dose that proved invariably fatal in the case of the No. 1 bacillus. The young rats, however, were not in the least affected by it, and were still alive at the end of a month, proving conclusively that this bacillus differs widely in its pathogenicity from the No. 1 bacillus.

TOXIN FORMATION.

The next point of investigation was to determine whether Nos. 1, 3, and 4 produced a toxin. For this purpose flasks of alkaline peptone beef broth were inoculated with each of these cultures, and incubated for four weeks at 37° C. At the end of this time they were filtered through a Berkefeld filter, and the filtrate was injected in doses of ½, 1, 2, and 5 c.cm. intravenously into rabbits.

The first experiment was made with No. 3 filtrate, which failed to produce any effect on a rabbit even when injected intravenously in so large a dose as 5 c.cm. The filtrate of No. 4 produced no effect in any dose less than 5 c.cm., when it caused death, preceded by diarrhoea; so that, although in the feeding experiments this bacillus caused no diarrhoea before death, its toxin when injected intravenously seems to have that effect.

The filtrate from the broth culture of No. 1 bacillus was found to cause death in about twelve hours when injected intravenously into a rabbit in a dose of 2 c.cm., in some instances causing diarrhoea before death. The toxin developed seems to be somewhat unstable, as the same dose failed to produce any symptoms after having been kept in a cold room at a temperature of about 0° C. for a week. The toxicity of the filtrate was found to be no greater at the end of four weeks than it was at the end of one week, the minimum lethal dose being also 2 c.cm. It may be that we are here dealing, as in the case of dysentery, with toxins of an intracellular type.

CONCLUSIONS.

These results, together with the evidence drawn from other sources, indicate that the bacillus No. 1 is not an organism normally present in human stools, sewage, or in drinking water. From its prevalence in the stools and intestines of cases of infantile diarrhoea (in 28 cases out of 58), also from the fact that it causes death, preceded by diarrhoea in young animals, in the spleens of which it is invariably found in pure culture, one is led to conclude that this bacillus may possibly be a factor, or one of the factors, in the disease. A very significant fact is that the bacillus was isolated in pure culture (together with lactose-fermenting bacilli) from the stools of a nurse who had contracted diarrhoea from the patients in the ward set apart for this disease in the Hospital for Sick Children, Great Ormond Street.

The evidence in favour of bacillus No. 3 being either the cause, or a cause of the disease, is perhaps almost equally strong, for although it was isolated in only 5 cases out of 58, one of these cases points very strongly to its being an infective agent, from the fact that the patient's blood agglutinated this bacillus, and also the typhoid bacillus. It will be remembered that it was proved by experiment that this bacillus was agglutinated equally well by dysentery (Flexner) serum, and by typhoid serum. That this patient's serum, therefore, should agglutinate typhoid bacilli, as well as its own bacillus, is a very strong point in favour of this bacillus being infective in this particular patient at least. It will be remembered, too, that it proved pathogenic to young rats which were fed on it, although no diarrhoea was produced.

The evidence in favour of bacillus No. 4 being a factor in the disease is based principally on its resemblance to the Flexner group of dysentery bacilli, which have been assigned as the cause of the disease in America. This bacillus, too, proved pathogenic to young rats when they were fed on it.

Bacillus No. 1 and bacilli Nos. 3 and 4 were found with about the same relative frequency in the cases clinically diagnosed as "acute infective diarrhoea" and "catarrhal diarrhoea." Bacillus No. 1 was found in 11 out of the 28 cases of the former type, and in 17 out of the 30 cases of the latter type of the disease. Bacillus No. 3 was found in 2 cases of "acute infective diarrhoea" and in 3 cases of "catarrhal diarrhoea," and bacillus No. 4 in 1 of the former and 2 of the latter. It will thus be seen that the relationship between the clinical diagnosis and the

bacteriology of the disease is not well marked. Dr. Batten informs me in this respect that the distinction between these two types is not very definite, and it may very well be that the acute infective type is merely an aggravated form of the catarrhal.

The presence of a certain bacillus in the stools, or in the intestines, in cases of disease, even although that bacillus be not found in the stools of healthy individuals, hardly proves it to be the cause of the disease. If, however, it has been found to invade the blood or the spleen, this evidence is very strongly in its favour. Unfortunately, neither the blood nor the spleen of fatal cases were examined bacteriologically, so that the presence or absence of bacteria in these localities are matters for future investigation by me next summer.

My thanks are due to Dr. F. E. Batten, who took the greatest interest in this research, and supplied me with the material from the Hospital for Sick Children, Great Ormond Street; also to Dr. Allan Macfadyen, at whose suggestion and in whose laboratory the work was done, for his most valuable assistance and advice.

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REPORT XCVI.

THE INFLUENCE OF INCREASED BARO-METRIC PRESSURE ON MAN.*

BY M. GREENWOOD, JUN., M.R.C.S., L.R.C.P.,

RESEARCH SCHOLAR OF THE BRITISH MEDICAL ASSOCIATION.

ALTHOUGH the idea of employing compressed air for submarine work is at least as old as the sixteenth century (Sturmius, Halley, etc.), the subject hardly possessed much industrial importance until the application of the principle to well-sinking and pile-driving by Triger, a French engineer, towards the middle of the last century. Triger's memoir was presented to the Academy of Sciences in 1841, his methods were speedily applied on a large scale, and since his time caisson works have been in operation all over the world. The pathological effects of living in compressed air are therefore of practical importance, and their scientific investigation worthy of attention.

The earliest description of caisson disease by medical men is that of MM. Pol and Watelle, published in 1854, but written in 1847, and founded on observations of caisson works in connexion with mining operations at Douchy. The theories of these authors, being destitute of experimental foundation, are erroneous; their practical conclusions, on the other hand, are much sounder than those of many subsequent writers. Thus they noted the importance of slow decompression in obviating accidents, a factor neglected or even derided by other workers. They say:[1]

Compression up to 4¼ atmospheres is not in itself dangerous; it is quite well borne, far better than a proportionally much less rarefaction. The return to normal pressure is alone the dangerous part, this danger being proportional at once to the amount of compression and rapidity of decompression; the rate of the latter should accordingly be much reduced.

For the next twenty years although a good deal was written little of it has any value. In 1877, Paul Bert's treatise, *La Pression Barométrique*, was published. This work deserves to be regarded as one of the classics of physiological science, and proves its author to have been

an experimenter and reasoner worthy to be ranked with his illustrious countrymen—Claude Bernard and Louis Pasteur. By a series of well-planned and accurately-conducted experiments Bert proved that:

1. Increased air pressures up to + 7 or + 8 atmospheres do not produce any unfavourable symptoms.

2. Pathological accidents occur during decompression only, and are due to the liberation of nitrogen bubbles in the blood and other tissue fluids, such bubbles leading in some cases to embolism, in others to transitory nerve irritation.

3. These symptoms can be prevented if the rate of decompression be sufficiently slow—that is, at the rate of about twenty minutes for each atmosphere of positive pressure.

These conclusions have received abundant confirmation from the more recent experiments of Hill and Macleod in England, and Heller, Mager, and von Schrötter in Austria.

In view of the attitude taken up by a small section of the British public with regard to experimental physiology, it is worth noting that Bert's epoch-making discoveries—discoveries which, if adequately recognized, would be the means of saving many lives—resulted from numerous experiments on animals.

It is somewhat discreditable to our intellectual culture that Bert's work has been little regarded. Those responsible for caisson works since his time have almost invariably allowed far too short periods for decompression, and many preventable deaths have occurred. Even in Austria, where scientific research is in closer touch with commerce than in many countries, Heller, Mager, and v. Schrötter speak bitterly of the obstacles placed in the way of an attempt to adopt suitable hygienic measures at the caisson works to which they were attached. They say[2]:

Unfortunately during our work we were able to convince ourselves how difficult it is, without possessing special authority, to carry out the necessary arrangements, with the result that well-thought-out and energetically-urged advice remains merely a pious expression of opinion, in that it is frustrated by external factors and opposition.

This and other experiences indicate that the co-operation of pure and applied science is still lamentably incomplete.

If we consider the effects of pressures greater than those employed in caissons, we shall see that the same conclusions hold. Deep-sea divers have been exposed to very high pressures, and in practice accidents similar to those already noted have occurred.

The following are some "record" dives. Lambert, the famous diver employed by Messrs. Siebe and Gorman, salved £100,000 at a depth of 160 ft., staying twenty minutes below and occupying about the same time in his ascent. On his last journey he stayed rather longer below, and became affected subsequently with symptoms pointing to a lesion of the spinal cord—permanent incontinence of urine, etc. Another diver, Walker, has descended to a depth of 189 ft. This man, to whom I have spoken, attributes his immunity from accidents to his habit of ascending slowly. Perhaps the greatest depth ever attained is one of 204 ft. (approximately equal to +88½ lb. air pressure); the diver in this case expired on returning to the surface, probably owing to a very rapid ascent.

The experimental study of these higher pressures, as applied to man was first undertaken by Hersent, who, in 1893, compressed a workman to +71.2 lb., and subsequently to +76.8 lb., without misadventure. As the subject of these experiments was not a trained observer, it seemed desirable to reinvestigate the subject, and to furnish yet another proof of the accuracy of Paul Bert's work. This has been done in the course of some researches by Leonard Hill and the writer.

Messrs. Siebe and Gorman kindly placed their workshop at our disposal and constructed the pressure chamber, of which a photograph is reproduced. In addition to the fittings represented in the figure, the chamber was provided with a mattress, blankets and electric light; further, it was of such a size as to permit the observer to sit up or lie down comfortably.

We first studied the effect of air pressures greater than those formerly employed.

In an experiment carried out in November last, the