A CASE OF CHOLERAIC DIARRHŒA CAUSED BY AN ORGANISM OF THE *BACILLUS PROTEUS* GROUP.

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The clinical history of the case was as follows :—

The patient, a Greek labourer, was admitted one morning to Khartoum North Dispensary in a collapsed condition, suffering from abdominal cramps, vomiting and diarrhœa. The previous night he had partaken of a meal obtained at a native kitchen, and, up till within an hour of admission to the dispensary, was in apparently good health.

When seen by the medical officer in charge of the dispensary, the patient's symptoms simulated those of Asiatic cholera and the case was reported as such to the Medical Officer of Health. Peripheral blood-films were asked for and examination of them showed that malaria parasites were not present. A specimen of the patient's stools was also sent to the Wellcome Laboratories for a bacteriological examination.

The stools were greyish in colour, very offensive in odour, acholic, of a watery consistence and contained large flakes of mucus with but little fæculent material. Macroscopically they resembled closely "the rice-water stools" of Asiatic cholera.

Hanging-drop preparations of the stools showed the presence of epithelial flakes and large numbers of short motile bacilli. Neither vibrios nor intestinal parasites were present. Stained preparations showed practically nothing but the presence of short bacilli.

The usual technique of inoculating and incubating peptone flasks for the purpose of encouraging the growth of cholera vibrios was carried out. The inoculated flasks were carefully examined for evidence of cholera vibrios, but with negative results. They contained almost a pure culture of short, motile bacilli. It was noted, too, that a very penetrating and offensive odour was emitted from the inoculated flasks.

Samples of the stools were also suitably diluted and inoculated into MacConkey's bile salt broth tubes and after these had been incubated for twenty-four hours sub-cultures were made on Drigalski-Conradi media. Other portions of the stools were diluted and plated out directly on Drigalski-Conradi media. All the plates were examined at the end of forty-eight hours' incubation at 37° C. and found to contain a pure culture of large, bluish, globular colonies of a very viscid consistence. By transmitted light, each colony showed a pink centre.

A very heavy and penetrating odour emanated from the plates,

extremely suggestive of the odour given off by organisms of the *B. proteus fluorescens* type. At the end of seventy-two hours many of the colonies had coalesced and produced a characteristic, greenish-yellow fluorescence in the Drigalski-Conradi media.

Colonies were picked off and inoculated into broth, and after suitable incubation subcultures were made on agar slopes; colonies were also subcultured from agar into the various sugar media.

The broth culture was examined at the end of eighteen hours' incubation and found to contain a short, motile bacillus which was Gram negative and which stained readily with the usual aniline dyes. Its average length and breadth was 2·4 μ by 0·5 μ. The shorter forms measured 1·3 μ and resembled cocco-bacilli. Slight turbidity was produced in broth. On agar plates, the colonies were circular and of a greyish-yellow colour with a granular centre and opaque margin. On agar slopes, a greyish-yellow viscid growth was produced. The bacillus was an indol producer, the reaction being definitely obtained in an hour by the paradimethylbenzaldehyde test recommended by Marshall [1]. Owing to the prevailing climatic conditions, it was not possible to use gelatin as a culture medium.

A positive Voges-Proskauer reaction [2] was obtained in glucose peptone.

Acid and gas were produced in glucose, mannite, lævulose, maltose, galactose and dextrin, whereas no change occurred in lactose, cane sugar, dulcite, adonite, inulin and raffinose. Acid, but no clot, was produced in litmus milk; but, three days later, this medium became alkaline and permanently remained so. Blood serum was not liquefied.

On potato, a creamy, viscid growth was apparent in forty-eight hours. The bacillus was a facultative anaerobe, non-acid fast and non-spore forming; it stained readily with acid and basic dyes.

Pathogenicity.—A guinea-pig was injected intraperitoneally with 2 c.c. of a young broth culture of this bacillus. Four hours afterwards this animal died with a well-marked and severe peritonitis, and intense hyperæmia of the liver and spleen. The bacillus was isolated in pure culture from the heart's blood and spleen. Subcutaneous injection of 0·5 c.c. of a broth culture produced no ill-effects in a guinea-pig.

Some experiments were then carried out to ascertain whether guinea-pigs would develop symptoms of enteritis when fed with broth cultures of this bacillus. Apart, however, from causing a rise in temperature, no ill-effects were observed. In one experiment opium was given *per os* to a guinea-pig, so as to inhibit its intestinal peristaltic movements, and it was then fed with a young broth

culture. As the animal's health was apparently unimpaired at the end of a week, it was chloroformed. The small intestine in its lower part was hyperæmic, and the spleen was congested. The bacillus was isolated in pure culture from the spleen. Similar feeding experiments were carried out on a *Cercopithecus sebæus* monkey, but unfortunately the animal succumbed two days afterwards from pneumonia.

The pathogenic properties of this bacillus were also tested on a rabbit. Intraperitoneal inoculation with 0·2 c.c. of a broth culture caused death in twelve hours; the peritoneum, spleen and liver showing intense congestion. Subcutaneous injection of 1 c.c. of a broth culture produced no untoward symptoms, and feeding experiments *per os* gave the same results as in the guinea-pig.

Several agglutination tests with the bacillus were carried out with the sera of the animals used in the feeding experiments. The highest agglutination reached was a dilution of 1 in 40.

The patient's serum had no specific agglutinins either for this bacillus or for any of the *Bacillus typhosus* and *coli* group.

In all probability, the absence of a systemic infection of the patient would account for a deficiency in specific agglutinins; unfortunately, blood cultures were not carried out to prove the presence or absence of a septicæmia.

After frequent subcultures on agar, it was found that the virulence of this bacillus was somewhat attenuated. Its cultural reactions were tested again in the various sugars, and at the end of a month these reactions had in no way differed from the original ones carried out.

The further clinical history of the patient was as follows:—

After admission to hospital, he remained in a very critical and collapsed state for a period of twelve hours, but eventually responded to the potassium permanganate treatment recommended by Rogers [3] in cases of cholera.

For the sake of convenience a table of the cultural reactions of this bacillus is appended.

Glucose	Mannite	Lævulose	Maltose	Galactose	Dextrine	Lactose	Cane sugar	Dulcite	Adonite	Inulin	Raffinose	Litmus milk	Indol	Voges proskauer
+	+	+	+	+	+	−	−	−	−	−	−	A. Alk.	+	+

+ = Acid and gas. A. = Acid.
− = No acid and no gas. Alk. = Alkaline.

Conclusions.

If reference be made to the above table, it is apparent that the organism described possesses different cultural reactions from many of the pathogenic intestinal organisms that have been isolated from human excreta. It does not conform in all cultural reactions to any of the intestinal organisms mentioned in a very complete list in a recent paper by Castellani [4]. During the last four years the writer has had many opportunities of working out the various intestinal bacteria found in man and animals in the Sudan, and this organism has never previously been met with.

The clinical picture of the patient certainly indicated an intestinal infection with either the cholera vibrio or with an organism of the food-poisoning group. Bacteriological examination excluded the former, and cultural reactions and serum agglutination tests proved that the bacillus isolated did not correspond to those of the *B. Gaertner* or *B. paratyphosus* groups.

The fact that it produced a positive Voges-Proskauer reaction would, according to MacConkey [5], place it in either the *B. lactis aerogenes* or the *B. cloacæ* group.

Orr [6], however, in his work on milk, states that the organisms of the *B. proteus* group frequently give a positive Voges-Proskauer reaction. The writer is inclined to assign this bacillus to a position in the *B. proteus* group. Its chromogenic characters in certain media and its power of producing such an offensive odour during its growth, rather favour this view. The fluorescent properties rather resembled those of *B. proteus fluorescens*. Unlike most of the members of this group, this bacillus showed no tendency to grow in the form of filaments.

The proteus type of micro-organism was first isolated by Hauser [7], who considered it as the chief one concerned in the process of putrefaction. Since then, it has been studied by many observers.

Glenn [8] has worked at the cultural reactions of some of the proteus group. His observations, however, are chiefly confined to a study of the acid and gas-producing powers in sugar media and to the ferments produced.

Schnitzer [9] isolated a member of the proteus group from a case of cystitis, and Flexner [10] and Reed [11] have obtained a proteus organism in cases of peritonitis and pneumonia.

Booker [12], in his elaborate work on cholera infantum, came to the conclusion that *B. proteus vulgaris* played an important part in the etiology of that disease.

Metchnikoff [13] found *B. proteus* very commonly in the stools of children suffering from diarrhœa.

At the Bombay Medical Congress, Macy [14] read a useful paper on tropical diarrhœa, and stated that under certain predisposing conditions many of the proteus group of organisms might excite violent intestinal symptoms.

Recently, Cantu [15] has made an extensive investigation into the distribution of *B. proteus* in nature, and has noted its constant presence in vegetable products which are in direct contact with soil. According to this observer, the summer season appeared to favour its growth, a fact borne out by Metchnikoff, who found *B. proteus* frequently in the stools of adults during the late summer months. Metchnikoff considered that flies acted as the transmitting agents in infecting food, especially cheese and grapes.

Bernstein [16] found that by the use of boric acid to the extent of 0·3 per cent (20 grm. to the pound) a selective inhibitory effect was produced on all organisms of the *B. proteus* group. Advantage might therefore be taken of this as a prophylactic measure to be adopted in epidemic gastro-enteritis caused by organisms of this group.

Sufficient references have been given to show that the *B. proteus* group justifies a certain claim in being considered pathogenic to man, a claim which the writer feels has not aroused sufficient attention from bacteriologists, particularly in tropical countries.

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