UNUSUAL DYSENTERIC INFECTIONS.

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From time to time certain organisms, all closely similar, have been encountered in this laboratory which from their unusual occurrence and unfamiliar behaviour presented some difficulty in their identification. An account of some of them and of the cases from which they were derived is here given, and an attempt is made to correlate the organisms. If the result of the investigation shows that they belong to a single group of pathogenic bacilli, the important fact will be illustrated from the short accounts of the cases that a wide range of clinical manifestations may be met with in association with this group of bacteria.

I. Clinical and other Data.

(1) *Cases T.* Towards the end of April, 1925, an outbreak of acute illness occurred in the County of Ayr, 11 persons being affected. Nine of these, of whom three were adults, were resident in an hotel, and the remaining two were children belonging to a neighbouring house. The onset in all cases was sudden and was characterised by abdominal pain and diarrhoea, and in some of the cases by vomiting. The number of stools in the first 24 hours was approximately seven or eight. Temperatures of 103° F. and 104° F. were recorded at the onset in some cases. Blood and mucus were seen in a few of the stools. The illness lasted about five days, though in the cases of one or two delicate children it was longer. It appeared clear to the Health Authorities who made the investigation that food could be excluded as the vehicle of infection, because two cases occurred contemporaneously outside the hotel while none of the food of the hotel was derived from local sources. The only supplies common to the hotel and the neighbouring houses were milk and water, and nothing suspicious was found in connection with the milk. Although no pollution was found in the water when examined it was ascertained that the supply at its fountain head was not properly protected and was near a place used by travelling gipsies. Human excreta were found in the vicinity. On the ground that a similar outbreak lasting a few days occurred in the same hotel almost exactly a year previously the idea was entertained that the annual visit of the caravans, which takes place at a fairly constant time of the year, might have significance in relation to the occurrence. The causative organism was found in the stools of four of the patients, the eldest 11 years old, from two to four days after the onset. These organisms will be referred to here as Bacillus T. 1, T. 2, T. 3, and T. 4.

(2) *Case K.* This patient, a young man of 22 years, became suddenly ill with abdominal pain on August 28th, 1925. He felt sick and vomited, and complained of headache. The tongue was furred. Previous to this he was apt to be constipated and readily noticed some blood in his stool on the first day of illness. On admission to hospital on August 29th the patient complained of pain mostly on the right side of the abdomen and there was rigidity over the right iliac region, there being also some pain on pressure on the left side. The temperature and pulse were elevated and respiration moderately accelerated. An enema was given, the ensuing discharge consisting solely of blood and mucus. Diarrhoea followed. The appendix was removed on the evening of admission to hospital and was noted to be slightly inflamed, with the vessels in that region engorged. There was no other abnormality observed in the abdomen. There was no sign of ulceration of the bowel. The appendix, which contained much mucinous material, yielded a great variety of organisms on culture but not the bacillus that is about to be described. In the forenoon of the day following the operation the patient's temperature was 101·2° F., and pulse 108, and on the evening of the succeeding day (August 31st) the temperature was 101° F. On September 1st it was 99° F. and descending to normal. There were accordingly four days on which the temperature was elevated in this case. The faeces were submitted for examination on the third day from the onset and a bacillus was isolated which will be shown to have had an intimate connection with the illness. Examination of the faeces 12 days from the onset gave a negative result. This organism is referred to as Bacillus K.

(3) *Case L.* The organism from this case, referred to as Bacillus L., was found in large number in the lower part of the great intestine of a young man *post mortem.* Though reported to have been somewhat out of sorts on a certain day this patient continued at work. In the evening he complained of thirst and vomiting. He retired to bed early and then had attacks of diarrhoea which continued through the night. In the morning he collapsed and died. The mucous membrane of the stomach was swollen and the greater curvature exhibited a large area of ecchymoses without ulceration. The lower part of the small intestine was lightly pink in colour, while the whole lining of the great intestine was intensely red. There were traces of blood but no ulceration of the bowel. The signs of disease were confined to the alimentary tract.

(4) *Case D.* The symptoms in this case were milder than in any of the cases here dealt with. The patient, a medical man, had an illness of sudden onset, with rise of temperature to 101° F. and diarrhoea. The temperature subsided after 24 hours. Blood or mucus was not observed by the patient nor was either present in the faeces from which Bacillus D. was cultivated. The chief feature of this case was that for a period of three weeks afterwards diarrhoea was induced on slight provocation by the mere taking of food. The patient was otherwise well after the first two days or so.

(5) *Case R.* This case differs considerably from those already described.

The organism to be referred to as Bacillus R. was isolated a year after the first attack of dysentery, but as it had not been found previously an important link in the chain of events is missing whereby to implicate it with certainty in the first attack. Nevertheless, at the time when the organism was found there were no grounds for supposing that the patient was suffering from any infection other than that contracted twelve months previously, the symptoms having been more or less continuous. The patient, a doctor of medicine, had gone to Portugal for improvement of health and during the first week's residence there contracted an illness which was diagnosed as acute colitis. For about five days the temperature remained elevated and the stools contained mucus and streaks of blood. There was no vomiting. The pain in the abdomen is described as of a griping character. The patient continued to have intermittent attacks of discomfort and looseness of the bowels for 12 months after returning to this country and to suffer from considerable debility, and at the end of that time, during an acute attack accompanied by fever, Bacillus R. was cultivated from the first stool examined, the faeces being typically dysenteric in character, *i.e.* consisting of blood, mucus and pus cells with no faecal matter. No protozoa were found on examination, nor any other pathogenic organism. The occasion did not present itself for further examination of the stools in this case because the patient straightway entered a nursing home, where a thorough course of bowel irrigations contributed to an immense improvement of the condition. This improvement has been so far maintained that for a period of 18 months there has been nothing in the nature of a recurrence of symptoms.

II. Bacteriological Examinations.

In making a detailed study of the before-mentioned bacteria two strains of Sonne's Group III bacilli, derived from the National Collection of Type Cultures, have been used in closely parallel tests throughout. These are Nos. 268 and 269 of the Catalogue. I have also had the privilege, through the kindness of Dr Bamforth, St Thomas's Hospital, of carrying out comparative tests with three of the organisms cultivated and described by him from cases occurring in a certain institution. Dr Bamforth intimated to me that he considered his organisms to be Sonne's bacillus, subject however to serological determination. The cultures from the National Collection and from St Thomas's Hospital will, for the purposes of this investigation, be referred to as S. 268, S. 269, B. 1, B. 3 and B. 4. The cultural and other characteristics about to be described apply, however, exclusively to the organisms that have been isolated in this laboratory, unless otherwise stated. The organisms about to be compared, then, are, in accordance with the above description, referred to as T. 1, T. 2, T. 3, T. 4, K., L., D. and R. (which constitute the present series), and S. 268, S. 269, B. 1, B. 3 and B. 4.

The members of our series, the T.'s, K., L., D. and R., under the preliminary tests applied at the time of their isolation, temporarily gave rise to

a suspicion that they were bacilli of the Flexner dysentery group, but their strong divergence from this group became very apparent during their examination. They are all non-motile, Gram-negative bacilli and none of them forms indol in five days.

Morphology. The organisms are of the colon-typhoid type. A feature common to them all is that as young cultures on agar they are mostly minute, slender rods. Longer forms, up to the size of *B. typhosus*, appear and set off the general picture as being that of minute rods. In this respect bacillus B. 4 conforms to this series. An inconstant feature is the appearance of filamentous forms. These were observed with bacillus K. (frequent) and with the bacilli T. (rare) but were not seen with D., L. or R. A notable appearance observed with each member of this series is that when films are stained with dilute carbol fuchsin many of the organisms exhibit a very lightly staining transverse central band, which in the minute forms gives a diplococcal appearance. The suggestion is that of bipolar staining.

On solid media. The growths on agar and on taurocholate lactose agar are similar in appearance. They are larger than that of *B. dysenteriae* Flexner. On agar the colonies show no regularity of size. The majority are 3–5 mm. in diameter on 20 hours' incubation at 37° C. While a few are almost circular, the general appearance is one of irregularity of outline. The centre of the colony is elevated but flat. The margins shelve out to a thin edge, the edge being finely crenate. Colonies well separated from one another on taurocholate agar may grow to a large size when left for some days at bench temperature, a diameter of as much as 1 cm. having been observed. Lobulation of outline, which may be present to some extent in the younger colony, becomes a marked feature of these large colonies.

Veal broth. Incubation of each of this series of bacilli in this medium results uniformly in the production, after 18 hours, of a heavy growth, the bulk of which has sedimented, forming a thick layer at the bottom of the tube. There is no pellicle.

Fermentations. The medium used in these tests was peptone water containing 1 per cent. of "sugar" and Andrade's indicator. The initial reaction of the medium was *p*H 7·8. No gas is produced in any of the media.

Glucose and *Mannite.* Fermentation of these substances appears within 24 hours and the acidity is permanent.

Dulcite. No fermentation was observed.

Lactose. With lactose, as with saccharose and maltose, acidification is first observed in the Durham tube, and in the case of those organisms where the acidity of those three media is not permanent the Durham tube is the last portion of the medium to preserve it. While each organism of the series showed some degree of fermentation of lactose in from seven to nine days, the progress towards fermentation of the whole tube was slow with some of them, the T. bacilli, K., L., D. and R. taking 11 to 13 days. S. 268 and the B. bacilli reached this stage more rapidly than the others. The organisms showed differences

in the permanence of the acidity in lactose. T. 2 and T. 3 were observed to have returned to alkalinity in three to four weeks, and B. 1 and B. 4 in three weeks. S. 268, S. 269, D. and K. exhibited no change in five weeks, the tubes remaining acid, and were then discarded.

Saccharose. The fermentation of this sugar precedes that of lactose by a short interval with most of these organisms, and in every case there is a return to alkalinity in three weeks to a month. K. was alkaline on the 13th day. Residence or subculture on agar appeared to diminish the duration of the acidity.

Maltose. All the organisms showed fermentation of maltose in 24 hours. With the exception of bacillus K. an interval of three weeks sufficed for the production of partial or complete alkalinity, the change appearing first at the surface of the liquid exposed to the air. The Durham tube may retain its acid reaction for a long period. No change was observed in the K. tube in five weeks. *B. typhosus* was used as the control. The sugars galactose, laevulose, adonite, dextrin, raffinose, inulin and arabinose were used to ascertain if there was any feature that might serve to contrast these organisms with *B. dysenteriae* Flexner Y. Bacilli S. 269, T. 1, K., L., D. and R. gave fermentations identical with those of bacillus Y. (Hiss and Russell).

Litmus milk. All the organisms of this series produced acid and clot in this medium if given sufficient time. The period varied with the different bacilli from 11 to 22 days. Clotting, which is preceded by a long period of strong acidity, begins at the bottom of the tube and extends upwards, the lower portion of the medium becoming colourless. Bacilli S. 268 and S. 269 behaved similarly.

Gelatin. None of the organisms liquefied this medium. Along the line of the stab tuft-like outgrowths appeared and there was usually a well-developed mop-like tuft at the lower end of the stab. The outgrowths were so well developed with bacillus R. that the appearance resembled that of the antenna of the male anopheline mosquito. This phenomenon is, however, not peculiar to this group of colon bacilli.

Haemolysis. The test was made with tubes of 5 c.c. peptone water containing 2 per cent. rabbit's corpuscles, with parallel negative and positive controls. All the organisms, including the N.C.T.C. strains, were non-haemolytic.

Serological relations.

These comprised agglutination tests with the sera of two patients upon the various organisms and agglutination and saturation tests with immune sera prepared by the inoculation of rabbits with certain of the strains.

(*a*) Patients' sera. It was early found that the use of formalised emulsions from agar was unsatisfactory. They gave no well-defined end-point, and the reaction was at a lower dilution than it was with the use of formalised veal-broth cultures. The latter medium gave clear and higher end-points and was accordingly used in all the subsequent serological work. In all agglutination

tests throughout the investigation the reaction was allowed to proceed for four hours in a water-bath at 52° C., and was read 15 minutes later. The two patients' sera were those of T. 1 and K., that of T. 1 being taken ten days after the onset of illness. In case K. two specimens were taken, the first on the third day and the second on the ninth day after the onset. In case R. blood was taken during a relapse 12 months after the onset and the serum gave no reaction with bacillus R. which was just then isolated. This serum was not available against any of the other organisms. It has been suggested by Thjøtta in his account of the Sonne III Group of bacilli that reactions in comparatively low dilutions of patients' sera may be taken as diagnostic of this Group, since none of his organisms reacted with normal human serum. If it should eventuate that each organism of the present series may properly be subsumed under that group, a note ought to be made here that bacilli R. and L. for some period after isolation gave not a little trouble through their reactions with normal human sera, a considerable number of these being employed at the time. This property had disappeared when the present investigation was undertaken, and no other of the series showed the same property at any time provided the reading of the reactions were made with the naked eye and not with a lens. With the possibility in view that our series may be examples of the Sonne III Group in connection with which somewhat low titres of agglutination have been recorded, and in consideration of the paucity of records regarding the agglutination titres which a patient's serum may be expected to possess in infection with this type of organism, the end-points found with this series by the use of the technique already mentioned may with advantage be detailed.

End-points of agglutination in 4 *hours at* 52° C. *Patients' sera.*

Organism	K. 1st spec. 3rd day	K. 2nd spec. 9th day	T. 1 10th day
K.	12	50	50
T. 4	50	100	100
T. 1	50	200	250
T. 2	Not done	200	200
T. 3	50	250	250
D.	50	200	200
L.	50	100	50
R.	50	100	100
B. 3	50	200	250
B. 4	25	100	100
S. 268	25	200	200
S. 269	25	100	100

These readings were made with the naked eye and were distinct. Normal human serum controls under the same circumstances and employed in each case were uniformly negative. The T. bacilli, K., R., D. and L. gave no reaction in five hours in dilution $\frac{1}{25}$ to agglutinating sera of *B. flexneri* V., X., Z. or W. (titres 250), and the same result was observed with a typhosus serum (titre 400) put for five hours against organisms T. 3, L. and R. Bacillus K. had just been isolated when the test was done, and T. 3 had been passed

through a guinea-pig. The very finely granular reaction which characterised S. 268 and S. 269 and was noted with K. could not be said to be a feature of the other organisms.

(*b*) Immune sera for the saturation tests were prepared by inoculating rabbits with 24-hour broth cultures, it having been previously ascertained that the animals' sera gave no appreciable reaction with any of the organisms. In the case of only one of the untreated animals' sera was there a trace of agglutination in dilution $\frac{1}{25}$ with bacilli S. 269 and B. 1 when read by a hand lens, the other organisms giving no reaction. In the case of rabbits' sera, accordingly, the lens could be used with advantage in these tests without any complication such as appeared with its use in the case of certain "normal" human sera. Bacilli D. and T. 4 were selected as antigens on no other grounds than that case D. was much the mildest of the cases, and that case T. 4 was typical of the outbreak while bacillus T. 4 was somewhat less agglutinable with the patients' sera than the other T. organisms. Sera of suitable agglutinating power were obtained by inoculating intravenously 500 million bacilli of a formalised broth culture and following up at weekly intervals with 250, 500, 1250 and 2000 millions of the living organisms grown in broth, all the injections being intravenous. With slight variation in dosage immune sera were similarly prepared with bacilli S. 268 and S. 269. All the organisms under review were found to be agglutinable by each of these four sera in high dilution, by sera D. and S. 269 in very high dilution and by sera S. 268 and T. 4 at never less than half titre.

Saturation experiments. Heavy suspensions of the bacilli from agar plate cultures were made in saline containing 0·2 per cent. phenol. A portion of the immune serum under test was diluted 1 in 5, and to 1 c.c. of the diluted serum was added 2 c.c. of one of the bacillary emulsions. The mixture having been made complete was placed in the incubator at 37° C. for two hours and then kept in the ice chest overnight. Next day the treated serum was separated off in the centrifuge and tested for agglutinin content along with the untreated or control serum. The results of these tests, taken with the microscopical and cultural characteristics and biological properties, already described, serve to place all the organisms under examination in one and the same group. The results are tabulated after Dudgeon's method. The result is given as a fraction whose numerator is the end-point of agglutination with the treated serum while the denominator is the end-point of reaction with untreated serum. The readings were made in all cases with a hand lens, and a dilution of 1 in 200 of the saturated serum was taken as the lowest limit of test in each case. (See Table I, next page.)

Pathogenicity for animals. (1 *a*) Bacillus "T. 1," having been a month on agar, was injected subcutaneously into a guinea-pig weighing 300 grm. Dose, 0·25 c.c. of a 24-hour broth culture. In four days the weight was 280 and in six days 272 grm. The animal was killed on the seventh day. At the site of inoculation there was a thick-walled circumscribed abscess containing

Table I. *Relating to saturation experiments* (see p. 193).

1. Immune serum T. 4 saturated with bacillus "S. 269." Emulsion 110,000 million per c.c.

On T. 4, T. 1 and K., each ...	0/5000
On L., R. and B. 1, each ...	0/5000
On D.	0/4000
On B. 3 and S. 268, each ...	0/2500

2. I.S. T. 4 saturated with bacillus "L." Emulsion 90,000 million per c.c.

On T. 4, K. and R., each ...	0/5000
On D.	0/4000
On S. 269 and B. 4, each ...	0/2500

3. I.S. D. saturated with bacillus "S. 269." Emulsion 150,000 million per c.c.

On D., K. and L., each ...	0/12500
On T. 1, R. and B. 1, each ...	0/12500
On T. 4	0/8000

4. I.S. D. saturated with bacillus "R." Emulsion 180,000 million per c.c.

On T. 2, K. and L., each ...	0/12500
On D. and B. 3, each ...	0/12500
On S. 269	0/8000

5. I.S. S. 269 saturated with bacillus "T. 1." Emulsion 150,000 million per c.c.

On S. 269, K. and D., each ...	200/12500
On R., L. and B. 1, each ...	200/12500
On B. 3, T. 1 and S. 268, each	200/12500

6. I.S. S. 269 saturated with bacillus "D." Emulsion 100,000 million per c.c.

On S. 269, S. 268 and B. 3, each	200/12500
On L., K. and R., each ...	500/12500
On T. 2	0/12500

7. I.S. S. 269 saturated with bacillus "R." Emulsion 100,000 million per c.c.

On S. 269, K., L., D., R., T. 1, B. 3 B. 4 and S. 268, each	0/12500

8. I.S. S. 268 saturated with bacillus "L." Emulsion 100,000 million per c.c.

On K. and D., each	0/2500
On B. 4	0/4000

9. I.S. S. 268 saturated with bacillus "B. 1." Emulsion 100,000 million per c.c.

On S. 268 and T. 1, each ...	0/5000
On K. and L., each	0/2500
On R.	0/4000

pus cells in a grey fluid. The small intestine was oedematous and its vessels congested, the mesenteric glands near the caecum being enlarged. The liver was deeply and the adrenals slightly congested. The spleen appeared normal. The lungs for the most part were pale with here and there in both small patches of deep congestion. The organism was recovered from the liver. (1 *b*) A guinea-pig fed with 0·6 c.c. of a 24-hour broth culture of bacillus "T. 1" remained well.

(2) 1 c.c. of a very light emulsion of the faeces of case T. 3 was inoculated subcutaneously in a guinea-pig. The animal was killed on the fifth day when it was ill and showed an indurated swelling extending from abdomen to thorax. Subcutaneously there were dense adhesions and extensive haemorrhagic oedema over the anterior body wall, with enlargement of the axillary glands. The liver was congested and the spleen slightly enlarged but of normal colour. The organism was recovered from the subcutaneous fluid, blood and spleen.

(3 *a*) A guinea-pig was given an intraperitoneal inoculation of 0·05 c.c. of a 48-hour broth culture of the freshly isolated bacillus "K." It died the following day. There was haemorrhage at the point of inoculation and subcutaneous watery oedema over abdomen and thorax. The peritoneal cavity showed an excess of turbid fluid containing a culture of bacilli and pus cells. The liver was covered with flakes of lymph and the spleen was moderately enlarged. The adrenals were slightly congested. The peritoneum, blood and spleen yielded bacillus "K." in pure culture. (3 *b*) A guinea-pig weighing 425 grm. received a subcutaneous inoculation of 0·05 c.c. of a 22-hour broth culture of bacillus "K.," freshly isolated. In three days the weight was 340, in seven days, 304, in eight days 290 grm. The animal was killed on the ninth

day. There was a subcutaneous abscess with necrotic tissue lining its wall and proceeding to ulceration. The liver was markedly congested and the spleen normal in size. The organism was present in pure culture in the abscess while the blood and spleen were sterile. (3 *c*) A guinea-pig fed with 0·5 c.c. of a 22-hour broth culture of bacillus "K." three days after isolation remained well.

(4) Tests by feeding and subcutaneous inoculation were made on guinea-pigs with bacillus "R." within a fortnight after its isolation. Feeding produced no apparent effect. The inoculation resulted in a very tender, indurated swelling but the animal was well otherwise and recovered.

(5) Experiments done with certain of the organisms two to three months after isolation indicated that this type of organism loses its virulence comparatively soon on artificial culture.

DISCUSSION.

Sufficient proof has been adduced to show that all the organisms dealt with in this enquiry fall into Group III of Sonne's classification of dysentery bacilli, since their essential identity with *B. dysenteriae* No. 268 and No. 269 of the National Collection of Type Cultures has been shown. The cases described illustrate the wide difference in virulence for man shown by these organisms. The length of time required for the production of strong acidity and clotting of litmus milk appears to vary considerably with different strains and may be the factor which accounts for the remark of Thjøtta, that "in litmus milk there was as a rule a slight production of acid but never coagulation" with this group. In any case, bacilli S. 268 and S. 269 now behave similarly to the bacilli of the present series when grown in this medium. With regard to agglutination tests with the sera of patients it may be noted that the end-points obtained by Bamforth, when the reactions were in any way definite, approximated to those obtained with the present series. The titres of such sera given in Thjøtta's communication are, on the whole, definitely lower than those found in the present enquiry. His reactions were allowed to proceed for two hours in the incubator at 37° C. Sera with low titres (1 in 40) towards the homologous organism in this type of dysentery were observed by the writer in certain cases occurring after the present account was completed. In those cases the bacilli were inagglutinable on isolation but after being ten weeks on agar they reacted to the same samples of homologous sera as were used at first, the flocculations being very fine and the end-points 1 in 40. The low end-points of the Norwegian sera were likewise associated with fine flocculi. The coarser flocculations and higher serum titres obtained from *e.g.* the "T." bacilli were associated with organisms which gave clear, well-marked agglutinations on isolation. The group apparently contains strains which vary much in agglutinogenic power as that is exhibited in a patient's serum towards a stock organism. A minimum of four hours is recommended for these tests.

The first of the two types of Mita's "paradysentery" bacilli has a strong resemblance to the members of the present series in respect both of bacteriological properties and of the clinical course of the disease. The organisms found by D'Hérelle in an outbreak of dysentery in 1916 are regarded by Thjøtta as being Sonne Group III bacilli. It appears, however, that D'Hérelle's bacilli fermented neither lactose nor saccharose, nor did they coagulate milk, so that unless there are other grounds for so regarding them than are contained in the original description of them, it is uncertain what, if any, the relationship may be. The Medical Research Council, reporting in 1920, state that Sonne's Group III had not yet been recognised in England. Since that time the few reports that have appeared indicate that the distribution of these organisms is wide. In addition to the earlier known occurrences in Denmark and Norway, Mita was very probably dealing with them in Japan previous to 1921. It is claimed that they have been found in Southern Australia (Patterson and Williams) though the account given does not amount to proof. It is considered possible that the above described case R. was infected in Portugal. Bamforth handled members of this group in London in 1923 and made useful comments on their features, while their presence in Aberdeen has been found by Smith. In Glasgow the Public Health Department of the city has no cognisance of any epidemic or outbreak within the city known to be due to these organisms, only isolated cases having been met with. Some of these cases along with an outbreak in Ayrshire are the subject of the present communication. The area of distribution is accordingly so wide that these bacilli may be expected to occur anywhere. Their recognition however is apt to be elusive when sporadic cases occur at wide intervals. Hitherto it would appear that attention has been fixed upon them only in the midst of what might be called an outbreak. Isolated and mild cases coming under observation are not so likely to be missed if it is fully realised that this type of organism may be inagglutinable either by the homologous patient's serum or by immune sera for some little time after isolation. There is a difficulty to be surmounted here if diagnosis is to be made reasonably early. For example, in an outbreak of enteritis outside this city toward the end of 1926, an outbreak spread over some weeks and affecting over 80 people, six stools and two patients' sera were submitted to the writer for examination. From five of the stools bacilli were cultivated which were eventually proved to be Sonne Group III organisms. (It may be noted in passing that two of these were from people on a farm supplying milk to the area, and one of the two, the farmer's wife, had never exhibited any unusual symptoms.) These five organisms were inagglutinable by either of the sera (which were taken at a suitable time) in dilution $\frac{1}{25}$ in 22 hours, and also by three immune sera (titres 12,000, 5000 and 12,500) in dilution $\frac{1}{500}$ in four hours, while eight weeks afterwards they fully satisfied the applied saturation tests and were agglutinated by an immune serum to full titre (12,500) and also by the original samples of the patients' sera (1 in 40). On the other hand it was observed that when received the two patients' sera gave

reactions with two out of three stock organisms (dilutions 1 in 50 to 1 in 125). Accordingly in cases of enteritis where one encounters a Flexner-like bacillus which is negative to Flexner sera and to the patient's serum (the time being ripe), since the full biological characters of the Sonne III group are so long in showing themselves, it will probably avoid loss of time if one is attentive to certain of the features that can be had early. The heavy sedimentation of the growth in the peptone-water fermentation tests will readily be noticed, and if microscopically the organism is a small bacillus with a tendency to bipolar staining with carbol fuchsin and with perhaps a few filamentous forms, the quickest path to a proved diagnosis may be through a resort to agglutination or absorption tests with patients' sera and stock Sonne III bacilli. Two or three of these strains should be used in simple agglutination tests. In all serological work with these organisms adequate human serum controls are held to be quite indispensable.

I wish to record my indebtedness to Dr Bamforth for the use of his three cultures for comparative purposes.

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ADDENDUM.

Subcultures of Mita's *B. paradysenteriae* Types A and B, recently obtained from the National Collection of Type Cultures, were found to possess the following immunological properties: Type A is agglutinated by immune sera D. and S. 269 to full titre (12,500 in each case), and Type B by these sera in dilutions 1 in 10,000 and 1 in 8000 respectively, the serum controls being negative. The results of saturation tests performed and read as above described are as follows:

I.S. "D." saturated with Type A bacillus. 150,000 million per c.c.		I.S. "D." saturated with Type B bacillus. 120,000 million per c.c.	
On D.	200/12,500	On D.	200/12,500
On S. 269	200/8000	On S. 269	200/8000

The relation of Mita's organisms to the Sonne III group is accordingly substantiated.

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