Microscopically the intestine showed nothing beyond intense congestion and catarrh. Death was therefore due to an intense intoxication, there being no time for structural changes to be produced.

Cultures from the contents of the large intestine and from the ileo-caecal lymphatic glands yielded abundant and almost pure growths of the Sonne bacillus, but cultures from the contents of the ileum, spleen, and heart blood were negative.

The bacillus had the following characters. On the MacConkey plate it formed rather large, opaque, rounded colonies, which at first remained colourless. Subcultures into the fluid sugar media gave the results tabulated below:

Lactose	...	...	...	...	Acid on 6th day.
Glucose	...	...	...	...	Acid in 24 hours.
Mannite	...	...	...	...	Acid in 24 hours.
Maltose	...	...	...	...	Acid in 72 hours.
Saccharose	...	...	...	...	Unchanged.
Gelatin	...	...	...	...	No liquefaction.
Indol	...	...	...	...	Negative.
Motility	...	...	...	...	Negative.

It was not agglutinated by Shiga, Flexner, typhoid, and paratyphoid antisera, but it was strongly agglutinated in the first subculture by a Sonne antiserum obtained by the kindness of Professor Cruickshank of Aberdeen. Later, at the request of Dr. A. D. Gardner of the Standards Laboratory, Oxford, the strain was sent to him and its serological identity independently confirmed.

There are two difficulties in connexion with this organism which probably account for its infrequent recognition in the past. When first isolated the majority of the strains ferment lactose very late—six to ten days—and are therefore regarded as non-lactose fermenters. As they are not agglutinated by the usual standard antisera, as detailed above, they have been frequently regarded as “inagglutinable Flexner” strains. On the other hand, as pointed out by Thjötta, the strain may ferment lactose in two or three days, and may therefore not be distinguished from colon bacilli growing on the same plate. Final proof can only be obtained by serological tests, and fortunately the group specificity is very high.

Inquiries entirely failed to elicit the source of the infection. No further case occurred in the child's family or apparently in the school.

REFERENCES.

- Channon, H. A. (1926): *Journ. Path. and Bact.*, xxix, 496.
 d'Herelle, H. (1916): *Ann. de l'Inst. Pasteur*, xxx, 145.
 Fraser, A. M., Kinloch, J. P., and Smith, J. (1926): *Journ. Hygiene*, xxv, 455.
 Mita, K. (1921): *Journ. Infect. Dis.*, xxix, 580.
 Nabarro, D. (1927): *Journ. Path. and Bact.*, xxx, 176.
 Ohnell, H. (1918): Quoted by Thjötta, infra.
 Patterson, S. W., and Williams, F. E. (1922): *Journ. Path. and Bact.*, xxv, 393.
 Smith, J. (1924-25): *Journ. Hygiene*, xxiii, 94.
 Sonne, C. (1915): *Centrall. f. Bakt.*, lxxv, 408.
 Thjötta, Th. (1919): *Journ. Bact.*, iv, 355.

LIMITATIONS OF THE AGGLUTINATION REACTION IN THE DIAGNOSIS OF BACILLARY DYSENTERY.

BY

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A RECENT outbreak involving 120 cases afforded an opportunity of further investigating the reliability of the usual routine agglutination tests in the diagnosis of bacillary dysentery. Standardized cultures and the technique recommended by Professor Dreyer were employed for estimating the agglutinins; in the absorption tests the procedures detailed in the Medical Research Council Special Report Series No. 51 were followed, and each absorption experiment was controlled by comparison with known dysentery strains. Blood samples were examined from 47 affected individuals, of which 19 were positive to the Flexner group and 28 were negative.

Ten cases, positive later, were negative at the first examination. The duration of disease at the first and second tests in these ten cases was as illustrated in the following table.

Table showing Duration of Disease at First and Second Tests in Ten Cases of Bacillary Dysentery.

At Time of Negative Reaction.				At Time of Positive Reaction.			
Duration of Disease.		No. of Cases.		Duration of Disease.		No. of Cases.	
3 days	...	...	1	5 days	...	...	1
8	...	...	3	16	...	...	2
11	...	...	1	30	...	...	1
12	...	...	1	32	...	...	1
15	...	...	2	33	...	...	1
16	...	...	1	34	...	...	1
17	...	...	1	38	...	...	2
				39	...	...	1

Predominant agglutinins for Flexner's bacillus Type V were present in the ten specimens, and ranged from 4 to 114 units per cubic centimetre of serum; five of them which showed 23 units or over were from patients who had been ill for thirty days or longer. One case of thirty-eight days' duration showed only 4 units, and the remainder reacted in the neighbourhood of 11 units. Agglutinins were also present for Type W, but to a less degree in 7 of these cases. Two of the sera showing 114 units to Type V contained 65 units to Type W. Of the three samples negative to Type W one showed 4 units and two 11 to Type V. Agglutinins to Type Y were present in all ten, and ranged from 4.4 to 8.8 units. Completely negative results were obtained with Sonne cultures and with Types X and Z. Absorption experiments pointed to a Type V infection, the reactions to the other types being attributed to the phenomenon of coagglutination. This diagnosis was supported by the isolation of Flexner's bacillus Type V from some of the cases in the same outbreak.

As the investigation proceeded it was noticed that all the specimens did not react in a similar manner to the first batch, and although a Type V culture was still agglutinated in the highest dilution, on translating the results into agglutinin units it was found that some of the specimens contained more agglutinins to Type W than to Type V; the suspension factor for the V culture was 11, while that for the W culture was only 1.9.

A Flexner bacillus was now isolated from the faeces of one of the cases, which proved on absorption to be Type W and not Type V. This point was verified by immunization tests. It was agglutinated by several of the positive sera, whether from the Type V or Type W cases, but without marked predilection, except that the serum of the patient from which it was recovered agglutinated it up to 1 in 5,000. This serum presented a peculiar anomaly—showing more agglutinin units to Type V than to Type W in the routine test, but absorption tests conducted on it with known V and W cultures were inconclusive. There was insufficient serum to repeat the investigation, and, owing to circumstances over which there was no control, a month elapsed before another specimen could be obtained; by that time the agglutinins had greatly diminished, and those to Type W were principal and predominant. This isolated bacillus only agglutinated the three stock standard antisera V, W, and Y up to a dilution of 1 in 125, but all the agglutinins were removed from the W antiserum by absorption with it, while the V and Y antisera were but slightly affected.

An important fallacy in the routine test was discernible in some of the early and late cases in which the agglutinins were not present in high amount. For instance, an established Type W infection reacted in late convalescence to Type V 1 in 25 and 1 in 50, and was negative to Type W in these dilutions, but reacted to it in lower dilutions. This effect was due to the great difference in the agglutinability of the respective cultures. The discrepancies which may arise when using dilutions of serum below 1 in 25 are recognized, but the example is tentatively put forward to illustrate what may happen when working with cultures possessing widely different suspension factors.

Taking the total 19 positives, 10 worked out as a Type V infection, and 2 were established as due to Type W. There were probably other cases of Type W among the 7 remaining positives and the 28 negatives, but owing to difficulties in procuring repeat specimens, and the fact that the Type W infection was not diagnosed until late in the

epidemic, further investigations in this direction were hampered. The 7 remaining serums, though positive for the Flexner group, were equivocal as to type by ordinary agglutination methods, and the amounts of the serums did not permit of absorption tests.

In four instances bacilli showing the morphological and cultural characteristics of the dysentery group, but not agglutinating with any of the stock antiserums except the patients', were isolated. One of these organisms was also well agglutinated by another patient's serum. None of the serums in this epidemic agglutinated Shiga cultures, and none of the principal bacilli isolated were agglutinated with Shiga serum. In a few cases typhoid infection was co-incident with the dysentery, but the agglutinins of the one infection were uninfluenced by absorption with the other micro-organisms.

COMMENT.

It will be seen that one negative agglutination reaction does not exclude acute bacillary dysentery, and this remark applies much more to these infections than to those of the typhoid-paratyphoid group. In typhoid infections the appearance of agglutinins with a few exceptions is constant about the seventh or eighth day, but in acute dysentery they may appear earlier in a few cases, though in many instances they are much later. Sir Leonard Rogers¹ states that "agglutination may occasionally be obtained as early as the third or fourth day, but it is not until about the tenth or fourteenth days that a larger proportion of cases, such as 50 per cent., yield positive results, while by the third or fourth week as many as 82 per cent. of successes have been reported." Keersmakers² obtained positive agglutination tests in 75 out of 170 affected cases. W. Moodie³ infers that agglutination may be delayed for several weeks, and is of little diagnostic use. The literature abounds with divergent opinions as to the value of agglutination tests, and one is obliged to judge by one's own experience. In the epidemic under review the tentative diagnosis of a Flexner infection was made on repeated agglutination tests from a fair number of cases, but while a main group diagnosis, such as that of Shiga or Flexner, can be arrived at by this means, it is obvious that agglutination alone is not a valuable means of distinguishing between the various types of Flexner's bacillus, and even when the varying agglutinability of the cultures are known and taken into account such procedures must be supported by isolation of the micro-organism and absorption tests.

The duration of agglutinins resulting from these infections varies enormously, and in some of the cases under consideration they had practically disappeared within six weeks of recovery. They may, however, last for years and cause confusion; but such persistent agglutination can be distinguished from that resulting from active disease by repeated estimation of the titre, when it will be found to vary very slightly, if at all, when resulting from an old infection which is not active.

The presence of different types of Flexner's bacillus in this outbreak is not unique. Difficulty has at times been experienced by some observers in fitting the causative organism into the present recognized types even by absorption tests. This difficulty was experienced in one of the cases; in another, from which a definite Type W bacillus was isolated, agglutinins to Type V appeared to be the most prominent in the patient's serum, and the absorption tests were inconclusive. This may have been a double infection, but the last word has not been said on typing, and it is quite likely that organisms can exist showing a mixture of the antigenic properties of the several strains at present recognized.

The difficulty of giving an outbreak a type designation may be realized by the experience of Amako,⁴ who recovered five different types from a local epidemic, but the organisms isolated in his outbreak were so far removed from one another as to include members of the main Shiga and Flexner groups, and their main means of separation was by cultural reactions. While this observer's findings support the possibility of multiplicity of types in the same outbreak the development and improvement of serological technique since the time of this investigation must not be ignored. Modern serological procedures show that cultural characteristics must take second place.

In this outbreak the occurrence of several types could be reasonably expected, since dysentery has been endemic in the institution for years, and there are probably several sources of infection.

With reference to the strength of the agglutination reaction which is deemed diagnostic the Medical Research Council⁵ states that the presence of 10 or more "standard agglutinin units" per cubic centimetre of serum in a male, and of 20 or more units in a female, is for all practical purposes diagnostic of acute bacillary dysentery infection caused by the bacillus agglutinated—that is, if old infection or previous inoculation can be excluded. The presence of 6 to 8 units in a male and 12 to 16 units in a female justifies a strong suspicion of dysenteric infection.

CONCLUSIONS.

1. Owing to lack of any correlation between the day of disease and appearance of agglutinins a negative agglutination reaction, even if shown by several affected cases, does not exclude bacillary dysentery.

2. If the serums from a number of cases are repeatedly examined for the presence of agglutinins it is often possible in the event of positives to place the cases in the main Shiga or Flexner group, and such findings may be further refined by absorption tests on these serums with known dysentery bacilli, but for exact work isolation of the causative micro-organism from the patient is essential.

3. The possibility of the multiplicity of types of Flexner's bacillus in an outbreak is again established, but it has not been settled whether the lack of correspondence between the agglutinins as found in some of the patients' serums and the type of bacillus recovered from the faeces was the result of a double infection or the outcome of the close relationship of the W and V strains. The possibility of intermediate strains must also be borne in mind, but further work in this direction is necessary.

My thanks are due to Mr. F. W. Duke for the assiduous manner in which he helped in this investigation.

REFERENCES.

- ¹ Sir Leonard Rogers: *Bowel Diseases in the Tropics*, p. 393.
- ² Keersmakers: *Arch. Med. Belges*, January 1st, 1921.
- ³ W. Moodie: *Lancet*, 1921, ii, p. 225.
- ⁴ Amako: *Zeit. f. Hyg.*, 60, 1906, 93.
- ⁵ Medical Research Council, Special Report Services No. 51, p. 134.

PATHOGEN-SELECTIVE CULTURES AS AN AID TO THE DIAGNOSIS OF INFECTIVE FOCI.*

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(Abridged.)

THE bacteriological examinations carried out in this work do not refer to findings in acute infective or septic conditions, in which the causal organism is indisputably present in the pathological material and frequently isolated in pure culture, but to the more difficult matter of attempting to determine in a subacute or chronic case if any bacteria pathogenic to the patient are present in the specimens examined. In the bacteriological overhaul of any one case many specimens of possibly infective material may be examined in the search for the infecting organism. Improved technique and differentiating media have increased the possibility of isolating with greater precision organisms which may have pathogenic importance. This elaboration means that an increasing number of bacteria have to be considered in any case under examination, and the result of this has been to add to the difficulty of deciding as to which, if any, of such an assortment of isolated organisms are pathogenic to the patient and which are not.

Many serological tests are available to decide this question, such as agglutination, precipitation, haemolysin, and absorption tests, etc., but, except for special conditions, are either too elaborate or indefinite to be applied as a routine test in clinical bacteriological examinations. They all, however, indicate that serum or blood, preferably that

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