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.

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

BY

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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

* Read before the Liverpool Medical Institution on March 29th, 1928.

of the patient concerned, does contain some factor which is utilizable to decide this question of pathogenicity, if it can be applied practically.

Various workers¹ have shown that natural immunity in animals is associated with the presence in their blood of bactericidal action to the organism concerned.

Black, Fowler, and Pierce² conclude that an estimation of the bactericidal power of the blood "is the most dependable criterion of immunity in an animal."

In 1927 Solis-Cohen³ of Philadelphia published a paper on "Accentuating pathogenic organisms in culture," which utilized this bactericidal power of blood as a means of differentiating the pathogenic from the non-pathogenic bacteria present in any specimen as far as the patient under examination was concerned, and called his method of doing this "Pathogen-Selective Culture."

During the past year, with the hope of determining the relative pathogenic importance of organisms isolated from various foci in the same patient, this pathogen-selective culturing method has been resorted to in over 600 specimens. As far as it has gone the findings of Solis-Cohen have been fully confirmed, and one or two other points which were not mentioned in his paper have been emphasized.

By this method an equal quantity of any specimen to be examined is inoculated, as far as possible simultaneously, on to suitable media on the one hand, from which, after incubation, will be obtained the ordinary direct or "A" cultures, and on the other hand into 5 c.cm. of the patient's own blood (freshly drawn), which, after incubation and subsequent subculture on to similar media, will provide the pathogen-selective or "B" cultures, according to whether the patient's blood happens to be able to inhibit, or otherwise, any or all of the bacteria shown by the "A" cultures to have been present in the specimen.

The principal point of difference between this method of pathogen-selective culturing and others which attempt to obtain evidence of pathogenicity of the organisms present is that:

1. The original material under suspicion is used as the inoculum of the patient's blood, and not the isolated bacteria from such infected material.
2. That the whole blood, and not the serum, of the patient is used.
3. It is important that equality of distribution of inoculum between the "A" and "B" cultures should be ensured, so that the results may be reasonably comparable, for it is obvious that the implantation of infected material into the patient's blood specimens should not be unduly heavy.

One has, therefore, as far as possible, standardized the emulsion of faecal and urinary deposits to be not greater than a strength approximately similar to a bacterial emulsion of 2,000 million organisms per cubic centimetre, from which three loops are inoculated into 5 c.cm. of blood freshly drawn from the patient.

In the case of sputum, catarrhal discharge, and tonsillar and dental infective material, the specimen to be inoculated has been thoroughly mixed in a few drops of broth, and from this equal quantities have been inoculated on to the direct "A" plates and into the freshly drawn blood. An analysis of 400 specimens shows that the results of the pathogen-selective cultures fall into one of the following five groups.

1. Similarity of results in the "A" and "B" cultures. This might be expected to be the most common result of such a procedure, and the opinion has been expressed by several critics that such findings would be usual. On the contrary, it has occurred in only 6 per cent. of examinations in this series. The explanation of this is that the blood of the patients in this series of tests was deficient in normal bactericidal power, and failed, therefore, to inhibit any of the organisms present, thereby suggesting marked absence of general immunity.

2. A selection of more than one organism out of the many present in the specimen, as shown by the results of the direct cultures. This result was obtained in 30 per cent. of the tests.

3. The selection by the "B" cultures of a single organism out of the several shown to be present in the

direct "A" cultures. This result was found in 35 per cent. of examinations and constitutes the largest group of findings, which means that the most common result of culturing pathological specimens by this method is that the patient's blood will suggest that only one of the many organisms present is pathogenic to him, and that the others are non-pathogenic, in that the specific bactericidal action of the blood is adequate to inhibit them.

4. That in 18 per cent. of the specimens examined by this blood selective method organisms were isolated in the "B" cultures which did not appear in the direct "A" cultures. Solis-Cohen says of these findings—that "ordinary cultures may fail to contain the etiological factor in an infection," and suggests that these are either outgrown by the more luxuriant non-pathogenic organisms present in the specimen, or that the inhibition by the patient's blood of such non-pathogens has actually encouraged the growth of the pathogenic organisms, which are thereby readily recognized.

5. In the fifth group of findings, in spite of there being many possible pathogenic organisms present in the specimens as shown by the direct "A" cultures, all of these were completely inhibited by the patient's blood cultures, suggesting that as far as bacterial contents of such specimens were concerned, the organisms were not pathogenic to the patient. This result obtained in 22 per cent. of the examinations, and is of equal clinical importance to the previous positive findings, for when it comes to evaluating the pathogenic importance of the bacteriological findings of several specimens from one patient, it would appear reasonable to exonerate areas from which no pathogenic organisms can be isolated, and blame those in which such can be demonstrated as harbouring foci of infection capable of producing systemic infection in the patient concerned.

In classifying the findings of 400 examinations of various specimens into the five groups already detailed it is found that in over 50 per cent. of the examinations pure single cultures of organisms pathogenic to the patient were differentiated by this method from the many present in the specimens, and in 88 intestinal examinations the striking result appears that all the organisms present in the faecal emulsions of 32 per cent. of these were completely inhibited, suggesting that the intestinal flora, however important its bacterial content might appear to be, was not likely to be a source of infection to the patient concerned.

The same applies to the urine cultures; out of 20 cases showing bacilluria in carefully collected catheter specimens, in 14, or 70 per cent., the organisms present were shown to be non-pathogenic to the patient. This is quite in keeping with clinical experience, and while it is probable that in such cases the bacteria may be producing local irritation of the urinary passages, which requires local treatment, these organisms are not causal of systemic infection if such exists, and vaccine treatment prepared from such cultures (which are thus proved non-pathogenic to the patient) is not likely to be of any value, which probably accounts for the occasional disappointment attending vaccine treatment of such cases.

Several control experiments have been made to ascertain whether this pathogen-selection would allow of accurate repetition, and equal quantities of the same specimen were planted into more than one sample of the respective patient's blood. The result was the selective isolation of the same bacteria only in each case. These findings appear to confirm the contention that the pathogen-selection on the part of the patient's blood is associated with some want of specific bactericidal power.

The clinical importance of this method of bacteriological examination is the possibility of utilizing it to differentiate the pathogenic importance of organisms isolated from various areas which are suspected of harbouring a focus causing generalized infection. Space will only allow of three examples being given.

The examination of post-nasal, tonsillar, sputum, and faecal specimens in a case of *chronic catarrhal asthma* demonstrates that the patient suffers from infection of the respiratory tract, probably associated with some dental condition requiring treatment, but with no evidence of intestinal infection.

In a further case *asthma* alternated with urticaria and vaginitis, and the examination of sputum, urethral, vaginal, urine, and faecal specimens suggested an intestinal origin of the whole condition as probable; subsequent treatment seemed to confirm this conclusion.

A case of severe subacute *infective arthritis* gave a history of commencing as a result of puerperal infection accidentally contracted at a first confinement. Post-nasal, tonsil, cervical, urine, and faecal specimens were examined, and it was found that in spite of there being many organisms present in the ordinary cultures which might have been accredited as likely infective agents in such a case, the only strain that the patient's blood selected from them all was the comparatively simple *Streptococcus faecalis* from the faeces. It is, however, most likely that this would be the organism capable of infecting her at the confinement, and against which she had not yet regained an adequate immunity. It is further suggestive that this is so, in that she has proved disturbingly sensitive to the smallest doses of a simple autogenous vaccine prepared from this organism, but is slowly improving under this treatment.

In conclusion, the findings in over 600 examinations by the pathogen-selective culturing method confirm the opinion expressed by Solis-Cohen, that:

1. Cultures as ordinarily made fail to differentiate organisms that are pathogenic for the host from those that are non-pathogenic for him.
2. Ordinary cultures may fail to isolate the etiologic factor in an infection in certain cases.
3. In the pathogen-selective culture the patient's fresh blood is used to inhibit organisms that are non-pathogenic for him, thus permitting the free growth of organisms that are pathogenic for him.
4. To these I would add, that this form of culture will also differentiate between foci in the same patient, and indicate those which contain organisms that are pathogenic for the patient and which are only of local infective importance.
5. That such positive pathogen-selective cultures when obtained from dental, tonsillar, or sinus material indicate the need for radical removal of such a focus of infection, if this is clinically possible.
6. That when similar positive findings in intestinal specimens are associated with any clinical evidence of appendicular trouble the same radical attitude should be adopted.
7. That negative pathogen-selective cultures suggest that the focus of infection from which the specimen was collected, while probably requiring local treatment, is not causal of systemic infection if such exists.
8. That this pathogen-selective culturing method is *only* applicable to the bacteriological examination of cases in which some focal infection is causal of a systemic infection. Where the condition is a purely local infection, as occurs in many cases of chronic catarrh, cystitis, or colitis, the negative selective cultures will demonstrate this fact, and for such, if vaccine treatment is to be employed at all, the preparation should be made from the ordinary direct "A" cultures. Nor does this method apply to the preparation of ordinary prophylactic vaccines. It is essentially a means by which the selected pathogen represents the required antigen for the patient concerned.

The pathogen-selective culture method is therefore suggested as a valuable contribution to clinical bacteriological investigation, both as regards the presence of focal infection and as a guide to the treatment required for such a condition.

I wish to thank my colleagues of the Southport Infirmary and the Liverpool Eye and Ear Infirmary, and the many clinicians who have allowed me to examine their cases by this method; also, I wish to express my appreciation of the detailed and accurate work of my two assistants, Mr. P. H. Osmond and Mr. L. H. Bennett, which has added value to the conclusions drawn from these "pathogen-selective cultures."

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A CASE OF PERTHES'S DISEASE OF THE HIP.

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THE patient in the case here recorded was a boy, aged 8½ years, who attended for examination on June 20th of this year.

The family history is good. He has a brother aged 5, and a sister aged 4, both of whom are fit. His father and mother also are well. There is no history of a similar condition in any other member of the family.

History of Present Condition.—His mother states that eighteen months ago, while running and playing about, he strained his left thigh and complained of pain in his left knee, and limped a little. He was kept resting in the house for a week, and had massage. At the end of a fortnight he appeared quite well again. Three weeks ago he stumbled over a molehill, and has complained of pain in the left thigh and knee ever since, and the limp returned. Some nights the pain has been severe enough to make him cry. Eight weeks ago he walked a distance of two miles, and the pain and the limp have since been worse.

The general condition of the patient on June 20th was good. He is a little undersized for his age. He walks with a definite limp on the left side, but he is able to walk and even run about with comparative ease. His mother states that he complains of pain around the knee, and over the middle of the thigh in front. There is a quarter of an inch wasting of the left calf, and three-quarters of an inch wasting of the left thigh. No definite shortening of the left lower limb could be made out. While resting on the couch it was noticed that the left thigh was held slightly flexed and rotated outwards. Marked muscular spasm could be detected on manipulating the left hip, but the patient did not complain of any pain, and appeared quite cheerful. Flexion and extension of the hip were good, but definite limitation, though slight in extent, could be made out on careful examination. Abduction and external and internal rotation of the hip were very limited.

X-Ray Examination.—The x-ray appearance of the left hip-joint, as shown in the radiograph, is typical of Perthes's disease. The epiphysis of the head of the femur is much flattened and elongated; it appears to creep over the outer part of the neck of the femur. There is x-ray evidence of fragmentation of this epiphysis, the normal single ossific nucleus being broken up into numerous nuclei, which vary in density and shape. Towards the centre of the epiphysis can be made out a large and markedly dense bony nucleus, and on either side are tiny nuclei scattered irregularly. When compared with the normal