mass into five or six somewhat irregular and often rod-shaped bodies appeared to follow, and finally a further subdivision into numerous small granules, each surrounded by a clear capsule. With the rupture of the original limiting capsule these small bodies which seem to form the starting-point of the cycle are again set free.

We have not as yet been able to follow these changes in the living organisms, and are unwilling to interpret them as indicating sporulation until further studies have been made. The appearances, however, are suggestive, especially in view of the forms seen in the intestinal tract of the louse, reference to which will be made later.

Transplants have been made from the condensation water upon the following media: hydrocele-agar, dextrose, lactose, saccharose, maltose and mannite broth, lactose-bile broth and Loeffler's blood serum. In none of these media has a growth of the organism been obtained. A transplant, however, made upon human citrated blood broth after one week developed an increasing turbidity, and examination of fresh and stained preparations showed typical organisms in great abundance.

The original cultures from periosteal muscle tissues have also been transplanted to tubes containing sterile human tissue obtained at autopsy, but with varying success. The experiments are being continued, but so far we have not obtained regularly a growth in the second generation. We have attempted, without success, to substitute sterile rat connective tissue and muscle.

IV. Culture from the Circulating Blood.

In two cases we have succeeded in cultivating the organism directly from the circulating blood. A small amount—less than 1 c.cm.—of citrated blood was poured into agar tubes containing sterile human periosteal tissue obtained at autopsy. Growth occurred slowly, but showed the same characteristic clouding of the media that was found in the periosteal and fascial cultures.

Two other blood cultures obtained at the same time from cases of supposed trench fever upon the same medium remained sterile.

V. Animal Inoculations.

Rabbits, guinea-pigs, and white rats have been inoculated with the blood of patients and with suspensions of the tissue. In none of these animals were there obvious signs of illness; temperature charts of guinea-pigs and rabbits were continued for four and a half weeks without showing a febrile reaction, and examination of the blood on numerous occasions has failed, thus far, to show the presence of the parasite. Further experiments on animals are being undertaken.

VI. The Possible Occurrence of the Organism in the Louse.

Upon the arrival of convoys at the hospital lice were collected from the clothing of the patients and placed in small tubes labelled with the diagnosis upon the admitting card. Smears were made by teasing out the tissues and the intestinal contents on a slide, and stained with Wright's stain. A certain number of lice have also been sectioned and stained. While it is too early to report definitely upon this phase of our work, it may be said that there are present in a number of the smears small ring-shaped bodies identical in appearance with those observed in our cultures and blood smears. We have also observed larger forms, surrounded by a capsule, and transitions between the small discs and these larger bodies. In sections we have found within the alimentary canal large encapsulated globular bodies resembling the sporulating (?) encysted forms observed in the cultures. We are not yet, however, ready to present in detail our findings, but regard this merely as a suggestive observation.

General Discussion.

It will be seen from the above that we have up to the present succeeded in finding a characteristic organism in blood smears, in cultures from the blood, in sections of periosteum and fascia, and in cultures from these tissues.

The morphology of the organism obtained varies somewhat under different conditions. It is most definite and

constant in the blood smears, whereas in the sections of the tissue and in the cultures smaller forms are met with the relation of which to the organisms present in the blood requires further study. So also the significance of the larger encapsulated forms seen in certain of our cultures. Since, however, we have repeatedly found, both in the cultures and in the sections of muscle, forms practically identical with those observed in the blood, we are of opinion that we are dealing with varying forms of the same organism.

As to the nature of the organism, we cannot yet commit ourselves definitely. That it is not bacterial seems probable, (1) because of its characteristic morphology and the changes which it undergoes in culture; (2) because of its peculiar staining reaction, which distinguishes it from ordinary bacteria; (3) because of its failure to grow on the usual bacterial media; (4) because of the absence of all inflammatory reaction about the organisms in the tissue.

While it is impossible to classify the organism finally until its life-cycle shall have been more completely studied, we would provisionally assign it to the genus *Piroplasma*.

We are much indebted to Miss May G. Robson and Mdle. Scheu for their valuable assistance in the collection of histories and preparation of specimens.

A METHOD TO FACILITATE THE ISOLATION OF THE CHOLERA VIBRIO AND OTHER ORGANISMS.

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THE method here described is based on the action of specific and non-specific agglutinins on the bacteria with which the cholera vibrio is most frequently mixed in the material (stools) to be examined.

To explain the general principle on which the method is based the following experiments may be made:

Action of Specific Agglutinins.

I.

Take two tubes of peptone water: inoculate Tube No. 1 with *B. typhosus*, *B. paratyphosus* A, *B. paratyphosus* B. Inoculate No. 2 tube with the same germs, but immediately before or after inoculation add:

3 to 5 drops agglutinating typhoid serum,
3 to 5 drops agglutinating paratyphoid A serum,
3 to 5 drops agglutinating paratyphoid B serum, and shake well.

Instead of the individual mono-serums, 3 to 5 drops of a high titre trivalent serum may be used derived from a rabbit inoculated several times with typhoid, paratyphoid A, paratyphoid B. Incubate both tubes at 37° C. for twelve hours and examine. No. 1 tube will show diffuse cloudiness; in No. 2 tube, on the other hand, there will be no diffuse turbidity, the growth taking place at the bottom; the medium will be quite clear, or will contain only a few flocculi, which will very quickly go to the bottom on centrifuging.

II.

To a tube of peptone water add

3 drops typhoid serum,
3 drops paratyphoid A serum,
3 drops paratyphoid B serum.

Or instead of the mono-serums 3 to 5 drops of high titre trivalent serum may be added, derived from a rabbit repeatedly inoculated with typhoid, paratyphoid A, and paratyphoid B.

Shake well; inoculate the tube with *B. typhosus*, *B. paratyphosus* A, *B. paratyphosus* B, and *Vibrio cholerae*.

Incubate at 37° C., and examine after twelve hours. The medium will show diffuse cloudiness, often more marked towards the surface (with at times a pellicle), due to the growth of *V. cholerae*, and also a certain amount of bottom growth, and occasionally some flocculi due to agglutinated typhoid, paratyphoid A and B bacilli. These three bacteria have been influenced by the specific serums, which have had no action on the cholera vibrio. In carrying out this experiment care of course should be taken to use typhoid and paratyphoid serums containing no coagglutinin for the cholera vibrio, or only in very small amount. Certain powerful typhoid and especially paratyphoid A serums may contain quite an appreciable amount of cholera coagglutinin, which is, however, in very small amount in comparison with the other coagglutinins, and may be removed by absorption.

III.

To a tube of peptone water add 3 to 10 drops of polyvalent *B. coli*, *B. pseudo-coli*, *B. coli tropicalis*, *B. neapolitanus* serum, or 3 to 5 drops of each of the respective mono-serums. Inoculate with *B. coli*, *B. pseudo-coli*, *B. coli tropicalis*, *B. neapolitanus*, and shake well. Incubate at 37° C., and examine after twelve hours; the medium will be clear. Repeat the experiment, inoculating the medium with the same bacteria plus *V. cholerae*, and examine after twelve hours' incubation at 37° C.; the medium will show diffuse cloudiness, often more marked towards the surface (and at times a pellicle), due to the growth of the cholera vibrio, which has not been influenced by the serums added. Care of course should be taken to use serums containing no cholera coagglutinin, or only in minute amount.

Action of Non-specific Agglutinins.

I.

Add to a peptone water tube 3 to 5 drops of a very powerful agglutinating paratyphoid B serum, rich in specific and non-specific agglutinins. Inoculate the tube with *B. typhosus*, *B. paratyphosus A*, *B. paratyphosus B*, *B. aertrycke*, *B. enteritidis* (Gaertner), and examine after twelve hours; there will be no homogeneous growth, and no diffuse turbidity. The growth is at the bottom, and at times under the form of flocculi in the medium, which will quickly go to the bottom on centrifuging.

If, instead of a very powerful agglutinating paratyphoid B serum, one of low titre is used, containing only a very small amount of coagglutinins, the medium will show a diffuse turbidity, due to the growth of the *B. typhosus*, *B. paratyphosus A*, *B. enteritidis*, while bottom growth and flocculi will be present, composed of agglutinated paratyphoid B bacilli, and also *aertrycke* bacillus, because generally any paratyphoid B serum contains nearly as much agglutinin for *B. aertrycke* as for *B. paratyphosus*. These two bacteria can be differentiated only by my absorption method, as demonstrated by Boycott, Bainbridge, O'Brien, and others.

II.

Add to a tube of peptone water 3 to 5 drops of a very powerful agglutinating paratyphoid B serum containing coagglutinins for the typhoid-paratyphoid group of micro-organisms.

Inoculate with *B. typhosus*, *B. paratyphosus A*, *B. paratyphosus B*, *B. aertrycke*, *V. cholerae*. Inoculate at 37° C., and examine after twelve hours. There will be diffuse turbidity, due to the growth of the *V. cholerae*, and flocculi and bottom growth due to the typhoid, paratyphoid, and *aertrycke* germs; the flocculi may easily be got rid of by a short centrifugation which will have no effect whatever on the non-agglutinated cholera vibrios. After a few hours longer there may be a distinct pellicle composed of vibrios. Of course, in carrying out this experiment, care should be taken to use a paratyphoid B serum which contains no coagglutinin for *V. cholerae*, or only in very small amount.

From the above experiments, and similar ones, a general method can be devised to facilitate the isolation of the cholera vibrio, the typhoid bacillus, and other germs. The method consists simply in adding to the medium in which the material is going to be sown, serums influencing—that is, agglutinating, and delaying the growth of—the germs we do not intend to isolate, while they do not hinder appreciably the growth of the particular germ, or group of germs, we want to grow.

Cholera.

For the isolation of the cholera and paracholera vibrios from stools the following technique may be followed:

Inoculate peptone water tubes with the faecal matter in the usual way, but before or immediately after making the inoculation add to each tube:

- 3 to 5 drops polyvalent lactose fermenters faecal bacteria serum (*B. coli*, *B. pseudo-coli*, *B. coli tropicalis*, etc.), or the respective mono-serums may be used.
- 3 to 5 drops polyvalent non-lactose fermenter faecal bacteria serum (*B. proteus* group, etc.).
- 3 to 5 drops paratyphoid B serum.

The addition of the last named serum is made with the object of agglutinating and delaying the growth of the bacilli of the paratyphoid B and *aertrycke* type, which, in the tropics at least, are not very rarely found in the intestinal fluid. Care should be taken, of course, to use serums containing no coagglutinin for the cholera vibrio, or only in very small amount. Or serums can be used from which the cholera coagglutinin—which is always in very much smaller amount than the other coagglutinins—has been removed by absorption.

The tubes are placed in the incubator, and the further steps in the investigation are carried out in exactly the same manner as with the ordinary methods.

1. From the very surface of the medium a loopful of liquid is taken, examined microscopically, and the agglu-

tion reaction with cholera serum carried out straightway.

2. A small loopful from the very surface is smeared in the usual way on gelatine plates, bile salt neutral red agar plates, etc., and any suspicious colonies which will grow are further investigated.

The method may be applied to the isolation of the typhoid group of bacilli.

Typhoid.

To facilitate the isolation of bacilli of the typhoid-paratyphoid group the following technique may be used:

1. Inoculate with the faecal matter to be investigated several tubes of taurocholate of soda peptone water, or Browning, Gilmore, and Mackie's telluric acid peptone water might be used.

2. Immediately after, or better immediately before, the inoculation, add 5 drops polyvalent lactose fermenter intestinal bacteria serum, 5 drops polyvalent non-lactose fermenter faecal bacteria serum (*B. proteus* group, etc.), taking care to use serums containing only a very small amount of typhoid coagglutinin; or serums can be used from which the typhoid coagglutinin has been removed by absorption.

3. Incubate for twelve or twenty-four hours, then make plates on MacConkey, Conradi-Drigalsky, or similar media, from the most superficial portion of the liquid medium, and investigate further any suspicious colonies which may develop, testing them with typhoid, paratyphoid A, and paratyphoid B serums, etc. When there are many flocculi of agglutinated bacilli also in the upper portion of the tube, these may be got rid of by a short centrifugation. A short centrifugation with an ordinary electric centrifuge will cause the agglutinated bacilli to fall to the bottom, while it has practically no effect on the non-agglutinated germs in young cultures.

REMARKS.

In theory the method I have described is simple, and it should be possible by means of it to isolate in absolutely pure cultures the germs we want to grow to the exclusion of all those we do not want to isolate. In practice things are not so easy, and several difficulties are encountered: (1) The intestinal bacterial flora found in association with the cholera vibrio varies to a certain extent from country to country, from epidemic to epidemic, and even from individual to individual. Therefore, the serums used may have to be varied, and serums have to be prepared influencing a large number of intestinal bacteria. (2) The preparation of powerful agglutinating serums against certain intestinal germs is far from easy. (3) Occasionally certain intestinal micro-organisms are at first inagglutinable by their homologous serums, becoming agglutinable only after several transplantations on artificial media.

In practice, therefore, we seldom succeed in agglutinating and retarding the growth of all the species of germs we do not want to grow, but merely succeed in agglutinating and retarding the growth of a certain number of them. Even this result is, however, of practical importance, and greatly facilitates the isolation of the germs we want to grow.

ADAPTATION OF THOMAS AND JONES SPLINTS TO OBTAIN FIXATION OF THE ARM IN AN ABDUCTED POSITION WHILE THE PATIENT IS AMBULATORY.

BY

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THE war has demonstrated quite clearly that the ring and wire splints of Hugh Owen Thomas and those which Sir Robert Jones has devised are wellnigh universally adaptable. As surgeons become familiar with their use, and appreciate the soundness of their simple mechanical principles, they are becoming more and more the method of choice, and the results obtained are becoming more perfect.

The principle of extension by traction and counter pressure makes of these splints a unit apparatus which provides easy and comfortable transportation, and allows the detailed treatment in the base and home hospital to be continued with complete satisfaction without change of