NOTES ON BLOOD CULTURE TECHNIQUE.

BY
R. L. THORNLEY, M.D.LOND., D.P.H.,
TEMPORARY LIEUTENANT R.A.M.C.

A FAIRLY extensive experience of blood cultivation during the past few months, while in charge of a mobile laboratory in France, has shown that, although the method usually employed of drawing blood from a vein direct into ox bile, and thence plating on to special media, is excellent for the typhoid-coli group, it does not detect the numerous other organisms which may be found in the blood in cases of pyrexia of unknown origin. The special media, or the ox bile, appear to have an inhibitory effect on these. For this reason I have for some time used, in addition to ox bile, two other fluid media, one similar in chemical composition to ox bile, and the other a broth prepared from trypsinized bullock's heart. Instead of plating out on to Drigalski-Conradi and MacConkey media only I use also Conradi's brilliant green, Fawcett's brilliant green, and a modification of the pea extract tryptic agar used at the Royal Army Medical College, Millbank.

In every case of vein puncture it is absolutely necessary first thoroughly to cleanse the skin in the vicinity with alcohol before painting with tincture of iodine. A piece of rubber tubing, which is so fastened round the arm that it can be instantly released, is used to constrict the blood flow, and a wide-bore 20 c.cm. needle passed into any suitable vein at the bend of the elbow or its vicinity. Not less than 5 c.cm. of blood should be drawn direct into each of the three liquid media, and an additional 2 c.cm. into a test tube for a Widal reaction for typhoid, paratyphoid A and B by Dreyer's method.

The tubes are incubated, and next morning plated out in the following manner: The five plates are each marked with a grease pencil into three sectors, and the names of the three fluid media marked on the sectors, one on each. The fluid media are then in turn taken up and a loopful of fluid transferred to the corresponding marked sector of each plate. The plates are then returned to the incubator for examination the following day. If no growth has occurred, the process must be repeated, if necessary, for three successive days.

The extra time taken up in transferring in all fifteen loopfuls of fluid from the three media as compared with one or two only when using ox bile, is well repaid by the increased number of positive results obtained. It happens not infrequently in the case of the delicate organisms like pneumococci and the feebly virulent streptococci that growths may only appear on one or two of the whole fifteen sectors, although in these cases growths may usually be seen in the broth medium.

Already a large number of cases of pyrexia of undefined origin, a term which includes cases of so-called "trench fever," have been shown by this extended method of blood culture to be due to bacterial infections of the blood, but it is essential to examine the blood when the fever is at its height.

For the composition of the two liquid media which I use with the ox bile when collecting the blood, and for perfectly prepared media generally, I am indebted to Sergeant H. A. H. Howard, B.Sc.Lond., R.A.M.C., my laboratory attendant.

My analysis of the cultivation of the blood of the last 100 cases of fever of unknown origin examined by me gives the following results:

Negative	...	...	42	<i>Micrococcus tetragenus</i>	...	23
Typhoid	...	...	3	<i>Pneumococcus</i>	...	9
Paratyphoid B	...	...	7	<i>Staphylococcus</i>	...	2
Flexner	...	...	2	<i>Streptococcus</i>	...	5
Shiga	...	...	1	<i>Gram-negative coccus</i>	...	2
<i>Coli communis</i>	...	...	4			100

Dr. CHARLES JOHN GIBB of Newcastle-on-Tyne, the oldest medical practitioner in the district, for many years lecturer at the Newcastle College of Medicine, who died on May 13th, aged 89, left estate of the gross value of £143,022, of which £120,384 is net personalty.

THROUGH gifts of Dr. Howard Kelly and Mr. Blanchard Randall, the Johns Hopkins Hospital, Baltimore, has now one of the finest medical portrait galleries in the world. The collection, which is valued at £20,000, consists of forty-eight portraits, some of which go back to the early part of the sixteenth century.

DIGESTED AND DILUTED SERUM AS
A SUBSTITUTE FOR BROTH FOR
BACTERIOLOGICAL PURPOSES.

By A. DISTASO, D.Sc.,
HONORARY BACTERIOLOGIST, WELSH NATIONAL MEMORIAL
ASSOCIATION.
(From University College, Cardiff.)

THE nourishment of microbes is a passive one, at any rate at the beginning of their life. It depends upon such substances as can pass through the walls of the microbes and be absorbed by the protoplasm. The best example of this principle is the growth of microbes in media made with sugar. A small quantity of sugar makes the growth quicker and richer; if the percentage of sugar is increased the growth is arrested. I have made experiments to ascertain whether serum, which in normal conditions is a bad pabulum for the majority of microbes, becomes a suitable medium for them when diluted or digested—when, in other words, the molecule of the albumin is broken down and becomes smaller.

The medium is prepared as follows:

(a) One volume of sheep or ox serum is mixed with one volume of tap water, and boiled till it becomes milky.

(b) A pancreas of a pig is minced and extracted with 400 c.cm. of distilled water in the presence of chloroform for twenty-four hours. (The first experiments were performed with 1 per cent. trypsin, but for the sake of economy I used afterwards pancreatic extract, which is equally suitable for the purpose); and

(c) A piece of the upper part of the small intestine is extracted in the same way in order to activate the pancreatic extract.

(d) To one litre of (a) is added 100 c.cm. of (b) and 10 c.cm. of (c), and digested at 60° C. for the night. Next morning the flask contains an amber-coloured liquid with fine flocculi floating in it. Filtered through Chardin paper, the amber-coloured liquid passes through, and the flocculi remain in the filter. The liquid is collected and sterilized at 120° C. for fifteen minutes, then tubed and re-sterilized.

In this medium the growth of the *B. coli* group, the streptococci, the staphylococci, *B. subtilis*, *B. proteus*, and *fluorescens*, is so luxuriant that in comparison normal broth may be said to yield a scanty growth. *Meningococcus* shows little or no growth.

Indol Formation.—Theoretically it is to be expected that with this medium indol should be detected, because the tryptic digestion gives rise to tryptophane, from which indol is formed by the microbes. As a control tryptophane medium was employed. The Böhme-Ehrlich reaction has always given similar results with the two media in twenty-four hours.

The Sugar Test.—When to this medium is added 1 per cent. of various sugars generally used for the identification of microbes, the same reactions are observed as with the control broth. But a better and more useful medium for sugar test is described later on.

Solid Media.—The utility of this medium is shown specially in the making of agar.

Three grams of thread agar are added to 100 c.cm. of tap water and sterilized at 120° C. for fifteen minutes, then 100 c.cm. of the digested serum above described is added, mixed thoroughly, tubed, and sterilized at 120° C. for fifteen minutes.

A beautiful transparent medium is obtained in which microbes of the *B. coli* group, *B. proteus*, *fluorescens*, *pyocyaneus*, staphylococcus, streptococcus, Nabarro's butter bacillus 5, Grassberger bacillus 1, *B. tuberculosis*, grow very heavily. The meningococcus again does not grow in this medium. Anaërobic bacilli, like *B. perfringens* and *B. sporogenes*, grow very well. Thus the *B. pyocyaneus* and *fluorescens*, at 37° C., give pigmentation in twenty four hours. The *B. proteus* shows in this agar also a pigmentation of oak-like colour.

This medium, employed for isolation of the microbe, I found to yield at least the same results as ordinary agar.

Diluted Serum for Sugar Test.

This is prepared as follows:

(a) To one volume of sheep or ox serum three volumes of tap water is added, and sterilized at 120° C. for fifteen minutes. A milky-coloured liquid is obtained.

(b) Solutions of various sugars are prepared and filtered through a Berkefeld filter. These solutions are diluted in (a) to 1 per cent.; litmus solution is added and sterilized at 100° C. for ten minutes.

This medium planted with various strains gives clotting