small intestine could be traced into the rectum at the pelvic brim, with no apparent trace of caecum or appendix.

The abdominal wound was closed in layers, and united by primary union. The patient made an uneventful recovery and left the hospital at the end of sixteen days. Quite recently I had a note from him to say that he went to work a fortnight after leaving the ward and has remained perfectly well since.

A SIMPLE HAEMOGLOBINIZED SALINE CULTURE MEDIUM

FOR THE GROWTH OF LEISHMANIA AND ALLIED PROTOZOA.*

By R. ROW, M.D. LOND., D.Sc. LOND.

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ROGERS, to whom belongs the credit of the discovery of *Leishmania donovani* of kala-azar going into fully formed flagellates in culture, made use of a saline solution containing a certain proportion of sodium citrate, to which he added the parasite-laden blood derived from the splenic puncture. Later the same author recommended the addition of a little citric acid, as this medium gave more abundant cultures. I believe a similar medium was used by workers who subsequently confirmed Rogers's discovery in India and in England: 'Nicolle' of Tunis made use of Novy-McNeal medium in his early cultures of *Leishmania infantum* of Algeria. This medium consists of the water of condensation of ordinary peptone agar slopes, into which, while yet fluid at 55° C., is incorporated 25 to 33 per cent. of defibrinated rabbit's blood. This medium was utilized also by Leishman and his collaborators in their culture work. Nicolle simplified the classical Novy-McNeal medium by using only agar and NaCl without peptone or meat extract. It is this medium which is now generally used in Leishmania culture work, and it goes by the name of Nicolle-Novy-McNeal or N.N.N. medium. It is also in this medium that Nicolle grew and demonstrated for the first time the cultural forms of *L. tropica* of the Tunisian oriental sore. Row of Bombay utilized dilute human blood (in saline) for his first cultures of *L. tropica* of the oriental sore of Cambay. Later, Carter used a similar medium for his cultures of *L. tropica* of the oriental sores of Northern India. Carter's formula consists of 4 parts of heated serum, 4 parts of red blood cells of the rabbit, and 3 parts of 10 per cent. sodium citrate in saline.

While working out the comparative merits of all these media, two things forced themselves on attention.

1. That haemoglobin seems to be essential for the growth and development of *Leishmania*.
2. That any changes in the character of the growth of these bodies seem to depend entirely on the way in which haemoglobin is present in the culture fluid, namely, according as it is free in solution or only in the red blood corpuscles unhaemolyzed.

Culture fluids containing red blood corpuscles unhaemolyzed offer a distinct resistance to a vigorous growth of flagellates, while those containing free haemoglobin in solution offer no such resistance. This is one of the reasons why it is difficult to obtain a series of successive cultures by using culture fluids containing unhaemolyzed red blood corpuscles, while vigorous cultures and subcultures are obtained indefinitely by the use of N.N.N. medium which contains free haemoglobin in abundance. The better results obtained in his later experiments by Rogers in the acidified citrate medium are perhaps to be accounted for by the haemoglobin set free by the citric acid. The presence of free haemoglobin in the alimentary canal of blood-sucking insects like the mosquito, the flea, or the bug (which retain the ingested blood for several days), explains the cultural forms of the parasites found in these, after they have been made to suck blood rich in the parasites.

Further, it may be of interest to note that while free haemoglobin in the saline medium of a certain density is eminently favourable for a rapid and vigorous growth of flagellates, the presence of unhaemolyzed red blood corpuscles seems to favour the multiplication of the original parasites as such. This is what takes place in Nature—for example, at the seat of disease, namely, the spleen or

liver in kala-azar, or the lesion in oriental sore—as well as *in vitro*. It is easy to demonstrate this by planting a clot, say, from the needle of a kala-azar splenic puncture in a saline solution, when an examination of the clot, even at the end of four days, will reveal an enormous number of $\odot$ bodies which have apparently multiplied in the meshes of the clot. A similar result is observed in an N.N.N. culture, as this becomes concentrated by age. Thus a culture containing vigorous flagellates about the fifth day shows about the twelfth to fifteenth day a large number and masses of $\odot$ bodies resulting from the post-flagellate growth of the parasite. This was referred to in another communication, and will form the subject of a separate memoir.

From the foregoing it is clear that the condensation fluid of Nicolle-Novy-McNeal medium fulfils all the conditions for a vigorous growth and multiplication of flagellates. But in practice in tropical laboratories the following difficulties present themselves:

1. The quality of blood available from a rabbit, even by a direct cardiac tapping, is so small that one cannot expect to obtain more than a dozen tubes for one rabbit sacrificed.
2. The quantity of condensation fluid obtained is not more than 1 to 1.5 c.cm.
3. This fluid gets concentrated in the course of a few days, owing to the tropical heat, but chiefly owing to the large surface of the agar slopes exposed to evaporation, so that the culture tubes are available from three to four days after they are prepared and before they are ten to twelve days old.

This gives an interval of only six days or so for subculturing. Further, it entails an enormous sacrifice of rabbits on the one hand, and on the other hand a great deal of labour in preparing the culture medium freshly every week.

It is to meet these difficulties that an attempt was made to simplify the process of making a suitable culture fluid, which would be like the condensation fluid of the N.N.N. medium in composition and be at the same time available readily and in larger quantity.

Details of Technique.

1. With the usual antiseptic and aseptic precautions about 5 c.cm. of blood are collected from the punctured marginal vein of a rabbit (blood can be aspirated easily to the extent of 5 to 8 c.cm. from one of the superficial veins of the forearm in man—thanks to Major Glen Lister's advice—when human blood is required) and poured into a sterile test tube containing a few glass beads. The tube is shaken to defibrinate the blood.
2. The defibrinated blood is now rendered laky by the addition of sterile distilled water in quantity requisite to dissolve all the haemoglobin available (1 in 8 to 10 of distilled water is found sufficient for the rabbit's blood in Bombay). The haemolysis may be also induced by repeated freezing and thawing, and is perhaps preferable when a stronger solution of haemoglobin is required.

3. The next step is to utilize the laky blood to dilute the NaCl solution so as to ultimately have a strength of 0.8 to 0.9 per cent. NaCl with a fair colour of the haemoglobin (after a great many experiments this strength has been found to be the best), and for this final admixture I follow the following routine:

From a stock solution of 1.2 per cent. NaCl two portions are sucked up with a sterile pipette and nipple and transferred to empty sterile test tubes of about $\frac{3}{4}$ in. diameter.

These tubes are kept in a water bath at 56° C., and when they are at this temperature one portion of the laky blood is added to each saline tube. This gives a strength of about 0.85 to 0.9 per cent. NaCl. The heating of the saline previously is done with the double object of ensuring sterility and of destroying any complement that may be present in the defibrinated blood.

By this method, with about 5 c.cm. of blood, as many or more culture tubes are made as can be prepared with the sacrifice of a rabbit, and the quantity of the culture fluid is at least 5 c.cm. in each tube, instead of being only 1 c.cm. to 1.5 c.cm. of the N.N.N. medium. The tubes thus prepared are ready for immediate use (after cooling), and from start to finish take twenty to thirty minutes to prepare.

In this culture fluid flagellates flourish and multiply even after five to six weeks, so that subcultures can be made at leisure any time during this period, as the fluid does not get so easily concentrated, as does the condensation water of the N.N.N. medium, which by this time completely dries up owing to the large surface exposed to evaporation by the agar slope.

The following advantages may in brief be claimed for the haemoglobinized saline medium:

1. The culture medium can be made at once when

* Communicated to the Bombay Branch of the British Medical Association.

urgently required, without any loss of time, as the technique is simple.

2. The technique involves less labour, and entails no sacrifice of rabbits—that is, it is economical.

3. Human blood can be used for the preparation of this medium when required for special purposes or for general use.

4. The fluid does not alter appreciably by age within the limit of four to six weeks, owing to the small surface exposed to evaporation.

5. A larger quantity of the culture fluid in each culture tube is available, so that when abundant cultures are required—for example, for the preparation of vaccine—this gives a decided advantage.

6. The culture fluid keeps sterile during the working period of six weeks more easily than does the N.N.N. medium in the tropical laboratories.

NOTES ON FOUR CAESAREAN SECTIONS.

BY

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The following four cases requiring Caesarean section fell to my lot within the short period of nine months; in each case, except No. 1, one or more craniotomies had been previously performed, the narrowed pelvis being caused by rickets.

Sterilization was done in each case by the following method: The tubes were clamped with small forceps close to the uterine cornua, the mesosalpinx transfixed by double ligature, one ligature tying off the proximal end of the tube close to the uterus, the other ligature being brought round the fimbriated end, thus removing the whole tube; the serous coat of the uterine end of the tube was stitched over. In two of the cases I was able to deliver the uterus, which I think is an advantage, although it needs a slightly longer incision; but there is less bleeding, as the uterine arteries can be easily controlled by an assistant and cloths are placed behind the uterus, completely shutting off the intestines. The placenta in all four cases was attached to the anterior uterine wall; this occurs in 75 per cent. of cases, but there is no difficulty in recognizing the placental tissue on incising the uterine wall. With regard to sutures for the uterus, I think No. 3 catgut for the main and No. 2 for the intermediate, both specially prepared to resist absorption for twenty-one days, is preferable to silk or Pagenstecher's material. Ligatures should not be tied too tightly, as the tissues of the gravid uterus are soft and easily cut through; a double hitch for the first half of the knot, and completed, followed by a single knot, is, I think, preferable to three single knots.

As to the time of operating, the best, in my opinion, is before the commencement of labour pains, the operation can then be conducted in daylight, adequate assistance secured, and the usual preparation for an abdominal operation can be carried out; the fear of haemorrhage and uterine inertia need not be considered seriously.

Sterilization was done in each case by the patient's desire, and I think the method described above is likely to prove satisfactory. It is stated that excision of a "portion" of the tube has been followed by pregnancy, even when the ovaries have been removed; this I can well understand, if a small portion of ovary has been left behind. This matter is fully referred to and discussed by A. J. Wallace and Sir W. Sinclair, in the *Journal of Obstetrics and Gynaecology of the British Empire*, 1907.

CASE 1.—S. L., aged 31, primipara, had arrived at full term. She was first seen by Dr. Anderson, of Bodmin, May 6th, 1911, who says, "There was no dilatation of the os, or other signs of labour. Next morning the pains were few and infrequent, and the os just admitted the tip of the index finger, but was too high up to make out the presentation; she was a short, rickety woman with a contracted pelvis; the antero-posterior diameter was $2\frac{1}{2}$ inches."

She was brought into the East Cornwall Hospital, Bodmin, and I was asked to see her with a view to Caesarean section. On May 8th I was able to confirm Dr. Anderson's notes, and performed an immediate Caesarean section. The child was dead, but the mother did well, the wound closing by primary

union. On the fourteenth day she developed slight thrombosis of the left femoral vein, which delayed her convalescence. She left hospital in six weeks quite well.

Case.	Age.	Date.	Doctor.	Previous Labours.	Convalescence.
1. S. L.	31	May 8, 1911	Dr. Anderson	Primipara	Good; child stillborn; in hospital six weeks.
2. L. W.	33	Sept. 28, 1911	Dr. Wagner	1 Craniotomy	Good; child strong; in hospital 24 days.
3. M. S.	30	Dec. 21, 1911	Dr. Hodge	3 Craniotomies	Uninterrupted recovery; in hospital 33 days.
4. L. G.	28	Feb. 3, 1912	Dr. Anderson	1 Craniotomy	Uninterrupted recovery; in hospital 30 days.

The Arris and Gale Lectures

ON

THE MECHANISM AND TREATMENT OF SHOCK.

DELIVERED AT THE ROYAL COLLEGE OF SURGEONS, MARCH 22ND, 1912.

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(Concluded from page 1072.)

LECTURE II.—PART II.

THE PELVIC ORGANS AND GENITO-URINARY SYSTEM.

Pelvic Viscera.

PELVIC and perineal structures do not share in the local vascular mechanism referred to in connexion with the abdomen proper. We have shown that all variations from these regions are eliminated by blocking afferent impulses. Blood-pressure variations, therefore, from interference with these structures are a true indication of the shock value. In the human subject sigmoidoscopy, with the accompanying inflation of the bowel, causes a definite rise in blood pressure of the pressor type. A study of the blood pressure, pulse, and respiratory-rate shows: (1) That the vasomotor variations are not due mechanically to increased pressure in the abdominal cavity. For the pulse and respiratory-rate are increased instead of being slowed, as

Diagram 2.—Proportion of afferent and efferent splanchnic nerve fibres mechanically stimulated by traction on mesentery in different situations.

would then be the case. (2) That direct stimulus to the bowel wall is not responsible. For our experiments have repeatedly demonstrated that manipulation of the bowel wall is without any ascertainable effects on the blood pressure. (3) That distension of the intestine induces tension upon the mesentery, and from the stimulus to this