importation into, or sale within, their districts of any shell-fish the safety of which there is reasonable ground to suspect, and to make it the duty of any authority which permits the exportation of shell-fish from its district to show e

that every reasonable care has been taken to keep such shell-fish as are to be found within its jurisdiction free from pollution.

**SOME COMPARATIVE MEASUREMENTS OF THE LIVES OF LEUCOCYTES**

**WHEN THE CELLS ARE RESTING IN THE PLASMATA OF DIFFERENT PERSONS, AND THE POSSIBLE APPLICATION OF SUCH MEASUREMENTS AS AN AID TO DIAGNOSIS IN INFECTIVE DISEASE.**

By Hugh C. Ross, L.R.C.P. Lond., M.R.C.S. Eng., Late Surgeon, Royal Navy; Pathologist to the Royal Southern Hospital, Liverpool.

Of recent years I have been endeavouring to ascertain the effect produced by one person's plasma on the life of another person's leucocytes. It appeared reasonable to suppose that the plasma of a person suffering from an infective disease would be poisonous to the leucocytes of healthy persons. If this is the case it might also be reasonable to suppose that the same plasma would not be so poisonous to the leucocytes of another person suffering from the same disease, because it is probable that the cells would be already used to, or immune against, the toxin, and furthermore that if the toxin of another infective disease differs from the toxin of another infective disease, it might be inferred that an immunity on the part of a leucocyte against one disease will not render it immune against another. Therefore, provided it is possible to tell accurately when a leucocyte is dead—that is, if one can differentiate a living from a dead cell—it also will become possible to measure the lengths of the lives of leucocytes after they have been removed from the body. And this will enable us to make comparative measurements of the lives of leucocytes when they are mixed with the plasmas of different persons. Supposing, therefore, it is true that an infected plasma shortens the lives of a healthy person's leucocytes but does not shorten the lives of the leucocytes of another person suffering from the same disease, it may be useful to reverse the process and assist in the diagnosis of infective disease by making measurements of the lives of such a patient's leucocytes when they are mixed with different plasmas. For instance, if the leucocytes of a person suffering from an indefinite infective disease are found to be easily killed by the plasmas of persons suffering from a variety of diseases but are not comparatively easily killed by the plasma of persons suffering from, say, typhoid fever, it might be inferred that the patient is suffering from, or has recently suffered from, typhoid fever, because his leucocytes are used to, or immune against, that disease.

The above is the enunciation of a problem which I set myself to solve several years ago, and the paper describes the experiments which have been conducted to investigate the last part of it—i.e., with the object of determining the actual measurements of the lives of leucocytes when they are placed in the plasmas of people who are suffering from various diseases. The earlier researches made in order to differentiate living from dead leucocytes have already been published in the *Journal of Physiology* (1), and the actual method employed to estimate how many living and how many dead cells there may be in a given volume of citrated blood has been described in The Lancet of Jan. 16th, 1909 (2). This method may be again briefly summarised thus:—

**Method for counting the number of living and dead leucocytes in a given sample of citrated blood.**—The following solutions are prepared and a jelly is made from them: 1. A volume of Unna's polyethylene methylene blue (Grübler) is diluted with two volumes of water. 2. A solution containing 2 per cent. of agar in water, filtered and sterilised. 3. An accurately neutralised solution containing 4.5 per cent. sodium citrate, 1.5 per cent. sodium chloride, and 0.225 per cent. atropine sulphate. 4. A 5 per cent. solution of sodium bicarbonate. In a test tube mix one cubic centimetre of the diluted stain, two cubic centimetres of the citrate solution, and three cubic centimetres of the molten agar solution. To this mixture a quantity of the alkaline sodium bicarbonate solution must be added in order to cause the excitant for leucocytes contained in the jelly to diffuse into the cells and the quantity added varies with the temperature of the room. If measurements are going to be made in a room the temperature of which is between 60° and 70°F., about 0.25 cubic centimetre of the alkaline solution should be added. The mixture is then boiled until it froths up the tube and a drop poured on to a slide and allowed to set so as to form a film. Supposing a given capillary tube contains the blood corpuscles of one person mixed with the plasma of another, the average number of living and dead leucocytes in the tube can be estimated by placing a drop of its contents on a cover-glass which is inverted and allowed to fall on the agar jelly. After two or three minutes the whole film but not the nuclei of the living leucocytes will stain and those cells will show exaggerated amoeboid movements, whereas the dead cells will remain immobile. Moreover, the dead cells may be achromatic (3), in which case they will not stain. Their nucleus may appear as a single nuclear mass, or their nuclei may even stain, or the dead cells may have undergone other changes which have been described in former papers (1, 2). Field after field can be rapidly passed in front of a 1-6th inch or equivalent objective and the number of the living and dead cells counted. Several preparations can be rapidly examined and an average struck so as to give an estimate of the number of living and dead cells in the given capillary tube. No difficulty is met with in making the counts, for living can be readily differentiated from dead cells by the presence or absence of exaggerated movements.

If all the leucocytes appear to be dead, and especially if the agar jelly has not previously been tested, it is as well to

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1 The word "leucocyte" refers to the polymorphonuclear leucocyte.

2 A scale has been given in the former paper.
control the measurement—that is, to see that the jelly will actually excite living cells by placing a drop of fresh citrated blood on to another part of the same film and noting whether stimulated movements of all the leucocytes occur.

**Procedure for the preparation of capillary tubes containing the leucocytes.**—It is best to use a long test-tube (having a diameter of one centimetre) and a series of small tubes which have such a diameter that blood will run into it by capillarity and at the same time its flow can be controlled by gravity. I use a tube with a lumen of about two millimetres. A neutral solution is made which contains 3 per cent. of sodium citrate and 1 per cent. of sodium chloride. Some of this solution is put into the test-tube, that is to say, its upper and meniscus stands at mark 6. Blood from the finger of the person whose plasma is going to be tested is added until the meniscus stands at mark 12, care being taken that no bubble of air separates the two fluids. Mixture is carried out by allowing the two fluids to gravitate up and down the tube six or seven times. The tube is sealed and centrifugalised; the blood being driven towards zero. The end remote from zero is then unsealed and the portion containing the precipitated corpuscles is separated and discarded by cutting the tube at 4. Eight portions of the tube now contain citrated plasma. If, owing to the sealing process, much of the tube has been lost, the unsealed portion can be easily restored. If the lower meniscus lies at a mark standing at 4 where the tube has been cut, and the upper meniscus standing at 12, blood from the finger of the person whose corpuscles are going to be tested is added until the upper meniscus stands at 13 (i.e., the mixture equals 1-9). Mixture is insured as before in the tube sealed. It will be seen that although the tube contains the plasma of both persons the corpuscles are bathed in a solution containing four times as much plasma as the first person as of the second. A series of tubes may thus be made.

**Appliance to insure continual mixture and to prevent the corpuscles from adhering to the glass.**—If a capillary tube prepared in the way which has been described is laid on a flat surface, the corpuscles will soon gravitate to the most dependent side and will ultimately adhere to the glass. The following appliance prevents this. By means of a simple clockwork movement a split drum is made to revolve once in about three minutes. The drum is so adapted that the mouth of a long test-tube (having a diameter of one centimetre and the cavity of which is lined with a roll of blotting paper) fits accurately on to it and revolves with it. The apparatus is so arranged that the tube is horizontal and is of such a size that it can be placed in the incubator if necessary. The capillary tubes inserted into the test-tube are continually tumbling over each other by gravity as the test-tube revolves, and so do the blood cells in their turn are continually gravitating in different directions through the citrated plasmata. It has been found that this device prevents them adhering to the glass and insures them being evenly distributed through the citrated plasmata provided the ends of the capillary tubes are not bent over when sealed. This apparatus also insures all capillary tubes being subjected to the same conditions of temperature.

**Procedure for measuring the lives of the leucocytes contained in the capillary tubes.**—The capillary tubes are examined on stimulating agar by the method already described. If all the cells are alive the tubes are resealed and returned to the revolving apparatus to be examined later, and so on. By this means the percentage of living and dead cells in a tube can be estimated. It is important to remember that in striking these averages only an approximate estimate can be obtained, and that therefore the greater the number of tubes made the better, as the error decreases with the greater number of leucocytes counted. In the experiments already described there were about 500 leucocytes in each case by making five films from each of five tubes, and counting about 20 leucocytes in each film. Since it is obvious that the greatest error may occur when the number of living approximates the number of dead cells in a tube, the following experiments would appear to be very erroneous, judging by the application of Poisson's formula, which shows that supposing there are half a million leucocytes in the five tubes, which is an excessive estimate, a count of 500 cells would give a possible error of not more than 6 per cent., or when the number is 50 it would be of the order of 10 per cent.

Before enumerating the actual measurements there is yet another question to be considered, a point upon which I wish to lay great emphasis—namely, that all measurements of the lives of leucocytes should necessarily be comparative. For this reason I have killed a person's leucocytes more rapidly than a septicaemic patient's plasma, when the typhoid measurement was made to-day and the septicaemic measurement made three days ago, for although there was a great difference in the length of the lives and the tests were made on the same person's plasma, it cannot be said that that person's leucocytes were in the same state to-day as they were three days ago, although the person is apparently in the same healthy condition.

Leucocytes appear to live longest at about 20°C. They will not live very long at 37°C and at 10°C will live longer than at 37°C but not so long as at 20°C. I have already suggested (4) that this may be due to the accelerated absorption of the poisonous salts in the citrate solution caused by heat, and this will also explain the early death in the presence of alkali which also accelerates diffusion. I prefer the last view to others because the factor heat affects the lives of the leucocytes, since the cells are necessary resting in a citrate solution which is itself poisonous to some extent, and even the temperature of incubators is variable. It is thus of the utmost importance that when the lives of a person's leucocytes, which have been placed in the plasma of a person suffering from an infective disease, are measured, a simultaneous measurement of the same leucocytes shed at the same time must be made in the plasma of a non-infected person. It is obvious that the difference between the two that the result can be determined. In other words, all measurements must be simultaneously controlled by other measurements and the contrast is the result. It is also obvious that since heat and the citrate solution affect the lives of a person's leucocytes, which have been placed in the plasma of a person suffering from an infective disease, are measured, a simultaneous measurement of the same leucocytes shed at the same time must be made in the plasma of a non-infected person. It is obvious that the difference between the two that the result can be determined. In other words, all measurements must be simultaneously controlled by other measurements and the contrast is the result.
these averages may be said to be about 30 hours. I conclude that the plasma of one person is poisonous to another person's leucocytes.

Healthy person's leucocytes; plasma from cases of typhoid fever. —All cells dead in 14 hours. Difference between test and control about six hours, which is the average out of four cases.

Healthy person's leucocytes; plasma from cases of malaria. —Majority dead in 15 hours, a few alive in 18 hours. Occasionally 50 per cent. were alive in 16 hours. Average difference between 12 cases and their controls about two hours. The differences in these cases and their controls were five hours and three and three-quarter hours respectively.

Healthy person's leucocytes; plasma from cases of phthisis. —Majority dead in 17 hours. Average difference between five cases and their controls about one hour. Sometimes it was as much as a day's difference, but in very chronic cases there was little difference.

Healthy person's leucocytes; plasma from a case of osteomyelitis. —50 per cent. dead in 14 hours. Repeated with a case of purpura haemorrhagica. —Majority dead in 15 hours; all dead in 20 hours. Practically no difference from controls.

Healthy person's leucocytes; plasma from a case of chorea. —All cells dead in 14 hours. Difference about six hours.

Leucocytes from cases of typhoid fever; plasma from other cases of typhoid fever. —Average from three groups of cases, all of which reacted to Widal's reaction and were in the third or fourth week of the disease except one which was convalescent. These groups include the cases mentioned above. There was never a difference of more than one and a half hours between the death of the majority of cells in test and control tubes.

Leucocytes from cases of malaria; plasma from other cases of malaria. —Five cases. The majority of cells in all cases were alive in 18 hours. Practically no difference from controls.

Leucocytes from cases of phthisis; plasma from other cases of phthisis. —Four experiments. 50 per cent. dead was the average in 18 hours; very little difference from controls, sometimes the cells lived longer than in the controls.

Leucocytes from cases of malaria; plasma from cases of typhoid fever. —The majority of the cells in most instances were dead in 14 hours. Differences varied from four to six hours.

Leucocytes from cases of typhoid fever; plasma from cases of malaria. —About 50 per cent. were usually dead in 15 hours and always dead in 20 hours. Five cases tried; average difference about three hours.

Healthy person's leucocytes; plasma from cases of cancer. —Seven cases; all cells alive in 16 hours; a large number alive in 20 hours. Usually there was little difference between the effect of cancer plasma and that of a healthy person.

From the foregoing measurements it would appear that in the cases which have been experimented with the plasma of persons suffering from infective diseases is poisonous to a healthy person's leucocytes and to the leucocytes of another person suffering from the same disease, but not so poisonous to the leucocytes of another person suffering from the same disease. I submit that it may be reasonable to suppose that such may be the case in other infective diseases.

In comparing the lengths of the lives of leucocytes of persons suffering from chronic infective diseases both in another infected person's plasma and in healthy plasma, I have frequently found that such cells will not live so long as the cells of healthy persons subjected to the same conditions. With no other infection in the tube the lives of leucocytes taken from cases of chronic illnesses in their own plasmas with the length of the lives of the cells of healthy persons in their own healthy plasmas. In cases of chronic phthisis, malaria, Hodgkin's disease, etc., I have found that the leucocytes will not live even in their own plasmas nearly as long as if they belonged to a healthy person, as much as a day's difference having been observed; and we may infer that these diseases, and probably others also, cause a loss of vitality in the patients' leucocytes, so that by this procedure the loss of vitality can be measured. It is important to remember this point, for if the making of a measurement is delayed it may be found that all the cells are dead in both control and test preparations. This method of determining the poisonous action of plasma may also prove of value in prognosis as well as in diagnosis.

I do not think that any difficulty will be met with in making the counts, with the exception of a possible one caused by the agglutination of one or more of the normally large clumps are met with. If the cells are clumped, however, it does not necessarily follow that they are dead, for from it, they may be very active, though I am of opinion that if clumped death will soon occur. The cells in a clump generally beclump the centers. Rouget's method is satisfactory. If bacteria are seen in large numbers in a film the capillary tube is discarded. The revolving apparatus is not essential but more constant results have been obtained by its use. As far as possible I have purposely avoided handling the blood of the person whose leucocytes are to be tested, for fear of injuring the cells. The variations of the alkalinity of the plasma may, I think, be neglected, as it is not sufficient materially to alter the length of the lives of the cells. This is borne out by the experiments with cancer plasma, because that plasma is more alkaline than normal and yet does not shorten life.

Summary.

I fear that it is too early to arrive at any definite conclusions from so small a number of experiments, but I think that the justifying results obtained are promising to warrant further investigation, though the work must still be regarded as being in the experimental stage. I hope that this method will be tried by others, as the problem of giving an exact and universal indication to diagnose an infective disease by this method, but a large amount of material will be required before one can determine its value in this direction, and I have mentioned its possibilities with reference to prognosis. The stage in a disease in which measurable immunity appears in a leucocyte also remains to be determined.

To summarise the method by which I endeavour to assist in a diagnosis in a case of infective disease, a small quantity of blood from a patient is mixed with eight times its volume of the citrated plasma of other persons who are known to be suffering from certain infective diseases and also with the citrated plasma of a healthy person. For this last purpose I sometimes use my own plasma. The method has been described. The capillary tubes are kept together in the revolving apparatus for about 14 hours. Then some films are prepared from jelly which will excite movements in living leucocytes and samples of the contents of the tubes are examined on these films. The number of living and dead cells are averaged and the difference between the lengths of the lives of the cells when resting in healthy and infected plasmas are determined. When an infected plasma is found which will not comparatively shorten the lives of the patient's leucocytes, it seems probable that the patient is suffering from the same disease as the person from whom the plasma was taken. I generally confirm this procedure by reversing the process and trying the patient's plasma on the leucocytes of other persons suffering from the disease determined, taking care to make controls in this case as well as the first by making experiments with healthy plasma and with the plasma of persons suffering from other diseases.

The method described in this paper has two disadvantages: first, it is not applicable to all cases of infections; second, since counting 500 leucocytes in each case, which is most tedious. The rest of the method takes very little time; collecting the plasma and mixing them with the patient's corpuscles is soon accomplished, and when the tubes are in the revolving apparatus, they require no attention, and the test has come to estimate the number of living and dead cells in them. The agar jelly can be made from stock solutions as specified and kept in test-tubes for months as moulds will

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4 It has been noticed that stain will diffuse more readily into the blood cells of those patients—that is, those diseases, and probably other chronic illnesses, cause a lowered 'coefficient of diffusion' in blood corpuscles.
not grow on it. Films are rapidly prepared by boiling the la
this temperature if fitted with a special capsule. Since my 4
practical purposes even away from the vicinity of a ne
such a temperature the leucocytes might live for a long time c -,
contrast. Consequently I deliberately shorten the life of n
before sufficient deaths occurred among the cells to make a st
solution it has been found that the majority of healthy t,
menting with a greater concentration of plasma with a view
bodies which have been lying in the mortuary for 24 hours or
attacked, it may be possible to state how long a person has 1
Bibliography.—H. C. Ross : (1) On the Death of Leucocytes, Journal
acute pain over the abdomen generally, which was tympanitic
enema yielded no result and peristaltic action was visible
nevertheless, at this stage suggested more an obstruction
Clinical Notes : MEDICAL, SURGICAL, OBSTETRICAL, AND THERAPEUTICAL.
A CASE OF OBSTRUCTION OF THE BOWELS, AT FIRST SIMULATING GASTRIC ULCER.
By D. M. MACDONALD, M.D. ABERD.

The patient was a thin, wiry-looking woman, aged 34 years, who had had no children. She complained of pain in the epigastrium with persistent vomiting. In February, 1907, she had an attack of dyspepsia with pain over the stomach which gradually abated under treatment. She was next given by me on Dec. 5th. She was taken ill on that day while out at farm work and too ill to bed complaining of pain in the epigastrium, which she said came out at the back, and constant vomiting. The vomit consisted first of food, then of partially digested blood, and ultimately of blood itself. She still liquid diet and lime-water for a few days. The pain soon became tolerable and the vomiting almost disappeared. The bowels were moved by injection. There was melena at the time. All this time the abdomen was exquisitely tender to touch in the left hypochondrium and left iliac region. A week after the initial illness the patient one morning developed acute pain over the abdomen generally, which was tympanitic and extremely tender. Vomiting was constant and consisted largely of bilious matter, brownish-black in colour, and of which a pint would sometimes be vomited at a time. The emesis yielded no result and peristaltic action was visible through the abdominal walls. The pulse was now 120 and the temperature was 96°F. I saw the patient about 4 o'clock that afternoon. Her abdomen was distended and her face had a sunken anxious look. The appearance, nevertheless, at this stage suggested more an obstruction than a rupture of the bowels. The pulse was of fair volume and she lay on her side with her legs drawn up. In consultation with Mr. A. Don of Dundee it was decided that the case was one for laparotomy. On his opening the abdominal cavity, under chloroform, there was a little free clear serous fluid which at once negatived a serious bowel rupture. The stomach and upper part of the cavity were first explored, but nothing was found to account for the toxid condition. The intestines were filled with gas, especially the descending colon. Attention was next directed to the lower part of the cavity where many adhesions in the pelvis were found. The omentum was first freed from the pelvis and ligatured and cut away opposite the abdominal wound. The bowels were then freed in the pelvic cavity, the adhesions being recent with the exception of a loop which seemed to be firmly fixed to the apex of the bladder. This was not interfered with. The appendix and other organs were normal and there did not seem to have been any peritonitis.

The probable explanation of the pain in the left side, which was what the patient complained of most when I saw her in the afternoon before operation, would seem to be that the colon, probably its sigmoid flexure, was bound down by the omentum or some of the adherent coils of intestine, and that a reverse peristaltic action was causing pain over the splenic flexure. Another explanation of the pain would be that the dragging on the omentum caused by the peristalsis of the bowels to which it was adherent would be exerted on its fixed point in the epigastrium and splenic flexure, and this would account for the most severe pain being in that region.

The patient made a complete recovery from her illness, though the pain continued for some days on the left side. There was no vomiting subsequent to the operation.

Dunkeld, N.B.

TRAUMATIC NASAL FISTULA.
By SOMERTON CLARK, F.R.C.S. EDIN., SURGEON TO THE MISSION HOSPITAL, DERA ISMAIL KHAN, INDIA.

The patient, a male Afghan, aged 50 years, was admitted to hospital on Nov. 28th, 1907, with the history that as he was grazing flocks near the Mohmand country six months previously, the Mohmands attempted to carry off his property and a fight ensued during which he received a wound in the left eye with a stone flying in the maxillary bone. On admission he was found to have a wedge-shaped piece of bone 1 inches long jammed in the junction of the nasal and superior maxillary bones. This was removed by lion forceps and the skin edges, which were very friable, were undermined and brought together. Owing to tension on the flaps union was only partial, and so six weeks later a second operation was performed. After this a fistula of the size of a pin's head remained and the patient went to his own country. On his return the fistula was of the size of a quill. He was again admitted. This time a piece of skin was dissected from over the lacrymal bone and turned over so as to form a lining membrane for the left nasal cavity. By this method a fistula of the size of a quill was formed. After this a fistula of the size of a quill was formed. He was again admitted. This time a piece of skin was dissected from over the lacrymal bone and turned over so as to form a lining membrane for the left nasal cavity. By this method a fistula of the size of a quill was formed. On his return the fistula was of the size of a quill. He was again admitted. This time a piece of skin was dissected from over the lacrymal bone and turned over so as to form a lining membrane for the left nasal cavity. By this method a fistula of the size of a quill was formed. On his return the fistula was of the size of a quill. He was again adm.