The curative value of these chemotherapeutic agents under very severe conditions is however seen from table II, which records results obtained in septicæmic cases. Plague septicæmia is a very serious condition indeed, as the control cases, of which nearly 95 per cent died, show. In septicæmic cases treated with sulphathiazole and sulphapyridine, however, the case mortality was reduced to about 40 per cent, *i.e.*, more than half of the cases treated recovered. Similar but slightly inferior results were obtained with the antiplague serum. This difference again may be due to unequal number of cases treated. Statistically no significant difference is apparent.

As regards the serum, we experienced serious difficulties in its administration. We wanted to give large dose but intravenous administration of even 20 c.cm. of the serum produced alarming symptoms in quite a number of cases. In our experiments with antiplague serum, it was found necessary to inject 0.4 c.cm. of the serum for a mouse of 25 g. weight. By analogy the equivalent dose for man weighing about 120 lb. would theoretically be in the neighbourhood of one litre and, even after allowing for the marked difference in the susceptibility to plague infection for the two species, a much larger dose than 200 c.cm., the maximum we used, would be needed.

It is now well recognized that, in the treatment with the sulphanilamides, it is the effective level of blood concentration of the drugs that decides the therapeutic result. This effective level varies with the nature of the infection, the degree of infection, and the animal to be treated. The defence mechanisms of the body are better developed in some animals than in others and it is generally conceded that these defence mechanisms co-operate with the chemotherapeutic agents in finally overcoming the infection. In the treatment of experimental plague infection in mice reported by Sokhey and Dikshit (*loc. cit.*), the best results were achieved with a concentration of free sulphathiazole in blood varying between 20 mg. and 10 mg. per 100 c.cm. (unreported figures). This concentration was maintained with an oral dose of 20 mg. given twice a day for 10 days for mice with an average weight of 25 g. In the case of the treatment of human cases reported in this paper, no effort was made to determine the level of the drug in blood reached, because we lacked the necessary facilities. But there is reason to believe that with the dosage of sulphathiazole and sulphapyridine used (about 3 g. a day), a concentration in blood of about 2 to 3 mg. per cent of the drug only could have been reached. Plague infection is much severer in mice than in men; 100 per cent of the mice infected with our standard infective dose die, while human case mortality in various epidemics varies between 50 and 80 per cent. In spite of this difference, a concentration higher than 2 to 3 mg.

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per cent is clearly indicated and we believe that with higher concentrations, results much superior to those obtained in the present series will be achieved. We propose to try this out in the field trials under way at present.

Summary

1. A report of treatment of 237 cases of plague with the Haffkine Institute antiplague serum, sulphapyridine and sulphathiazole in an epidemic at Bettiah is given.

2. In all cases taken together without differentiation into septicæmic or non-septicæmic cases, the case mortality in the serum, sulphapyridine, and sulphathiazole treatment was 28 per cent, 24 per cent and 15 per cent, respectively, as against 52 per cent in the controls. These reductions in case mortality are statistically significant, but not so the differences in the results of these different treatments.

3. Even in those severe cases in which septicæmia was present at the commencement of treatment all the three forms of treatment reduced the case mortality from 95 per cent in the controls to about 50 per cent in treated cases.

4. Six cases of primary pneumonic plague are reported.

5. The question of dosage employed has been discussed and it is suggested that a larger dose especially of the sulphanilamides used would reduce the case mortality still further.

Our thanks are due to Messrs. May and Baker for putting a liberal supply of sulphapyridine (M.&B. 693) at our disposal. Sulphathiazole was prepared in the Haffkine Institute and the powder was made into tablets by Cipla Laboratories, Bombay, to whom also our thanks are due.

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A NOTE ON DRIED BLOOD PLASMA AND ITS PREPARATION IN INDIA

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THE difficulties associated with blood transfusions in this country are many, the chief being that associated with the production of a donor of the correct group at the requisite time. Blood banks may overcome certain difficulties, but are wasteful in that stored blood occupies a large

amount of refrigerator space and only keeps for 30 days. They are only practicable in large towns. Recent work on the use of blood plasma (and serum) has altered the entire outlook of the treatment of shock and hæmorrhage.

Cannon, Fraser and Hooper (1919) showed that in shock there was a diminished blood volume and hæmo-concentration. Blalock (1931) pointed out that in experimental burns there was a loss of whole plasma from the circulation. Walther (1937) confirmed this and pointed out that the rational treatment was to supply plasma to the denuded circulation. It is clear that shock is associated with the loss of plasma from the capillaries producing a concentration of blood cells.

Walther's contention has been proved by all recent workers and the present method of election in the treatment of shock is by transfusion with plasma (or serum), the essential constituents being the blood proteins.

Best and Solandt (1940) have shown that both plasma and serum are equally efficacious. In hæmorrhage the obvious method of treatment is to replace the blood lost by blood of a suitable type and until recently this has been the method in use. Recent work however has emphasized the value of plasma and serum. In practice, hæmorrhage without shock is extremely rare. Edwards, Kay and Davie (1940) point out that it is very rare for a patient to lose more than three pints of blood in a severe hæmorrhage, as the resulting lowering of blood pressure causes cessation of the bleeding. In such cases, a patient is left with 75 per cent of the erythrocytes in some part of the circulation. Providing therefore that the blood pressure can be raised and maintained by the introduction of a fluid of a suitable osmotic pressure into the circulation, a three-pint loss of blood can be treated and favourable results expected. Plasma or serum provide such a fluid.

As hæmorrhage rarely occurs without shock and the accurate separation and estimation of either of these conditions are extremely difficult, it is more than probable that the cause of death in hæmorrhage cases is due to a combination of blood loss *plus* shock. One factor common to both is loss of blood proteins contained in the plasma. The obvious method of treatment is to restore and maintain the circulatory volume by the administration of the proteins. This can be done by transfusing with plasma or serum.

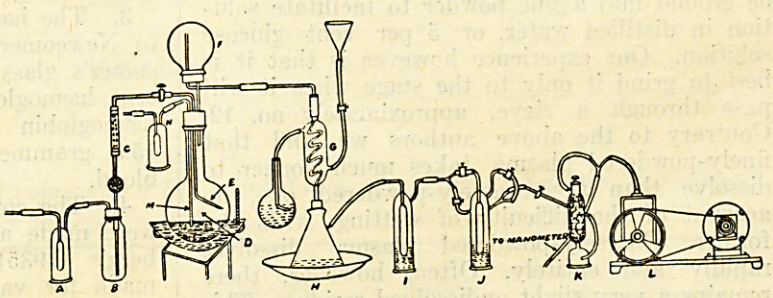
In earlier work, liquid plasma or serum were used and has the advantage that a certain amount could be given without reference to blood grouping. They were stable at room temperature. Blood plasma and serum can be solidified and in the west have been kept at room temperatures for 12 months without deterioration and with a considerable saving of storage space.

It appears that in solid plasma and serum we have substances for the treatment of shock and the majority of hæmorrhage cases possessing none of the disadvantage of whole blood transfusion and which should be of the greatest value in this country in that a valuable method of treatment is made easily available for outlying towns. Its use in the field during war is obvious.

The requirements for a transfusion are 20 grammes solid plasma dissolved in 250 c.cm. distilled water which is the equivalent of the plasma-protein value of one pint of citrated blood. Up to 500 c.cm. can be given regardless of blood grouping. Twenty grammes plasma fills an ordinary-sized test-tube.

Method of preparation

For converting liquid plasma into a crystalline solid, we have modified the procedure of Edwards, Kay and Davie (*loc. cit.*) and have employed an apparatus whose sketch is here reproduced :—



A is a bubbler containing 0.1 per cent mercuric chloride solution to sterilize the incoming air.

B is the liquid plasma bottle. This bottle has a screw top and a metallic screw cap with two small holes and with a rubber diaphragm through which liquid plasma can be introduced by means of a needle.

C is an arrangement of a bulb containing sterile gauze and a tower containing glass beads or short bits of thin glass tube for filtering the plasma.

D is the actual drying flask into which the plasma is introduced in the form of a strong thin jet or spray through the tube **E** which is drawn to a fine point.

F is a safety flask used to collect any froth which flows back into the flask **D**. The flask **D** is heated in a thermostat maintained at 37°C. The water vapours are condensed at **G** and collect in the receiver flask **H** which is surrounded with ice. By maintaining a flow of ice cold water through the condenser **G** and by keeping the receiver **H** also ice cold, the speed of distillation has been increased. The flask **H** is connected to the bubbler **I** containing 0.1 per cent mercuric chloride solution which eliminates all chances of infection if there is a back diffusion. The bubbler **I** is connected with the bubbler **J** in such a way that **J** can be put in or out of the circuit at will without interrupting the vacuum. A soda lime tower **R** is interposed between the whole apparatus and the vacuum pump **L**. Before starting, the entire apparatus from **B** to **H** is sterilized with a dummy empty bottle at **B** which is then replaced by a fresh bottle containing the liquid plasma, and the distillation is started and continued till all the available plasma has been dried. The flask **D** is provided with another inlet tube **M** to

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SEDIMENTATION RATE OF RED BLOOD CELLS IN EPIDEMIC DROPSY

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THE sedimentation rate of the red blood cells was determined of 74 epidemic dropsy patients and of 8 persons in whom symptoms resembling those of epidemic dropsy were produced after taking food cooked in mustard oil incriminated in natural outbreaks of the disease or in mustard oil to which known quantities of argemone oil had been added (Chopra *et al.*, 1939). The cell volume and the hæmoglobin percentage were also estimated. The methods employed were :—

1. *Sedimentation rate.*—Westergren's (1926) method was used. The readings were taken every 10 minutes for 2 hours. The tests were carried out at room temperature which varied from 29°C. to 34°C. during the period of these examinations. Although from observations made on parallel tests carried out at different temperatures, there is an acceleration in the rate of sedimentation at higher temperatures, the actual difference within the range of room temperature 29°C. to 34°C. is not sufficiently great to alter materially the results obtained.

2. The cell volume was determined by noting the volume of packed cells after centrifugalization at 3,500 revolutions for half an hour. The anticoagulant used was a mixture of potassium oxalate and ammonium oxalate in proportions recommended by Wintrobe and Landsberg (1935). The tubes after the addition of the solution of anticoagulant were dried over calcium chloride under vacuum in a desiccator.

3. The hæmoglobin was estimated according to Newcomer's method using the Klett-Newcomer's glass standard equivalent to 0.038 per cent hæmoglobin solution. The 100 per cent hæmoglobin by this method is equivalent to 15.3 grammes hæmoglobin per 100 c.cm. of blood.

4. The corrections of the sedimentation rate were made according to Wintrobe and Landsberg's (1935) graph in which corrections are made for variations in the cell volume. The average cell volume was taken as 43 instead of 47 used by Wintrobe and Landsberg.

As a control the blood sedimentation rate was estimated of 22 apparently healthy adults of the labouring class between the ages of 25 to 40. Individuals belonging to the labouring class were selected as the epidemic dropsy cases examined were mostly of that class. The results are given in table I.

The cell volume in this series of apparently healthy individuals ranged from 36.0 to 49.8 with an average of 43.1 and the hæmoglobin between 13.4 to 18.1 grammes per 100 c.cm. of blood with an average of 15.3 grammes.

A summary of the results of the sedimentation rate, the cell volume and the hæmoglobin content of 74 patients suffering from epidemic dropsy is given in table II. At the time of examination the patients were suffering from active disease and the degree of œdema is taken as an index of the clinical condition.

The increase in sedimentation rate is most marked in patients during the acute stage of the disease and is less in patients with slight or no œdema. The decrease in hæmoglobin content is most marked during the acute stage of the disease. The cell volume is also decreased during the acute stage.

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drive off vapours whenever desired. **M** is provided with another mercuric chloride bubbler **N**.

After drying, the mass of solid plasma is removed aseptically and packed in convenient-sized tubes after grinding. Edwards *et al.* (*loc. cit.*) recommend that the dried plasma should be ground into a fine powder to facilitate solution in distilled water, or 5 per cent glucose solution. Our experience however is that it is best to grind it only to the stage when it will pass through a sieve, approximately no. 12. Contrary to the above authors we find that finely-powdered plasma takes much longer to dissolve than the coarsely-powdered one, on account of the difficulty of wetting with the former. Coarsely-powdered plasma dissolves rapidly and entirely. Often, however, there remains a very slight undissolved residue. This residue is probably denatured fibrin or some other protein and rarely amounts to more than 0.1 to 0.5 per cent of the total dry plasma. This minute residue can be filtered through a Seitz, Berkfeld or Chamberland type of filter.

All published work up to the present has been undertaken in a temperate climate and nothing as yet is known as to the effects of variable conditions found in the tropics on plasma and serum. We are undertaking investigations into these questions, and other biochemical and clinical aspects of dried plasma production, properties and therapy. Results will be published in due course.

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