A METHOD OF RAPID STAINING OF INTESTINAL FLAGELLATES

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IT is recognized that the staining of flagella of flagellate protozoa is often no easy task. Fresh fæcal samples containing flagellates can be subjected to treatment with Donaldson's eosin-iodine solution in order to demonstrate the the flagella, but such a preparation is not of a permanent nature. Wet fixed films of flagellates can be stained with Heidenhain's iron-alum hæmatoxylin, but in this method too, one often experiences great difficulty in bringing about the proper differentiation of the different organelles and at the same time maintaining a proper depth of staining for the flagella. This is particularly so in the case of very minute flagellates.

Shortt (1923) fixed wet films of flagellates by exposing the film to the vapour of 4 per cent osmic acid solution and subsequently stained the dry film with Giemsa after the manner of blood films. This method produces excellent results, and the flagellates, however minute, stand out very clearly. This method, however, involves the use of an expensive material, *viz*, osmic acid. Noller's method, as quoted by Stitt *et al.* (1938), involves the use of a fixative and, after washing with water and saline, the film has to be immersed in clear sterile serum (*e.g.*, that of the horse) for 5 to 10 minutes and then fixed again in absolute alcohol before staining according to the blood film technique. This method takes time and, in addition, it is difficult to ensure that sterile serum shall be always ready to hand.

This article describes a process of staining which does not involve the use of an expensive reagent, but which produces excellent results. In less than half an hour the preparation is ready for microscopical examination. By this method one is able to count quickly the number of flagella, to see the disposition of the different organelles, and to ascertain the systematic position of the organism. The photomicrographs (*see* figures 1 to 12, plate V) illustrating this article are taken from preparations stained by the method described below.

Technique

With a long fine-bore pipette, remove the material (fæcal or culture) containing the flagellates, place it on a very small drop of dried mammalian blood* on a slide, allow the material to mix with the blood, and then draw it back into the same pipette. The sucked-up fluid should have a blood red colour; if it has not, the material may be pipetted back to another droplet of dried blood, and the process should

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*A worker's own blood can be used with great advantage. Prick the finger-tip with a sharp needle. Then press the bleeding finger on a clean slide so as to have several impressions of the blood on the same slide. Cover the slide and allow it to dry. This dried blood can be stored in some dry dust-proof place and can be used for a week or ten days without any appreciable change in the requisite reaction.

PROTEIN HYDROLYSATES AS TRANSFUSION MATERIAL

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DUE to the increasing demand for large quantities of transfusion material for combating shock and hypoproteinæmia, and due to the high cost and other difficulties associated with the

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be repeated. Now take a clean grease-free slide, warm it gently over a flame, and, with gentle pressure on the teat of the pipette, squeeze out the contents in long streaks on the slide. Only a small amount of the fluid should come out of the pipette at one time, so that the material dries up immediately it comes in contact with slide. With a little practice, a steady flow can be maintained so as to give parallel edges to the streaks. To accelerate the process of drying, the slide may be kept exposed to a gentle breeze from a table fan while the operation is being carried out. In this case, warming of the slide is not necessary. Stain the slide with either Leishman or Giemsa.

A thin film of this material can also be made after the manner of a blood film and stained as above. In such a preparation, the flagellates are always found at the 'fish-tail' end of the film. The writer, however, is of the opinion that the fine streak method described above is more convenient for rapid examination than the thin film method. Moreover, in the thin film method, the flagellates are slightly distorted, since they are dragged in the process in making the film; while in the streak method the organisms are allowed to flow on to the side in a steady stream and are allowed to dry immediately. This precludes any chance of distortion. Flagella stained by this method, however, appear thicker than normal owing to the flattening that takes place during drying.

This method, though unsuitable for detailed cytological studies, is, nevertheless, a great aid to the proper understanding of the morphology of the organism. The flagella of the non-parasitic flagellates can also be stained by this method with great ease.

In conclusion I should like to place on record my indebtedness to my teacher, Rai Bahadur Dr. G. C. Chatterjee, who about twenty years ago initiated me into this method of staining intestinal flagellates, which I propose to designate 'Chatterjee's method'.

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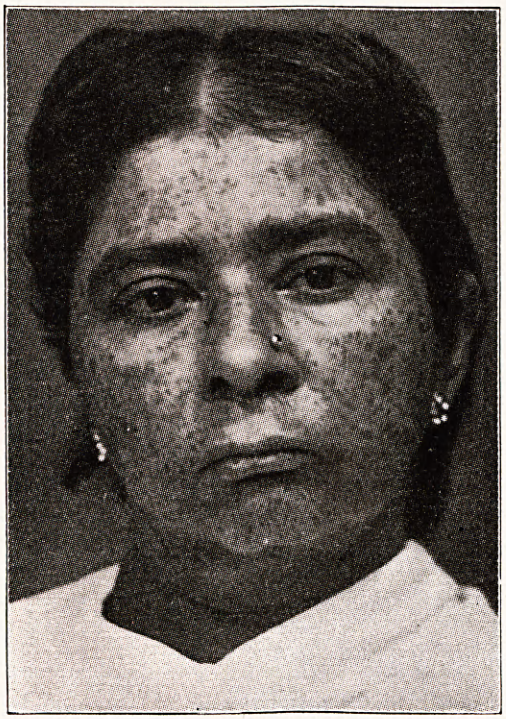

Case 18. Rash conspicuous on the face.

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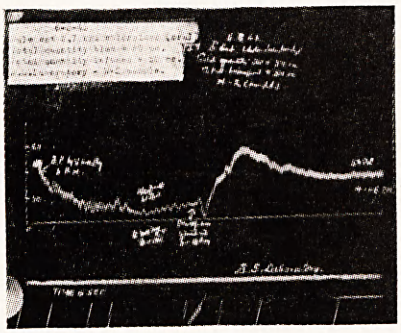
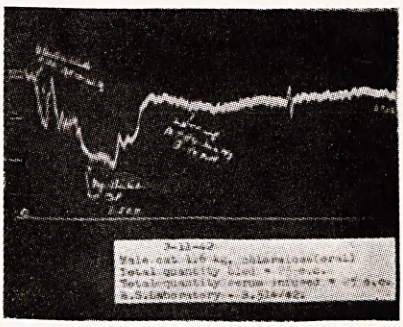
Fig. 1.



Kymograph picture of protein hydrolysate transfusion in cat.



Kymograph picture of serum transfusion in cat for comparison.

preparation of human plasma and serum for the purpose, various other cheaper and more easily prepared substitutes are being tried. One such is protein hydrolysates. Elman and Lischer (1943) have shown that 'in fatal surgical shock experimentally produced by repeated hæmorrhage, immediate replacement with a solution of hydrolysed protein containing amino-acids and polypeptides exerted a definite therapeutic effect as shown by prolongation of the survival time, and increase in the amount of hæmorrhage which could be sustained and a higher level of blood pressure as compared with the controls in which dextrose alone was used or in which there was no replacement'. When this publication appeared, we were already engaged in preparing protein hydrolysates for bacteriological purposes, we immediately intensified our efforts and attempted to make a product suitable for transfusion purposes. Success was achieved with a papain digest of meat. The present communication gives a brief account of the method of preparation and the results obtained in experimental animals and in man.

Method

The method recommended by Krishnan and Narayanan (1941) for hydrolysing proteins for bacterial nutrition was used with certain modifications. The papain digestion of meat was conducted at 50°C. for 24 hours and the filtrate made suitable for intravenous injection by removal of undigested proteins and meta-proteins (anaphylactogens) by repeated heat coagulation. To the standardized final filtrate, glucose and sodium chloride were added to yield a mixture containing 5 per cent protein hydrolysates, 5 per cent glucose and 0.85 per cent sodium chloride, and the mixture was finally sterilized in transfusion bottles.

Results

So far, 40 different batches have been prepared and tested on cats in a state of experimentally induced hæmorrhagic shock according to the technique of Krishnan, Mukherjee and Dutta (1944). In all of them the type of result obtained was exactly the same as shown in figure 1 (*see* plate VI), which is a photograph of the kymograph picture. It will be seen that, on transfusion with the protein hydrolysate mixture, there was no reaction (fall of blood pressure or respiratory distress), the rise in blood pressure was steady though not very high, and was well maintained for 3 hours—the period of the experiment. The report on the quality of this product by the Biochemical Standardization Laboratories (Dr. B. Mukherjee and Dr. N. K. Dutta, who very kindly conducted the cat experiments for us) is that it is safe for transfusion and that its value in the treatment of shock would probably come midway between that of serum and glucose saline. On receiving this report the product was administered to advanced inanition cases and the results were very satisfactory. So far, 106* cases have been treated, and the beneficial effects noted have led us to present the results in a separate communication (Krishnan, Narayanan and Sankaran, 1944). In this article the results obtained in the laboratory animals are alone discussed.

Discussion

The results recorded above show that hydrolysed proteins can safely be administered to cats (40 to 80 c.cm.) without any adverse reaction. The results however give this product a place lower than serum or plasma and higher than glucose saline in the treatment of shock. The reasons for this probably are two. Firstly, the amino-acids and peptides contained in the mixture take time to be converted into serum proteins. Elman and Weiner (1939) have pointed out that usually 3 to 6 hours elapse before an appreciable rise in the serum-protein level can be noted. The few tests conducted on the protein level of the sera of cats before and after transfusion in our experiments also confirm these findings. We are therefore led to presume that the immediate benefit from this transfusion is due chiefly to the glucose saline administered rather than to the protein hydrolysate present. The real benefit arising out of the increase in serum proteins which invariably follows the administration of protein hydrolysate takes time (a few hours) to appear. When this happens, the temporary benefit conferred by glucose saline in the product is continued and maintained for a longer period, due to the hydrolysates producing a rise in serum proteins. This fact may have to be considered in the selection of cases for this therapy. All cases of traumatic shock may not equally benefit by this treatment. While the less urgent cases may benefit by it as much as with serum, the more urgent cases may not show the same degree of improvement.

Secondly, the amino-acid mixture cannot be given too rapidly or in too large quantities. The experience with our product so far shows that administration at a rate over 4 c.cm. per minute may not be well tolerated, and that it may not be advisable to give more than a pint of our product at a time to man. Serum, on the other hand, has been administered in much larger quantities and in shorter periods in certain types of emergencies without any ill effects.

Conclusion

While our product has a definite value in the treatment of chronic hypoproteinæmic conditions such as starvation, it is not superior to serum or plasma in the treatment of traumatic shock. Unless it is further reinforced with suitable colloids to bring the blood pressure raising quality to the level of serum, it can occupy only a second place in the treatment of acute traumatic shock. Experiments are in progress to prepare a product which will not only be

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*The number of cases treated is now much greater.

PROTEIN HYDROLYSATES IN THE TREATMENT OF INANITION

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Introduction

WITH the prevalence of famine conditions in Bengal this year, starving destitutes from rural areas came to Calcutta in large numbers in search of food and work. Those that were unsuccessful in their quest made the open streets their home, and many of these in course of time reached the stage of advanced inanition. Arrangements had to be made to pick them up from the streets and send them to emergency hospitals for treatment and care. It was found that about 25 per cent of the cases removed to hospitals were in an almost moribund state. They were unable to take even liquids by mouth, and the death rate among them was very high. The immediate problem confronting the doctors in the emergency hospitals was to revive these collapsed cases by some suitable parenteral therapy and thereby prevent the high mortality. Intravenous injections of glucose saline were first tried and although improvement in the general condition was noted in most cases it was not sometimes sustained or uniform and the death rate was not markedly reduced. The administration of normal human serum was next tried in a few cases with some benefit. Yet the clinicians felt that there was urgent need for some better transfusion material, if the advanced starvation cases were to be saved from death. Being already engaged in the investigation of various transfusion materials used for combating shock including protein hydrolysates, it struck us that a mixture of protein hydrolysates, glucose and vitamins would, on theoretical grounds, be an ideal fluid for parenteral administration to cases of inanition. We had on hand a number of samples of hydrolysates prepared by us in our laboratory and one of these had been proved to be satisfactory for transfusion purposes by laboratory tests on animals (Narayanan and Krishnan, 1944). It was decided to try this product immediately on humans, and a case of inanition collapse was treated with it on 8th November, 1943. Since then many injections have been given and results were very encouraging. In this preliminary note we are presenting a brief account of this therapy.

Historical

Henrique and Andersen (1913) were the first to show that hydrolyzed proteins could be safely given intravenously to protein-starved animals, and that they supported life and growth, and maintained the animals in nitrogen equilibrium. Elman (1942) and Elman and Lischer (1943) found that solutions of amino-acids and peptides can be administered intravenously to man in fair amounts without any very serious reactions, and that patients suffering from cancer and ulceration of the intestinal tract and after surgical operations could be maintained in nitrogen equilibrium by their use. These findings stimulated us to prepare protein hydrolysates in different ways and in a form better suited for intravenous administration. This investigation was commenced in the early part of 1943.

Methods of preparation known.—Protein hydrolysates have been prepared by previous workers in one of two ways : (1) Acid hydrolysis and (2) Enzyme hydrolysis.

Acid hydrolysis is the easier method as it presents no great technical difficulties. But in this method at least one essential amino-acid (tryptophane) is known to be destroyed by the hot acid, and has to be separately prepared and added to the hydrolyzed mixture in order to make it efficacious. This extra step makes the product expensive. The delay and difficulty involved in obtaining pure tryptophane in sufficient quantities discouraged us from attempting this method of preparation in the present emergency. Acid hydrolysates have been used by Elman and others and their reports show that they are fairly satisfactory, though not absolutely safe in all cases.

Enzyme hydrolysis is also not a difficult method. It is quick and cheap. But in this method the hydrolysis is generally incomplete and the mixture may contain, in addition to

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cheap and easy to make but will also be as good as serum with regard to its osmotic properties. Should these experiments prove successful then the cost of treatment of traumatic shock with such a product will be only a fraction of that by whole blood, plasma or serum.

Acknowledgment

Our thanks are due to Dr. J. B. Grant, Director, All-India Institute of Hygiene and Public Health, for constant encouragement and help; to Dr. B. Mukherjee, Director, Biochemical Standardization Laboratories, for testing our product and certifying its non-toxicity.

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