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.

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amino-acids and simple peptides, higher polypeptides derived from the substrate as well as the enzyme. The latter have to be removed if they are found to be responsible for any allergic or other reactions. Elman (Mueller *et al.*, 1943) seems to have tried hydrolysis with trypsin. While his products gave fairly satisfactory results in a number of cases, in some they induced allergic and other reactions. We also found that the tryptic hydrolysates prepared by us invariably gave rise to toxic reactions in experimental animals. Although the degree of hydrolysis effected by trypsin is extensive, we felt that in view of the above finding it would be necessary to use other enzymes that would not give rise to toxic products. Success was achieved by the use of papain (*vide infra*).

Mixtures of the ten essential amino-acids separately prepared have also been used by some workers (Madden and others, 1943). While somewhat encouraging results have been obtained by some, others are of the opinion that pure amino-acid mixtures are not quite as good therapeutically as hydrolysate mixtures. The latter finding gains support from the view that in a patient with a negative nitrogen balance, a supply of essential amino-acids alone does not fully meet the need. Moreover, the preparation of pure amino-acid mixtures is time-consuming and expensive, and it may be a long time before they are made available in suitable amounts at a reasonable cost.

Choice of ferment and substrate

From a careful study of the methods of preparation described above, we came to the conclusion that a properly-prepared enzymic hydrolysate would be the cheapest and best product for parenteral therapy in this emergency. Pancreas trypsin which had mostly been used by previous workers, in our opinion, has two drawbacks. Firstly, the optimum temperature of its action being 37°C., it would be very difficult to avoid bacterial contamination and growth during digestion. This may be a possible explanation for the toxicity of some of the products. Secondly, despite all care in the collection and use of pancreas trypsin, there is always the risk of the presence of histamine or histamine-producing enzymes. In order to overcome these two drawbacks, a ferment capable of acting at higher temperatures and free from histamine and histamine-producing agents would be desirable. Although trypsin may act at higher temperatures, the degree of hydrolysis will be less extensive and the product will still be unsuitable for parenteral use due to the second drawback.

The vegetable proteolytic enzyme *papain*, which is readily available in large quantities at a low cost, answered the requirements, and was used. Its optimum temperature of action is 50°C. and its reaction products have been found to be free from histamine. While it is true that the hydrolysis produced by papain is not quite so extensive as that produced by trypsin, this does not appear to be a handicap from the immunological and therapeutic points of view. Having selected papain, the choice of the substrate necessarily fell on meat proteins, as its action on casein and vegetable proteins is more limited.

Our method of preparation

The protein hydrolysate that is at present being made in the Institute is a papain digest of meat (*vide* Narayanan and Krishnan, 1941). The digestion is conducted at 50°C. for 24 hours. The extent of digestion is then tested by determination of formol titre, total solids and total nitrogen. A fractional analysis of the product is also made. The values obtained are used as guides for standardization of the product. The undigested proteins and meta-proteins which are usually small in amount and which are likely to provoke allergic reactions are removed by repeated heat coagulation at pH7. During the process of preparation, bacterial contamination is avoided through the use of an aseptic technique and a preservative (toluol). The final product is mixed with glucose and sodium chloride so as to give a mixture containing 5 per cent protein hydrolysate, 5 per cent glucose and 0.85 per cent sodium chloride. The mixture is then sterilized and tested before issue, biologically on cats for toxicity, bacteriologically for sterility, and immunologically for allergic reaction.

Analysis of the mixture has incidentally revealed that it contains riboflavine, nicotinic acid and thiamin. The vitamins in the mixture are of additional value in increasing the therapeutic efficiency in promoting glucose utilization, particularly in vitamin-starved individuals such as the destitutes in Calcutta.

Rationale of therapy in inanition

Just as glucose is administered intravenously as a means of supplying predigested carbohydrates, so hydrolyzed proteins are injected for supplying the nitrogen needs of a patient. In cases of starvation, the nitrogen need is more urgent than in the normal subject. In the former, where body proteins are being rapidly broken down, it is absolutely essential to administer proteins to save life. Even if sufficient glucose is given to meet the calorie needs, protein break-down will not be completely stopped and continued life may not be rendered possible.

Protein food may be administered by mouth, but it will need to be digested and absorbed by the gastro-intestinal tract. In advanced cases of starvation, because of impairment of digestive function, this is often not possible; the only other alternative then is to administer hydrolyzed proteins parenterally. If this is done and if, along with it, glucose and vitamin-B complex are administered, the starving tissues will receive the more important requirements in a form ready for utilization. Both on theoretical grounds and

from the experience so far gained, it seems justifiable to state that administration of glucose protein hydrolysate in suitable amounts will not only prevent depletion of essential body and plasma proteins but will also help in the synthesis of these and in the restoration of the patients to normal condition.

Here two questions may be asked : (1) why not give these hydrolysates orally, and (2) why not give serum or plasma intravenously instead of the protein hydrolysate ? The answer to the first question is that protein hydrolysates can be given orally, but in patients with advanced inanition and collapse, absorption is slow and doubtful, and for quick effect parenteral administration is probably necessary. Also, after absorption from the intestine, the amino-acids first reach the liver and later only the tissues. If the liver is damaged, no synthesis will take place and also there will be delay in reaching the tissues which need them badly. Hence for the very advanced cases, intravenous injection is the method of choice. However, in children and in those cases where parenteral administration is difficult, oral or rectal administration of glucose protein hydrolysate may be resorted to with benefit. In the emergency children's hospital these methods have been found useful.

To the second question the answer is that tissue proteins and serum proteins are not directly interchangeable. The latter have to be autolyzed, and the amino-acids made available for synthesis. In advanced cases of starvation where there is dehydration, the need for tissue proteins is more urgent than the need for serum proteins which may be apparently normal due to the hæmoconcentration. Thus if serum is given, its utilization will depend upon the proper functioning of the enzyme systems in the liver and tissues responsible for the splitting up of the proteins and for reconverting them into tissue proteins. There is some evidence to suggest that the enzyme systems in inanition cases are not normal, and that the benefit with serum may not be attained to the same extent as with hydrolysates. This theoretical explanation is supported to some extent by actual experience with serum in starvation cases.

We are inclined to believe that administration of glucose protein hydrolysate intravenously to cases of inanition is superior to the administration of serum. The marked and rapid improvement noted in most cases denotes that the amino-acids and peptides in the mixture reach all tissues simultaneously and are being utilized by them very rapidly. The glucose present in the mixture acts by reducing liver damage and increasing its functional efficiency. It saves protein break-down and indirectly helps in the synthesis of tissue protein from the amino-acids supplied. The vitamins present help in the metabolism of glucose. These facts are the basis on which we recommend glucose protein hydrolysate treatment of advanced starvation cases.

Glucose protein hydrolysate.—The directions for the use of this glucose protein hydrolysate have already been published in this journal*.

Results of treatment.—Up to date something like 3,000 intravenous injections have been given in cases numbering about 1,000. Owing to the fact that the work has been scattered in several different hospitals and that patients have been treated at varying stages after admission into hospitals and in varying degrees of inanition, it is very difficult to give figures for death rates and recovery rates, etc., for patients treated by this treatment and by other methods of treatment. The general experience of clinicians is that in intravenous glucose protein hydrolysate we have a very useful method of treatment, but to assess accurately its value and its limitations more work with adequate controls is needed. The experience so far gained, however, would point to the following conclusions :—

(1) The intravenous injections of glucose with protein hydrolysates are well tolerated. In very few cases has any reaction been seen which could be attributed to the injection, and even in these cases other patients tolerated injections of the same batch without reaction. Moreover, the very few reactions seen have all been mild. There seems, therefore, to be no difficulty in applying this form of treatment widely. In some cases a rise of temperature soon after injection has been due not to the injection but to malaria. The only definite contra-indication to the use of this form of treatment appears to be the presence of nephritis. In such patients bad results have been seen. In patients with pneumonia, dysentery and other infections, the intravenous injections are not contra-indicated but should be accompanied by specific treatment for the infection.

The immediate reaction to the injection is usually good, the pulse and the patient's general condition improving and, in favourable cases, the patient has later been able to take food by mouth, but in severe cases this may be only after several intravenous injections. There are, however, some cases which in spite of repeated injections show only temporary improvement. The improvement is not maintained and the patient dies. Post-mortem examinations have revealed that in most of these patients there has been a serious complication such as pneumonia, malaria, severe anæmia, nephritis and, most common, dysentery with extensive bowel ulcerations. It is believed that with the use of this form of treatment a number of deaths in hospitals caused by inanition alone can be considerably reduced.

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THE OCCURRENCE OF MITES (ACARINA) IN HUMAN SPUTUM AND THEIR POSSIBLE SIGNIFICANCE

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IN this note the results of some preliminary observations on the presence of mites in human sputum are recorded. At the present stage of the investigations, the presence of mites of various species has been detected in the sputum of 17 out of 28 persons examined. Twenty-four of these persons were under observation or receiving treatment for respiratory disorders in a local hospital; they were of several different nationalities and none had resided in Ceylon for more than a few months. The remaining four persons (three Europeans and one Ceylonese) had lived in Ceylon for periods of from three to over twenty years; all of them gave histories of long-standing coughs and asthma.

Precautions against contamination of sputum samples

The presence of mites was first observed during the examination of a sample of sputum, from one of the hospital cases, for tubercle bacilli. This led to the examination of further samples of sputum from the same case, and from other cases suffering from chest complaints. The results were remarkable, and mites were found with such frequency that contamination from outside sources was seriously suspected. This suspicion was increased when it was found :

(*a*) that the mites which were being recovered from the sputum were not related to those species which are known to be true parasites in the lungs and air passages of monkeys and certain other mammals, but were species which are commonly present in stored products and debris in houses, shops, etc.;

(*b*) that mites of various kinds were present in dust and brushings obtained from the hospital and laboratory premises;

(*c*) that at least one of the mites present in the sputum samples was air-borne in the wards.

At an early stage in the investigations, therefore, precautions against contamination were taken whenever practicable, and results from samples of sputum to which the least doubt attached regarding the efficiency of such precautions were registered separately. In all cases in which the precautionary measures were considered to have been satisfactory, the samples of sputum were discharged direct into large test-tubes or screw-capped bottles which had been previously prepared for the purpose. These test-tubes and bottles had been thoroughly washed and cleaned and subsequently flushed for 5 minutes in running water. The tight-fitting rubber stoppers of the test-tubes and the caps of the bottles were subjected to the same treatment; they were removed only at the time of expectoration during which they were held in the hand, and after which they were immediately replaced.

Controls, using distilled water without precautionary measures, were carried out at times when specimens of sputum were being collected; and vessels containing distilled water were exposed overnight in the vicinity of mite-infected patients, and in other parts of the wards and in the laboratory. No mites were observed in any of these experiments.

The possibility of the mites being derived from the mouth or nasal passages of the patients was also investigated, and several samples of saliva and nasal washings were examined. So far, however, no mites have been recovered from these samples.

As previously stated, the results obtained from the samples of sputum taken with precautions of doubtful efficiency were registered separately. An analysis of the examinations made gave the following data :—

Efficiency of precautionary measures doubtful	.. ..	63 samples
Number with mites	.. ..	25 (39.7 per cent)
Efficiency of precautionary measures not in doubt	.. ..	44 samples
Number with mites	.. ..	21 (47.7 per cent)

Mites occurred, therefore, in both types of samples and were actually more prevalent in samples collected under precautions which rendered chances of contamination from outside

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