PARENTERAL REPLACEMENT OF PROTEIN WITH THE AMINO-ACIDS OF HYDROLYZED CASEIN *

ROBERT ELMAN, M.D.

ST. LOUIS, MO.

FROM THE DEPARTMENT OF SURGERY, WASHINGTON UNIVERSITY, AND THE BARNES AND HOMER PHILLIPS HOSPITALS, SAINT LOUIS, MO.

PARENTERAL REPLACEMENT of protein has become recognized as an important therapeutic need only during the past decade. This has been due largely to an increasing realization of the frequency and seriousness of hypoproteinemia in a variety of patients and in the apparent inability of the body to always correct this deficiency spontaneously. The problem is made more serious by the fact that transfusions frequently fail to correct the deficiency and for many other reasons; there is, in addition, a growing appreciation that protein needs other than those for the synthesis of serum protein may be important in many patients nutritionally depleted and unable to take and absorb nourishment adequately by mouth.

The possibility of supplying protein needs parenterally with amino-acids has been suggested from time to time, particularly by Rose,[1] in 1934, and was achieved experimentally as early as 1913 by Henrique and Andersen.[2] In the human, amino-acids were injected intravenously for purposes of protein alimentation for the first time in this clinic;[3] the amino-acid preparation used at that time was a mixture obtained by acid hydrolysis of casein, fortified with tryptophane which is destroyed during digestion by acids. Evidence of utilization, experimentally and clinically, as well as therapeutic effects in patients, was observed.[3] The necessity of adding tryptophane was, however, a disadvantage because of its cost.

The present observations are concerned with the injection of an enzymatic hydrolysate of casein,† containing *all* animo-acids present in casein, including tryptophane, and capable of maintaining nitrogen balance and promoting normal growth in rats.[4] This preparation has the power of provoking serum albumin restoration in experimentally produced acute hypoproteinemia.[5] Clinical observations in children have already been reported with this enzymatic product by Shohl, Butler, Blackfan and MacLachlan,[6] and by Farr and MacFayden.[7] Both of these groups of observers presented data indicating that the injected material was utilized. Although the former workers noted severe reactions (chills and fever) following the injection in several babies, the latter have injected large amounts without such reactions.[8] Undoubtedly, this difference was due to differences in the manner of preparation of the solutions for intravenous use.

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† This material has been generously supplied by Mead Johnson and Company, Evansville, Indiana.

The enzymatic hydrolysate, though a complete mixture of amino-acids, is, I am told, not as easy to prepare as the acid hydrolysate because of numerous technical difficulties. Unlike the latter, the former product is not completely hydrolyzed, only two-thirds being in the form of amino-acids, the remaining third being in a more complex form, presumably dipeptides. It is, however, not anaphylactogenic for guinea-pigs; moreover, the incompleteness of the hydrolysis is apparently of no biologic significance. This enzymatic preparation of casein (called 92–Z) was used in all of the observations reported herein. It should be mentioned, however, that successive batches of this product have been used, each of which has represented improvements in the method of manufacture as shown by easier solubility and less tendency toward reaction when injected intravenously, even when given at a faster rate than earlier products.

Method of Administration.—As received, the product is a dry, impalpable powder which is made up as a 10 per cent solution and heated to 90° C. For complete sterilization the solution is passed through a Seitz (EK) filter and 100 cc. amounts poured into flasks containing 400 cc. of sterile 10 per cent glucose; the mixture is then injected intravenously during one hour. Thus, in eight hours of continuous venoclysis (or two four-hour periods) 4,000 cc. can be injected—containing 1,600 calories and 80 Gm. of protein as amino-acids. Adequate electrolyte is also added, ordinarily as concentrated Ringer's solution—equivalent to 10 Gm. of salt per day. The nitrogen concentration of the 10 per cent solution is 1.2 Gm. per cent—so that when 4,000 cc. are administered the patient receives 9.6 Gm. of nitrogen. This is equivalent to the nitrogen in 3,000 Gm. (nearly ¾ lb.) of lean beef.

For injection the ordinary infusion flask has been used, the rate of flow being measured by the drops falling through an interpolated sealed glass trap. All connections were of glass and only stainless steel needles were used.

Difficulties Encountered.—Most of the earlier difficulties have disappeared with successive improvements in the manufacture of the hydrolyzed casein. The solubility has increased, so that precipitation of the solutions is no longer encountered. Obviously, a perfectly clear solution must be maintained before the material can be administered intravenously. Another difficulty has been the phlebitis induced when long periods of venoclysis were required. It has been my impression that the more recent preparations of hydrolyzed casein have shown less tendency toward thrombosis. This tendency, however, is not very great with the dilute (2 per cent) solutions.

The most serious difficulty has proved to be the occurrence of occasional reactions, similar to those reported by Shohl, *et al.*[6] Many patients had none even after two weeks of daily injections. In a few cases the reactions were undoubtedly due to the rapidity of injection. Thus, in Case 4, on March 24, 1939, 2,250 cc. of a 10 per cent glucose solution, containing 2 per cent of product 92–Z, was administered in five hours without reaction; later in the day, 1,500 cc. of the same solution was administered in two hours, and was followed by a chill and a temperature rise of 3° C. Whatever the cause of

reactions, there has been a complete absence, thus far, with the more recently received material. Indeed, the latest product (No. 143, which has already been brought into solution by the manufacturer) has been injected into three patients at a speed twice as fast as that already mentioned. Thus, 1,000 cc., containing 10 per cent glucose, 2½ per cent hydrolyzed casein, and 0.45 per cent NaCl, was given in one hour without reaction. In spite of the rapidity of the injection less than 0.1 Gm. of amino-acids of the 25 Gm. given appeared in the urine during the ensuing three hours. I believe that untoward reactions are due to one or more factors connected either with the manufacture of the hydrolyzed casein, with its method of solution and preparation for intravenous use, or with the technic of administration itself. These factors appear to be almost if not completely solved with the most recent product; only further experience will tell.

It must, of course, be admitted that uniformity in the composition of the amino-acid mixture is highly desirable if not essential. Whether such a uniformity can be or actually has been achieved is still incompletely answered and awaits further experience. Nevertheless, the preparation now available for experimental use has been administered to 35 patients, and data of much interest and great promise have been accumulated.

Clinical and Metabolic Observations.—The mixture of glucose, amino-acids and electrolyte described above was administered to 35 adults as their *sole* source of fluid and food; the period of treatment varied from one to 23 days and averaged over ten days. The total daily dose varied somewhat, but in most cases a maximum amount of 80 Gm. of 92–Z was given; this averaged between 1 and 2 Gm. per Kg. per day, dependent upon the body weight of the patient. As already mentioned, 80 Gm. of the hydrolyzed casein contains 9.6 Gm. of nitrogen. In 20 patients, nitrogen balance studies were carried out; complete collections of urine, feces and vomitus were analyzed for total nitrogen by the Kjeldahl method. Serum proteins were also determined in this way. The serum was fractionated by Howe's method. Of the following eight representative cases, the first two are normal controls, the next two preoperative and the last four postoperative patients.

REPORTS OF EIGHT REPRESENTATIVE CASES

Case 1.—Hosp. No. D–7721: P. W., Negro, male, age 55, was admitted, November 1, 1939, to the Homer Phillips Hospital. He was an asthmatic, and had been a patient many times previously with the complaint of respiratory distress. This time he complained more especially of epigastric pain and vomiting and had lost 20 pounds in weight. Examinations including cholecystogram and gastro-intestinal roentgenologic series were negative. All laboratory tests were negative; the serum protein was 7.01 Gm. per cent, albumin 4.2 Gm. per cent. Intravenous alimentation, with complete gastro-intestinal rest, was suggested and he accepted this regimen. During 15 days he took nothing but a little cracked ice by mouth, and was given each day 3,000 cc. of 10 per cent glucose containing the equivalent of 1,000 cc. of Ringer's solution, *i.e.*, two ampules of concentrated Ringer's (each sufficient for 500 cc.). After three days, a solution containing 60 Gm. of amino-acids (92–Z) was added to the glucose (Fig. 1). Only one reaction occurred during the intravenous therapy on the fifteenth day, when the patient had a slight chill but exhibited

no elevation of temperature. Subjectively the procedure was well tolerated; the patient's epigastric pain disappeared completely and when he was put on a regular diet it did not recur. He left the hospital asymptomatic and returned three months later with a request for a repetition of the experience.

Case 2.—Hosp. No. D–5541: Z. C., Negro, male, age 65, was admitted, November 5, 1939, to Homer Phillips Hospital. He was a frequent patient in the hospital, the diagnosis each time being bronchial asthma and hypertension; the electrocardiogram showed advanced myocardial damage. He had lost some weight and complained of difficulty in swallowing although all examinations including a cholecystogram and gastro-intestinal roentgenologic series were normal. Laboratory tests were all normal, the serum protein was 7.2 Gm. per cent, albumin 3.3 Gm. per cent. A period of complete gastro-intestinal

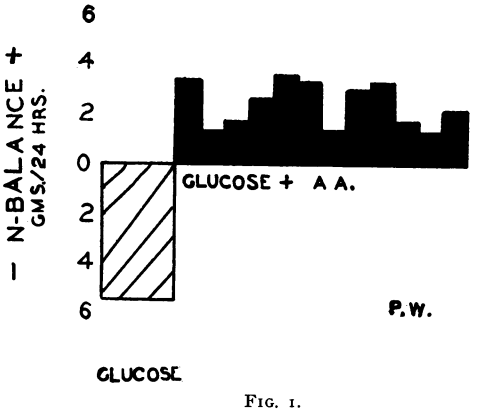

FIG. 1.

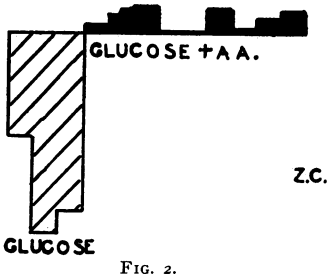

FIG. 2.

FIG. 1.—Case 1, and FIG. 2.—Case 2: In both cases represented above, the gastro-intestinal tract was normal (see Case Reports). Note, in both, the immediate achievement of positive nitrogen balance when amino-acids were added to the intravenous glucose. The cross-hatched columns represent the nitrogen output during three days, during which 3,000 cc. of 10 per cent glucose was injected daily. The solid black represents the period during which 60 Gm. of hydrolyzed casein (7.2 Gm. N.) was added daily. The metabolic study in Case 1 lasted 15 days, in Case 2, 12 days.

rest was suggested to him, which he accepted. For 12 days he was given only cracked ice by mouth and 3,000 cc. of 10 per cent glucose plus Ringer's solution for three days, and then amino-acids were added (Fig. 2) as described in Case 1. He had no reactions whatever during the experiment and complained of no hunger. When he was returned to a regular diet he was able to eat better and left the hospital much improved.

Case 3.—Hosp. No. 73309: G. A. H., white, male, age 48, was admitted, March 10, 1939, to Barnes Hospital. He had had several abdominal operations elsewhere for appendicitis, intestinal obstruction and ventral hernia, and presented a small intestinal fistula of four months' duration which developed following the last celiotomy. He had lost 40 pounds in weight during his illness. The wound was treated conservatively for several weeks when it was finally decided to close it surgically. This was done, April 5, 1939; it required resection of much diseased small intestine on either side of the fistula, but was successful. Preparatory to operation he was put on "nothing by mouth" and given intravenous injections of glucose, Ringer's solution and amino-acids, the amounts as indicated in the legend under Figure 3; vitamins C_1 and B_1 were also injected. It is interesting to note that during the period of gastro-intestinal rest the flow of intestinal contents from the fistula was active, the total nitrogen from this source varying between 0.2 and 0.9 Gm. per 24 hours. This patient had two reactions during the injection of the amino-acids but in each case it was associated with a rapid rate of flow and was transient. The serum protein was 6.5 Gm. per cent, albumin 4.2 Gm. per cent. Following his discharge from the hospital the patient regained his loss of weight and has remained well.

Case 4.—Hosp. No. 81491: J. B., white, male, age 37, was admitted, October 20, 1939, to Barnes Hospital. He had become ill three months before with severe diarrhea and cramps which resulted in a 51 pound weight loss. On admission, he was emaciated and miserable; in spite of various oral regimens he continued to have three to ten stools a day and much abdominal pain. His serum protein remained low, 5.1 Gm. per cent, albumin 3.0 Gm. per cent. A diagnosis of regional ileitis was made, largely with the aid of a gastro-intestinal roentgenologic series, and operation was advised. This was carried out several months later, after he had gained 30 pounds in weight and had a normal serum protein. The lesion at operation proved to be characteristic of regional ileitis. On November 15, 1939, a period of intravenous alimentation was started; nothing was taken by mouth but cracked ice. The metabolic findings are recorded in Figure 4. The clinical response was pronounced; his pain disappeared, stools ceased, abdominal distention diminished, and general well-being improved remarkably. On the twelfth day of the experiment he was started on glucose and amino-acids by mouth, without any return of symptoms, and later was gradually put on a regular diet; upon this regimen he gained 30 pounds before being operated upon. It is of interest to note the increase in serum protein in this patient from 5.1 Gm. per cent, on November 15, 1939, to 6.7 Gm. per cent, three weeks later; the increase was all in the albumin fraction. No reactions whatever occurred during the intravenous therapy in this patient. His sense of hunger was definitely satisfied during the days in which he was receiving the amino-acids in contrast to its presence while receiving glucose alone. The metabolic studies in this patient were carried out with the cooperation of Dr. Cyril MacBryde to whom I am indebted for the data obtained.

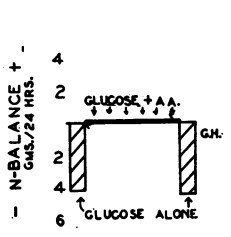

FIG. 3.

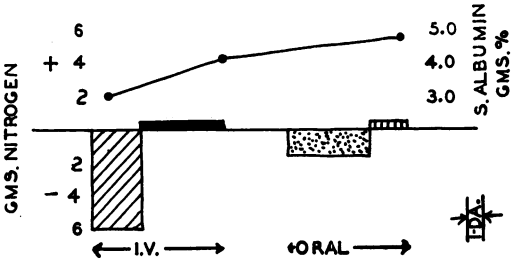

FIG. 4.

FIG. 3.—Case 3, and FIG. 4.—Case 4: These charts represent two preoperative cases (see Case Reports). Note, in both, the achievement of positive nitrogen balance during the periods in which hydrolyzed casein was added to the intravenous glucose (represented in solid black). In Case 3, on the first and sixth days (represented by the cross-hatched columns), 3,000 cc. of 10 per cent glucose was injected. On the second to fifth days, 50 Gm. of amino-acids (6.0 Gm. of N.) was added daily. In Case 4, on the first three days (represented by the cross-hatched columns), 3,000 cc. of 10 per cent glucose was injected daily; during the following five days, 60 Gm. (7.2 Gm. N.) of amino-acids was added. (The data on the ninth, tenth and eleventh days are omitted because of difficulties with the intravenous flow and loss of specimens.) Note the negative nitrogen balance during the next five days (represented by the dotted column) when the same amount of glucose and amino-acids was taken orally; this suggests the superiority of intravenous over oral administration. During the final two days, the dose of amino-acids was increased to 80 Gm. (9.6 Gm. N.), whereupon nitrogen balance was achieved. Note also in this case the increase in serum albumin from 3.0 to 4.6 Gm. per cent. The serum globulin remained unchanged at 2.1 Gm. per cent; as did the red cell count.

Case 5.—Hosp. No. D-10914: B. P., Negro, male, age 46, was admitted, February 8, 1940, to the Homer Phillips Hospital. He was operated upon soon after admission for a perforated peptic ulcer, which was found on the posterior surface of the pylorus, and was closed. The lesion had been present for over 24 hours, and a definite peritonitis was present. Intravenous glucose and Ringer's solution were given for five days; during this time his clinical course was stormy and, as can be seen in Figure 5, a large amount of nitrogen appeared in the urine. On the sixth day, amino-acids were added to the glucose and continued for 11 days. The sudden improvement in the general condition of the patient was striking and coincided with the addition of the amino-acids. Of interest, too, was the increase in the serum albumin and globulin, the

total serum protein changing from 6.11 Gm. per cent on February 10 to 7.4 Gm. per cent on February 27. That this change was not due to a concentration of the blood (decrease in plasma volume) was shown by the fact that the red cell count remained unchanged.

Case 6.—Hosp. No. D–12080: S. W., Negro, female, age 29, was admitted, March 14, 1940, to the Homer Phillips Hospital, and operated upon soon afterward for a strangulated umbilical hernia. The involved small intestine was gangrenous and a resection was carried out. During the first four postoperative days she received glucose and Ringer's solution alone, to which was added, during the next four days, 80 Gm. of hydrolyzed casein. Thereafter she was started on fluids by mouth and finally a regular diet. The patient weighed but 50 Kg., so that the amount of amino-acids she received was almost 2 Gm. per Kg. The clinical course was stormy for the first few days but improved remarkably with the onset of amino-acid injections. This was also evident, objectively, by a diminution of distention and the passage of gas and stool. It is important to note here, as in Case 5, the large urinary output of nitrogen in the postoperative period in spite of the administration of a large amount (1,600 calories) of glucose (Fig. 6). Laboratory tests were otherwise not notable; the serum protein was normal.

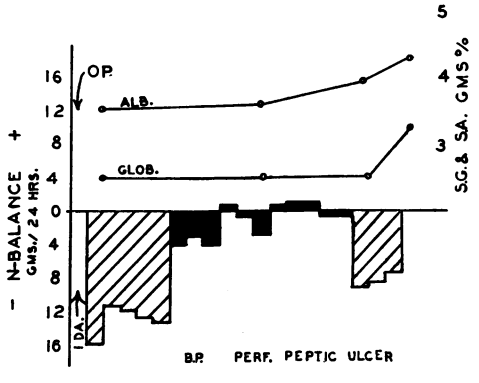

FIG. 5.

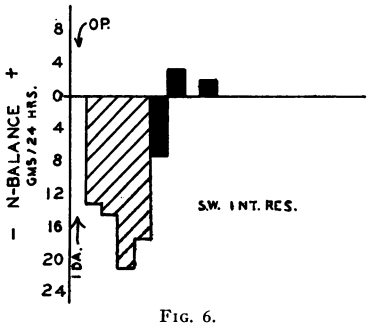

FIG. 6.

FIG. 5.—Case 5, and FIG. 6.—Case 6: These charts represent two postoperative cases (see Case Reports). Note the large output of nitrogen (12 to 20 Gm. per day) in both, in spite of the daily injection of 4,000 cc. of 10 per cent glucose (represented by the cross-hatched columns). The columns in solid black represent the addition of 80 Gm. (9.6 Gm. N.) of 92–Z to the glucose; note that nitrogen balance was achieved on several but not on all of these days. Note, too, the increase in serum albumin and globulin in Case 5.

Case 7.—Hosp. No. D–10954: J. H., Negro, male, age 73, was admitted, February 9, 1940, to the Homer Phillips Hospital. He was operated upon soon afterward and a large volvulus of the sigmoid was untwisted, replaced in the abdomen and the wound closed. Recovery was uneventful, particularly after the third day, coincident with the addition of hydrolyzed casein, of which he received 80 Gm. per day for 13 days together with 4,000 cc. of 10 per cent glucose and Ringer's solution. The period of intravenous therapy was especially long because of persistent distention which finally subsided with the passage of normal stools. Note the large output of urinary nitrogen in this case (Fig. 7). The laboratory data were not unusual. The serum protein showed no striking change varying between a low of 5.85 Gm. per cent to 6.85 Gm. per cent. On the last day of his intravenous regimen it was 6.66 Gm. per cent, albumin 3.96 Gm. per cent.

Case 8.—Hosp. No. D–10336: A. T., Negro, male, age 47, was admitted, January 22, 1940, to Homer Phillips Hospital. He had had a right lower quadrant mass of two weeks' duration; this was diagnosed as an appendiceal abscess and conservative therapy instituted. Because of increasing symptoms of extension he was operated upon five days later and a large spreading appendiceal abscess opened and drained. For the first four

postoperative days his course was quite stormy and repeated injections of caffeine were administered. Severe abdominal distention developed, the pulse was rapid, respirations labored. On the fifth postoperative day, amino-acids were added to the intravenous glucose, which resulted in a startling improvement, which continued thereafter. There was but one reaction during the course of the amino-acid therapy, consisting of a severe chill, unaccompanied, however, by any rise in temperature. The laboratory data were not significant. The serum protein four days after operation was 5.7 Gm. per cent, albumin 3.6 Gm. per cent; eight days later it rose to 6.7 Gm. per cent; albumin 3.8 Gm. per cent. There was no change in the red cell count. The metabolic data are represented in Figure 8, and show the high urinary output of nitrogen indicative of extensive "toxic destruction of protein."

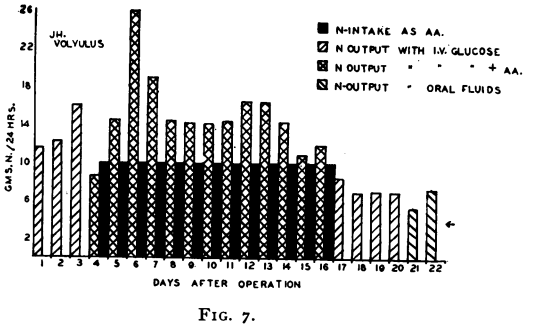

FIG. 7.

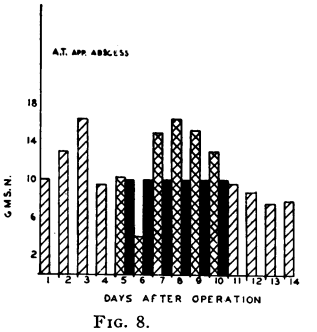

FIG. 8.

FIG. 7.—Case 7, and FIG. 8.—Case 8: The above charts represent two postoperative cases (see Case Reports). Note in both the large output of nitrogen in the urine (10 to 26 Gm. per day). The arrow in Figure 7 indicates what is about the normal output (5 Gm.). In both cases 4,000 cc. of 10 per cent glucose was administered each day; the amount of hydrolyzed casein was 80 Gm. (9.6 Gm. N.). Note that nitrogen balance was only occasionally achieved at this level of intake. (The low output in Case 8, on the sixth day, was due to loss of a specimen.)

There was no difficulty in achieving nitrogen balance in patients with low or moderate nitrogen excretion even when but 6.0 Gm. of nitrogen were injected per day (Figs. 1, 2, 3 and 4). As observed previously,[3] most fasting patients excrete about 4 to 5 Gm. of nitrogen a day when sufficient glucose is injected to meet caloric needs. Many of the cases described herein, however, showed a large output of urinary nitrogen, particularly following operations, amounting to as much as 26 Gm. per day; in these a positive nitrogen balance was, therefore, not achieved with regularity at the maximum intake of 9.6 Gm. of nitrogen per day (Figs. 5, 6, 7 and 8). Doubtless, with larger injections this could have been achieved. Nevertheless, favorable clinical effects were observed in several of these postoperative patients as soon as amino-acids were added to the intravenous glucose. This was particularly true in Cases 5, 6 and 8, which exhibited the largest output of nitrogen (see Case Reports).

In Cases 4 and 5, and less so in Case 8, definite evidence of serum protein regeneration was observed during the period of treatment. These observations are not decisive because the hypoproteinemia was not particularly severe. Indeed, few of the patients in the present series exhibited this defect to a significant degree.

COMMENT.—From the findings reported herein, the inference seems justified that a mixture of amino-acids prepared by the enzymatic hydrolysis

of casein, when injected intravenously, is retained and, presumably, utilized by the body, sometimes even in a demonstrable increase in serum protein. Although few of the patients studied showed significant hypoproteinemia, of those that did two, possibly three, exhibited definite increases in serum protein and albumin while under treatment (Cases 4, 5 and 8). In other patients the therapy may have prevented a fall in serum protein; such a fall is a not infrequent experience in severely sick patients after operation. No such fall occurred in any of the patients receiving amino-acids. Although the present observations permit no dogmatic inferences as to the effect of intravenous amino-acids on serum protein regeneration, the achievement of positive nitrogen balance as well as the clinical improvement noted in many instances points to the beneficial therapeutic results of nitrogenous nourishment administered intravenously in this way. On the other side of the ledger are the difficulties encountered, the most serious being the occasional reactions observed. These reactions, I believe, are not an inevitable part of the procedure but are due to factors which may even now have been overcome by newer methods of preparation and administration of the enzymatic hydrolysate. With the achievement of a constantly uniform product, the use of amino-acids as a method of parenteral replacement of protein will undoubtedly have widespread application. While this use may not solve the fundamental problem of serum protein regeneration, I believe that, with all other protein needs met with amino-acids, a relatively small amount of blood, when necessary, will permit restoration of serum protein which can then be maintained for a sufficient length of time to permit normal relations to become established. The purpose of most parenteral alimentation, in surgery at least, is to clear temporary hurdles; by bringing patients into a more normal preoperative nutritional state, by breaking into a vicious circle induced by nutritional edema, and by permitting temporary gastro-intestinal rest, intravenous alimentation finds its most clear-cut indications. In this field the addition of nitrogenous nourishment should fill a long felt want.

An incidental finding in the present study concerns the tremendous output of urinary nitrogen in many patients during the postoperative period (Figs. 5, 6, 7 and 8). It is of considerable interest and importance to note the magnitude of this loss; thus in Case 7, on the fourth day, 26 Gm. of nitrogen were excreted, which is equivalent to the loss of almost two pounds of muscle tissue a day. These losses were due to the disease itself and occurred in spite of the fact that each day 1,600 calories, as glucose, were injected, thus sparing protein as far as their use for caloric needs are concerned. Evidence of such "toxic destruction of protein" has been observed in a severe burn by Lucido,[9] in postoperative patients, by Touw,[10] and in patients following extensive trauma, by Cuthbertson.[11] Its significance in the production of symptoms is suggested by the beneficial effects observed in several patients after much of the nitrogen loss was met by the addition of amino-acids to the intravenous glucose. These findings would seem to add a definite indication for such treatment in the postoperative care of very sick patients.

SUMMARY

(1) In 35 adults a solution containing glucose, amino-acids and electrolyte was injected intravenously as the *sole* source of alimentation, with the particular purpose of parenteral protein replacement. The amino-acids consisted of a mixture obtained by the enzymatic hydrolysis of casein. The maximum amount of nitrogen administered was 9.6 Gm. per day, the calories, 1,600.

(2) Evidence of utilization was shown by: (a) The achievement of nitrogen balance; (b) increases in serum protein concentration; and (c) clinical improvement, particularly after serious operations.

(3) Large amounts of urinary nitrogen were excreted by many patients after serious operations, indicative of "toxic destruction of protein." The clinical improvement during treatment seemed to be associated with the partial or complete replacement of this loss of nitrogen by the intravenously administered amino-acids.

(4) Certain difficulties in the intravenous injection of the amino-acid mixture are described and discussed. These are being rapidly solved by newer methods of preparation of the amino-acid mixture and of the solutions made therewith.*

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* At the present time a 10% glucose 2½% amino-acid solution is prepared in the cold, immediately passed through a large capacity Berkfeld filter to remove pyrogens and autoclaved at once in 1000 cc. flasks for 30 minutes at 5 lbs. pressure. Thus far no febrile reactions have been observed with solutions prepared according to this much more simple method.