EFFECTS OF SIMULTANEOUS OR PRIOR INFUSION OF SUGARS ON THE FATE OF INFUSED PROTEIN HYDROLYSATES^{1, 2}

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Glucose usually is included in amino acid solutions for intravenous infusion. This has seemed desirable because the amino acids can spare body protein only when sufficient calories are provided. Although the calories so gained (usually 200 for a liter of hydrolysate) are far from enough for this purpose, they do add to the total and besides they might serve particularly efficiently being presented at the same time as the amino acids.

On the other hand Elman and his co-workers (1) have reported improved retention of the nitrogen of a partial hydrolysate when fructose replaced the usual glucose. In explanation these writers suggest that the cellular uptake of the glucose is relatively too late to spare the amino acids optimally, whereas the fructose is metabolized more quickly.

The present study was undertaken to compare the effects of glucose and fructose on the fate of the amino acids and peptides of infused hydrolysates. We have found that the presence of either sugar in certain commercial protein hydrolysates interferes with the tissue utilization of the peptides present; the effect increases in the order 10 per cent fructose, 5 per cent glucose, 10 per cent glucose. It is completely avoided if the sugars are added to the hydrolysate sterilely just before infusion or if the sugars are infused in advance of the hydrolysate. Therefore, we conclude that substances which interfere with peptide utilization are formed during autoclaving by interaction between the sugars and constituents of the hydrolysate.

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² A preliminary report of this work has been made to The Fundamental Forum, American College of Surgeons, Atlantic City, November, 1954.

Interference has been observed also with the utilization of the *free* amino acids especially at a 10 per cent glucose level. This interference was not due to autoclaving and therefore appeared to be due to the sugar itself acting upon the renal reabsorption of the amino acids.

If the advantage of fructose arises from its more rapid utilization, this advantage should also be attainable with either sugar by starting its infusion before the hydrolysate. In the course of seeking ways of avoiding the above interference, we have observed that the prior infusion of either sugar substantially diminishes the immediate augmentation of nitrogen excretion which otherwise occurs. Elman has reported, with unfavorable results, a single test of the reverse order of presentation, *i.e.*, amino acids in the forenoon and glucose in the afternoon (2).

EXPERIMENTAL

Commercially available hydrolysates were studied. The partial hydrolysate of fibrin (Aminosol®³) was supplied in four forms: a sugar-free preparation, one containing 5 per cent glucose, one containing 10 per cent glucose, and one containing 10 per cent fructose. The enzymatic partial hydrolysate of casein + pig pancreas (Amigen®³) was used as a sugar-free preparation, another with 5 per cent glucose, and a third with 10 per cent fructose. A partial hydrolysate of bovine plasma proteins (Travamin®³) was used as a sugar-free solution and one containing 5 per cent glucose. All preparations were indicated to be of 5 per cent strength, and analyzed by us for free and bound amino acids (Table I).

The sugar-free fibrin hydrolysate was supplemented in

³ The fibrin hydrolysate Aminosol® was kindly supplied by Dr. Douglas V. Frost of the Abbott Laboratories, in the several solutions listed. The casein-pig pancreas hydrolysate Amigen®, as well as fructose solutions, were supplied through the kindness of Dr. Warren Cox, Jr., Mead Johnson and Company. The plasma protein hydrolysate Travamin® is the product of the Baxter Laboratories, Inc., the samples having been obtained on the market.

TABLE I
Amino acids and peptides of hydrolysates studied—
Manometric ninhydrin method at pH 2.5

Protein of origin	Sugar content	α -Amino acids	
		Free mM/L.	Bound mM/L.
Fibrin	None	224	130
Fibrin	10% fructose	198	117
Fibrin	5% glucose	203	149
Fibrin	10% glucose	202	113
Casein	None	235	129
Casein	10% fructose	251	115
Casein	5% glucose	249	131
Plasma proteins	None	173	161
Plasma proteins	5% glucose	175	170
Fibrin	10% glucose, freshly added	216.4	126.4
Fibrin	Same solution, autoclaved	217.5	126.0

some cases by adding to 1 liter of it 200 ml. of sterile 50 per cent glucose, under sterile precautions. This mixture was infused at a 20 per cent faster rate to compensate for its higher water content. When it was autoclaved, this was done at a pressure of 10 to 11 pounds per square inch for 10 minutes under a carbon dioxide atmosphere. A sugar-free double-strength solution of the hydrolysate of casein plus pancreas was fortified with sugar by adding to 500 ml. of it 200 ml. of 50 per cent glucose and 300 ml. of sterile water under the same conditions. These solutions were infused promptly after their preparation.

A uniform rate of infusion equivalent to 7.2 ml. per kilogram per hour of the 5 per cent protein hydrolysates was obtained by means of a flowmeter (Fischer and Porter Company "Triflat" variable-area flowmeter). This meter was calibrated for each of the solutions used. The above rate corresponds to an infusion time of a little over two hours for a liter of solution. The comparisons were made in each case with the same subject over an interval of a few days. Ten of the subjects were convalescent surgical patients who were estimated to have minimal metabolic disturbances; the other five were normal young persons. Blood was taken from an antecubital vein at the beginning, midpoint, just before the end, and 30 minutes after the end of the infusion. The subjects fasted from the evening before to three hours after the infusion. A pretest urine sample was collected from 10 P.M. (or 2 A.M.) to about 8 A.M.; the experimental urine was collected from the beginning of the infusion to three hours after its end. Collecting urine over an additional four hours was found only to intensify somewhat the differences in the wastage of free and bound amino acids reported here. The collection of urine was under the supervision of a member of the investigating staff and with the close cooperation of the subject.

The plasma and urine samples were analyzed for free and bound amino acids after removal of proteins (3, 4). Some simplification of the technique should make the

determination of bound amino acids of plasma more attractive to other investigators. Accordingly the present procedure is described: Four aliquots of 6 ml. each of the picric acid filtrates were pipetted into four of the special Thunberg tubes described by Hamilton and Van Slyke (5). Two of these portions were analyzed immediately for amino acids by the carbon dioxide released by ninhydrin at pH 2.5 (5); 3 ml. of concentrated hydrochloric acid were added to each of the other two, and they were heated in a steam bath 24 hours. The stoppers were very nearly closed during the first 20 hours, and removed during the last few hours, until the volume had decreased to 0.5 to 1 ml. Evaporation was completed in a vacuum dessicator over potassium hydroxide pellets. Two ml. of water and a drop of bromcresol green were added, and the pH adjusted to a green color with 2 or 3 drops of N sodium hydroxide. Solid citrate buffer (6) was added in order to obtain a pH of 2.5, and the total amino acids were determined as usual. The urine (or in the presence of proteinuria, a dialysate of the urine) was subjected to similar analysis before (7) and after hydrolysis. A dialysis of the plasma filtrates before analysis would have made the exclusion of protein more rigorous (3), but changes during infusion of the small quantity of picric acid soluble protein were shown in the present instance to be insignificant, relative to the changes produced for plasma peptides.

The excretion rates for amino acids and peptides during and after the infusion were corrected for the comparatively small background (pretest) rates, to yield the extra excretion due to the infused material. The method of calculation has been illustrated earlier (4).

In part of the experiments the extra total urinary nitrogen excreted during and immediately after an infusion was estimated. In this case the infusions were separated by not less than 44 hours. The subject ate a supper of low and nearly constant protein content at 6 P.M. Abundant water was taken during the evening, at retiring, and upon being waked at 2 A.M., to promote a nearly maximal rate of urea elimination. A urine sample was collected from 2 A.M. for six to seven hours and the hourly rate of nitrogen excretion determined. The extra nitrogen above this background hourly rate excreted during the experimental period (infusion period + three subsequent hours) was then determined, and compared with the amount of nitrogen infused. Nitrogen excreted during a third period of four hours was also determined, but this quantity was not remarkably different for the various infused solutions. When a sugar was infused prior to the hydrolysate, the urine secreted during the sugar infusion was collected as a separate sample.

RESULTS AND DISCUSSION

Preliminary comparison of glucose and fructose

This study began as a comparison of the effects of glucose and fructose, each present in the hydrolysate at 10 per cent strength, on the fate of

FIG. 1. PLASMA AMINO ACID AND PEPTIDE LEVELS DURING AND AFTER INFUSION OF FIBRIN HYDROLYSATES AUTOCLAVED WITH FRUCTOSE OR GLUCOSE (CONVALESCENT PATIENT)

The amino acid concentrations are in millimoles per liter. They may be converted to milligrams per cent of amino acid nitrogen by multiplying by 1.4.

the amino acids of the fibrin hydrolysate. These solutions had been autoclaved by the manufacturer with the sugar present. The two preparations were infused on alternate days for four days. The plasma amino acid tolerance curves were found to be very closely reproducible in a given subject, although rather different in different subjects. The curve always lay slightly higher in the presence of fructose than in the presence of glucose (Figure 1; also Figure 3, below). This was the opposite of the effect reported by Elman and his co-workers (1). Accordingly, the same comparison was made with the casein hydrolysate used by these workers; in every one of eight comparisons with either preparation the higher plasma amino acid levels were obtained with fructose, whether the glucose was at a 5 or 10 per cent concentration.

The urine analyses showed a striking difference, the loss of amino acids and peptides being about twice as great in the presence of glucose. Our first thought was that the larger diuresis usually obtained with the glucose-containing hydrolysate might account for this finding; in agreement with others we have found much higher glucose levels in the blood during the infusions than for fructose, and much larger losses of glucose into the urine. Therefore, comparable degrees

of diuresis were induced by giving the subject water to drink during the fructose hydrolysate infusions. Figure 2 shows that the amino acid and peptide losses were unrelated to urine volume.

Much higher levels of peptides (that is, of non-protein, bound amino acids) in the plasma during the infusions containing glucose showed that the greater peptiduria was consequent to a higher peptidemia and, therefore, prerenal in origin (illustrated in Figures 1 and 3).

General study of the effect of sugars in hydrolysates

Subsequent trial with sugar-free hydrolysates showed amino acid and peptide losses even lower than for the fructose-containing preparation; hence, the advantage of fructose over glucose is really only a *smaller disadvantage*, that is, a smaller interference in the presence of this sugar with amino acid and peptide utilization. The study was, therefore, extended to include fibrin hydrolysate solutions containing a) no sugar, b) 10 per cent fructose, c) 5 per cent glucose, and d) 10 per cent glucose. Figures 3 and 4 show

FIG. 2. LACK OF RELATION BETWEEN THE LOSSES OF PEPTIDES AND AMINO ACIDS AND THE URINE VOLUME (CONVALESCENT PATIENT)

(G) indicates the results with a fibrin hydrolysate containing 10 per cent glucose, autoclaved by the Abbott Laboratories after mixing; (F) gives the results with a parallel hydrolysate containing 10 per cent fructose, likewise autoclaved. The subject drank 750 and 1500 ml. of water during the last two fructose-containing infusions to produce the diuresis. In this and the succeeding figures the urinary wastage is shown as the per cent of the amount infused of that particular category (free or bound) of amino acid.

FIG. 3. PLASMA LEVELS AND URINARY WASTAGES OF FREE AND BOUND AMINO ACIDS WITH FIBRIN HYDROLYSATES, AS AFFECTED BY THE PRESENCE OF FRUCTOSE OR GLUCOSE (NORMAL SUBJECT)

The preparations had been autoclaved by the manufacturer after addition of the sugar.

FIG. 4. PLASMA LEVELS AND URINARY WASTAGES OF FREE AND BOUND AMINO ACIDS WITH FIBRIN HYDROLYSATES, AS AFFECTED BY THE PRESENCE OF FRUCTOSE OR GLUCOSE (NORMAL SUBJECT)

The preparations had been autoclaved by the manufacturer after addition of the sugar.

that the degree of wastage of peptides increased in that order; the peptide loss passed from between 6 and 9 per cent to 30 per cent or more in this series. The amino acid wastage increased in roughly the same order. The accompanying tolerance curves show that the glucose solutions in every case resulted in distinctly more severe peptidemia; in general the peptide wastage was directly related to the level of bound amino acids reached in the plasma. Clearly it was the tissues in general which rejected the peptides, not the renal tubular cells specifically. Application of the biuret reaction has indicated that the conjugates excreted in connection with infusion of glucose-containing hydrolysates are actually peptides (8).

In contrast the level of free amino acids in the plasma had the opposite relationship to sugar infusion (Figures 3 and 4). The highest levels occurred with no sugar, next with fructose, followed in order by the 5 and 10 per cent glucose preparations. The increased loss of free amino acids with elevated blood sugar levels, therefore, appears in contrast to have mainly a renal origin.

Simple calculations show that one cannot account for the differences in the plasma levels of free amino acids by the differences in the rate of return of amino acids to the plasma by tubular reabsorption. These plasma differences therefore remain to be accounted for. If one argues as Elman and his co-workers have done (1), that a lower tolerance curve means a better cellular uptake of the amino acids, then one is forced to consider glucose more effective in this respect than fructose. Albanese, Belmont, Orto, DiLallo, and Rossy (9), in contrast, suggest that the action of a fructose infusion in elevating the fasting plasma amino acid level has a favorable portent, signifying an increase in the "amino acid pool" of the body. These diametrically opposed interpretations indicate how inadequate plasma amino acid levels alone are for such decisions. Glucose (10-12) and fructose (13) have been found effective in some instances, ineffective in others, in promoting the cellular accumulation of amino acids by isolated mammalian cells or tissues.

The results do not permit one to exclude the elevated plasma peptides as a factor in the depressed renal reabsorption of the amino acids. Peptides have been shown at least in some in-

stances to inhibit the concentrative uptake of amino acids by cells (14).

In samples of the commercial partial hydrolysate of bovine plasma proteins the presence of 5 per cent glucose likewise intensified the peptidemia and the urinary loss of peptides (an increase from 32 per cent to 42 per cent lost, in both of two experiments); the loss of free amino acids was likewise increased.

The interaction of sugars and protein-hydrolysate on autoclaving

Because the commercial casein-plus-pancreas hydrolysate was not available with a glucose content higher than 5 per cent, a sugar-free solution was brought to a 10 per cent glucose content by direct sterile addition as described in the experimental section. Surprisingly, this addition had very little effect on the urinary peptide wastage. This result suggested that the interfering agent might not be the sugar itself but a product of the interaction of sugar with amino acids or peptides. A similar suggestion was also received independently from Dr. Douglas V. Frost of the Abbott Laboratories, namely that the browning reaction might be responsible for the heavier wastage with the sugar-containing hydrolysates. The reader is referred to the discussions by Overby and Frost (15) and Danehy and Pigman (16) of the browning reaction. Although precautions are taken to minimize this interaction in the preparation of commercial hydrolysates containing sugar, no means have been found of avoiding it entirely. As far as oral feeding is concerned, no unfavorable nutritional consequences could be observed (15) for the extent of interaction ordinarily obtained. The following experiment shows that the situation is quite different when administration is by vein.

A given subject was infused on different days with a) a fibrin hydrolysate: glucose combination freshly mixed, and with b) the same solution autoclaved in a standard way. The results illustrated in Figure 5 for a normal subject show that the autoclaving is responsible for the high wastage. The same result was obtained for three convalescent patients.

Clearly the higher wastage of bound amino acids obtained with commercial hydrolysates containing sugar is due almost entirely to products of

FIG. 5. URINARY PEPTIDE WASTAGE STIMULATED BY AUTOCLAVING WITH SUGAR RATHER THAN BY THE PRESENCE OF SUGAR (NORMAL SUBJECT)

Glucose added to the equivalent of 10 per cent in a 5 per cent fibrin hydrolysate.

chemical reaction between the sugar and constituents of the hydrolysate. These wasted conjugates should not be supposed, however, to be products of interactions between sugar and free amino acids; autoclaving actually produced no perceptible increase in the proportion of the amino acids in the conjugated state (Table I). Another alternative was that an extensive reaction had occurred with the peptides during autoclaving, so as to make about 25 per cent of them unmetabolizable. This possibility is unlikely because the titration curves (Figure 6) of glucose-fortified hydrolysates obtained before and after autoclaving were entirely superimposable, not varying by more than 0.03 pH unit at any point. Reaction of the amino or carboxyl or other dissociating groups of the peptides should lead to a substantial change in the titration curve, especially between pH 4 and 8. Accordingly it is considered likely that small amounts of products of interaction between sugar and amino acids or peptides are interfering with the metabolism of substantial amounts of peptide. The smaller peptide wastage with fructose probably results from the weaker browning reaction which this sugar gives.

The grossly incomplete utilization of infused peptides has suggested that they were used either after extracellular cleavage or after passive dif-

fusion into the cells (14) followed by cleavage there. The present results may suggest a step in their utilization which is affected by other metabolic events. On the other hand perhaps the interaction products instead merely inhibit the cleavage of the peptides.

The extra losses of the *free* amino acids in contrast to the peptides were obtained in every case whether the mixtures were autoclaved or not, and appear to be due mainly to the presence of the sugar itself.

Decreased immediate excretion of nitrogen when sugars are infused first

The foregoing evidences of interference of the sugar with the utilization of the free and combined amino acids of the hydrolysate when the two were given together led us to try infusing the sugar solution first. This was given as 1 liter of a 10 per cent solution at the same standard rate used above; sugar-free fibrin hydrolysate was then infused without the slightest interruption of the flow into the vein. When the sugars were given in this way, they produced no increase whatever in

FIG. 6. TITRATION BEHAVIOR BEFORE AND AFTER AUTOCLAVING OF A PARTIAL HYDROLYSATE OF FIBRIN MADE TO 10 PER CENT IN GLUCOSE

The conditions of autoclaving were those described in the experimental section. Beginning at the lower left the even-numbered points (second, fourth, etc.) represent the unautoclaved solution, the odd-numbered points, the autoclaved solution. Correction has been made for a shift of the titration curve in the acid direction by 5 milliequivalents per liter during the autoclaving.

FIG. 7. EXTRA WASTAGE OF AMINO ACIDS AND PEPTIDES AVOIDED BY INFUSING SUGAR SOLUTION PRIOR TO FIBRIN HYDROLYSATE (NORMAL SUBJECT)

TABLE II

Decreased immediate amino acid catabolism when a sugar is infused before the hydrolysate *

Subject	Infusions		Nitrogen excretion		
	1.	2. Hydrolysate containing	Pretest g./hr.	Exptl. g./hr.	Extra g.
1.	—	—	0.33	0.67	1.85
	Glucose	—	0.31	0.48	0.97
	Fructose	—	0.38	0.54	0.85
2.	—	—	0.27	0.67	2.18
	—	Glucose 50 g.†	0.48	0.96	2.46
	—	Glucose 100 g.†	0.26	0.65	2.03
	—	Fructose 100 g.†	0.41	0.80	2.00
	Glucose	—	0.33	0.63	1.50
3.†	—	Glucose 100 g.†	0.40	1.03	3.53
	—	Glucose 100 g.†	0.37	1.00	3.23
	—	Glucose 100 g.†	0.31	0.92	3.05
	—	Glucose 100 g.†	0.37	0.75	2.12
	Glucose	Glucose 50 g.§	0.35	0.54	1.16
	Glucose	Glucose 50 g.§	0.31	0.54	1.41
	—	—	—	—	—

* Normal subjects; infusion No. 1 (if any) from 8 to 10 A.M., 1 liter of 10 per cent sugar; second infusion from 10 A.M. to 12 M., 1 liter of 5 per cent hydrolysate with or without sugar as shown. Infusions not on consecutive days except for subject 3.

† Six successive days during which subject received a constant oral intake representing 2.4 g. N and 1600 (first 4 days) or 1,500 calories (last 2 days) between the hours of 4 P.M. and 9 P.M. Infusions between 9 A.M. and 12 M. The successive daily N balances in grams were as follows: -4.2; -4.1; -2.2; -2.6; -0.95; -0.14.

‡ Autoclaved with sugar by Abbott Laboratories.

§ Added sterily just before infusion.

the urinary wastage of amino acids and peptides (Figure 7).

Table II shows the background rates of total nitrogen excretion during a pretest period (between 2 and 8 A.M. for subjects 1 and 2; between 3 P.M. and 8 A.M. for subject 3); and the higher rate during the hydrolysate infusion and the succeeding three hours (referred to as the experimental period). The "extra" nitrogen is the additional amount eliminated during this period of approximately five hours because of the faster rate of excretion. Urine collected during the No. 1 (sugar) infusion was not included in either period. Extra urinary total nitrogen excretion was little modified by inclusion of the sugar in the hydrolysate, but was substantially decreased by giving either sugar in advance. This diminution was highly significant statistically (P was well below 0.001, comparing all infusions not preceded by sugar with all those preceded by sugar). Analyses over an additional four-hour period did not modify the conclusion that the prior infusion of sugar has a much greater effect than the simultaneous infusion in slowing the catabolism of amino acids. The conclusion is also unchanged if the rates of nitrogen excretion during the experimental periods are compared without reference to the background rates.

This retardation of nitrogen excretion represents a retardation of amino acid catabolism, since it involved largely urea plus ammonia nitrogen; we have already seen (Figure 7) that the excretion of amino acids and peptides is unaffected by prior infusion of sugar. Although this retardation of catabolism represents the desired effect of a calorogenic supplement to amino acid nutrition, the effect is more conventionally shown by the method of nitrogen balance. Preliminary studies by this method (see for example, Table II) showed that prior glucose infusion gave as good or better results, compared to simultaneous infusion, but they emphasized the difficulty of demonstrating advantageous or disadvantageous features by this method with the human subject. The requirements for nitrogen balance study with the human are not easily attained, because positivity of nitrogen balance depends not only on the nature of the nutrients received but also on the magnitude of the existing nitrogen deficiency and on other preexisting catabolic or anabolic trends which vary enormously from patient to patient and which change from day to day for a given patient.

Practical considerations

Probably *both prior* and *simultaneous* infusion of sugar should be recommended, if one bears in mind that as many calories as presently possible should be supplied to a patient receiving amino acids by vein. Two reservations, however, are made about the simultaneously infused portion:

a) The present results indicate that the sugar probably should not be autoclaved with the hydrolysate. Storage of sugar and hydrolysate together also are known to cause browning. There is no evidence that this reservation applies to peptide-free hydrolysates.

b) The content in glucose probably should not be as high as 10 per cent, at least for the present rate of infusion.

Wherever autoclaving of a hydrolysate with a sugar seems inescapable, advantage may be taken of the lower reactivity of fructose. It remains to be seen whether fructose has any superiority to glucose when autoclaving is avoided and when the sugar infusion is started prior to the hydrolysate.

SUMMARY

1. The presence of 10 per cent fructose, 5 per cent glucose, or 10 per cent glucose in commercial partial hydrolysates of protein increases substantially the wastage of peptides and amino acids consequent to intravenous infusion. The effect increases in the order named.

2. The extra peptide wastage is associated with higher peptide levels in the plasma, indicating that the disturbance of peptide utilization is prerenal.

3. The extra amino acid wastage is not associated with higher plasma amino acid levels, and accordingly appears to be mainly renal in origin.

4. Slightly higher plasma amino acid levels are generally obtained during infusion of fructose-containing than glucose-containing hydrolysates.

5. The extra peptide wastage is avoided, if glucose is added sterilely immediately before infusion, but reappears if the mixture is autoclaved under usual conditions.

6. Although the presence of sugar in a commercial fibrin hydrolysate increases amino acid and peptide loss, it does not diminish significantly the immediate excretion of total nitrogen; on the other hand if the sugar (glucose or fructose) is infused just before the hydrolysate, the immediate nitrogen catabolism is substantially reduced.

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