SERUM ALBUMIN REGENERATION AS EFFECTED BY INTRAVENOUSLY AND ORALLY ADMINISTERED PROTEIN HYDROLYSATES [1]

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Observations on the ability of intravenously administered protein hydrolysates to regenerate plasma protein have been reported. One worker (1) employed an acid casein hydrolysate supplemented with tryptophane in acute hemorrhage in dogs and observed a very rapid, though small, increase in the plasma protein value. Later studies with pancreatic casein hydrolysate (2 to 4) indicated that the administration of adequate amounts by mouth or vein resulted in plasma protein regeneration. Using a papain casein digest, intravenously, in dogs made hypoproteinemic by plasmapheresis, others (5) have shown that plasma protein regeneration was effected.

Recently, there was observed (6) a small increase in the average plasma protein of 7 post-operative patients who were given an enzymic casein hydrolysate as the only source of nitrogen. This was coincident with a fall in the hematocrit value.

In the present study, we wished to determine, with as much exactitude as available methods permit, the comparative efficiency of different protein hydrolysates and their relative efficiency when given by mouth and by vein, in the regeneration of plasma albumin. Since wide differences have been shown (7) in the ability of different orally administered proteins to effect albumin regeneration, we felt it might be of interest to compare such proteins (as hydrolysates) given intraveneously.

EXPERIMENTAL

Three enzymic protein hydrolysates were very kindly prepared and analyzed for us by Dr. K. S. Kemmerer of this laboratory. Casein, lactalbumin, and beef serum protein were used. The casein was acid-precipitated from skim milk.[2] Lactalbumin was a low ash, readily soluble, dried preparation.[3] Beef serum protein was prepared by dehydrating beef serum,[4] and, as supplied to us, contained about 15 per cent ash. To remove the major portion of salts, it was dissolved in water and precipitated by heat coagulation.

The proteins were enzymically digested in the usual way, using fresh pork pancreas as a source of enzyme. Digestion was complete in 7 to 10 days. The digest solution was heated, filtered, treated with a small amount of norite for decolorization, evaporated, and spray-dried to a fine powder. Table I gives comparative data on the hydrolysates.

To compare the nutritive value of the hydrolysate with the original protein, and thus to determine whether essential amino acids had been lost during the digestion and the technical processes thereafter, they were fed as the sole source of nitrogen in the basal diet described earlier for rats (8). The hydrolysates were fed on the basis of equivalence in nitrogen. Three levels of each equivalent to 2.4, 1.7, and 1.2 per cent nitrogen of the diet were fed. No less than 10 rats, approximately 50 grams in weight and 21 days old, equally divided as to sex, were placed on each diet. Growth was observed for 8 weeks.

For comparative measurement of serum protein regeneration, we used the technic developed by Weech and Goettsch (9). A mild degree of hypoproteinemia was produced by feeding their low protein diet to normal dogs for 3 weeks. During the subsequent week, the hydrolysate was incorporated in the diet to give a total intake of 0.34

TABLE I

Chemical characteristics of protein hydrolysates

	Casein	Lactalbumin	Serum protein
Total nitrogen, *per cent*	12.1	11.9	12.1
Amino nitrogen, *per cent*	8.2	8.7	9.0
Amino nitrogen after acid hydrolysis, *per cent*	10.2	11.3	10.6
Possible enzymic cleavage, *per cent*	80.0	77.0	85.0
Tryptophane, *grams per cent*	0.62	1.77	0.91
Ash, *per cent*	5.2	5.9	4.2
Moisture, *per cent*	4.9	2.7	2.2
Total N from pancreas, *per cent*	18.4	13.5	12.3
pH of 10 per cent solution	4.5	5.7	4.4

[1] Presented at the annual meeting of the American Society of Biological Chemists, Boston, March 31–April 4, 1942.

[2] Purchased from the Casein Corporation of America. Quality "H-I-P."

[3] Purchased from The Borden Company Research Division. Labco L A. 7-H-A.

[4] Purchased from Armour and Company.

grams nitrogen per kgm. body weight. The basal diet supplied 16 per cent of this total nitrogen. The diet supplied 80 calories per kgm. body weight. Plasma protein was determined by micro-Kjeldahl, and plasma albumin by precipitation from 22 per cent anhydrous sodium sulfate (10). The change in serum albumin during the week of supplementation was measured and was taken as the basis for comparison. For intravenous administration, the hydrolysate was given rapidly in 10 per cent solution sterilized by Seitz filtration. An average rate of 3 ml. per minute was tolerated but when rates in excess of this were given, vomiting frequently occurred.

RESULTS

The rat growth experiments are summarized in Table II. Complete comparative data are available only for the 1.7 and 1.2 per cent nitrogen intake levels since our supply of beef serum protein, and especially its hydrolysate, was limited. These lower intake levels are most useful, however, in estimating nutritive efficacy, since they correspond to approximately 14 per cent and 10 per cent of the hydrolysates, respectively. The growth on casein was obtained by averaging growth records for all samples of casein assayed. The data indicate that all 3 hydrolysates were very similar nutritively to the original proteins, with the possible exception of the casein hydrolysate. Thus, lactalbumin hydrolysate at the 1.2 per cent level was, if anything, superior to the original protein, and the gain in weight for serum protein and its hydrolysate was identical. For casein and casein hydrolysate, a 16 gram weight difference at the 1.2 per cent intake level is of questionable significance, since only one assay of the hydrolysate was made. However, it may reflect the relatively low tryptophane value of this particular lot. Since later observations on albumin regeneration in the dogs were not prejudicial to the sample, the question may be raised whether the requirements for growth are the same as for serum protein synthesis.

TABLE II

Average gain in weight of young rats when protein hydrolysates or the corresponding proteins were fed as the sole source of nitrogen for 8 weeks

Nitrogen source	Level of nitrogen intake		
	2.4 grams per cent	1.7 grams per cent	1.2 grams per cent
	grams	*grams*	*grams*
Casein	162.8[1]	59.2[1,2]	100.4[1]
Casein hydrolysate	130.2	58.0[2]	83.9
Lactalbumin	168.7	142.8	95.6
Lactalbumin hydrolysate	179.8	126.5	115.8
Beef serum protein		107.0	84.6
Beef serum protein hydrolysate		115.3	87.6

[1] Several separate lots of casein were fed to different groups of 10 rats each. The values are averages of 4 samples fed at the 2.4 per cent level, 5 fed at the 1.7 per cent, and 14 fed at 1.2 per cent level.
[2] Gain in weight for 4 weeks only.

TABLE III

Serum albumin regeneration effected by casein hydrolysate given intravenously and orally

Values expressed in grams per 100 ml. of plasma.

Dog No.	Total N intake grams per kgm.	Initial plasma albumin	Decline during depletion	Increase during supplementation	Assay value
			Intravenously		
9	0.345	2.55	−0.96	+0.58	+0.73
32	0.345	3.05	−0.94	+0.42	+0.57
21	0.345	3.12	−0.82	+0.07	+0.22
31	0.345	3.68	−1.20	+0.41	+0.56
37	0.345	3.20	−0.70	+0.04	+0.19
44	0.345	2.57	−0.54	+0.16	+0.31
		Average			+0.430±0.061
			Orally		
2	0.345	3.17	−1.18	+0.21	+0.36
43	0.345	3.20	−0.75	−0.06	+0.09
10 dogs *	0.324	(3.52)	−0.93	+0.23	+0.38
9 dogs *	0.370	(3.43)	−1.26	+0.19	+0.34
		Average			+0.348±0.022

* Taken from published data (8).

Tables III, IV, and V give the findings on serum protein regeneration for the 3 hydrolysates given orally and by vein. In these tables, we record the initial albumin value, its decline during 3-week depletion, its increase during regeneration, and the final assay value. This latter value is obtained by adding 0.15 gram per cent to the observed change in albumin (9), and takes account of the average decline that would have been observed during the fourth week if the supplement had not been given. The second column in Table III gives the total grams N intake per kgm. body weight. The small recorded differences in this figure are not significant. They were caused by

TABLE IV

Comparative serum albumin regeneration effected by lactalbumin hydrolysate given intravenously and orally (0.336 gram N per kgm.)

Values expressed in grams per 100 ml. of plasma.

Dog No.	Initial albumin	Decline during depletion	Increase during supplementation	Assay value
		Intravenously		
9	2.65	−0.85	+0.46	+0.61
32	2.78	−0.44	+0.31	+0.46
37	3.44	−1.04	+0.06	+0.21
21	3.57	−0.73	+0.04	+0.19
44	3.58	−0.56	+0.08	+0.07
	Average			+0.308±0.067
		Orally		
2	2.92	−0.62	+0.27	+0.42
35	3.25	−0.97	+0.27	+0.42
60	3.07	−0.40	+0.17	+0.32
43	3.27	−0.65	−0.02	+0.13
	Average			+0.322±0.046

assumptions as to the protein equivalence of the nitrogen in the hydrolysate.

For casein hydrolysate given orally, an assay value of 0.348 was obtained. This is an average of 21 hypoproteinemic dogs, 19 of which have been previously reported (8). One investigator (11) reported 0.425 as the assay value for unhydrolyzed casein and this value does not differ significantly from our value for the hydrolysate given by mouth. When intravenously given, the average assay value for 6 dogs was 0.430, which again does not differ significantly from either casein or casein hydrolysate fed by mouth.

Five dogs were used for the intravenous injection of lactalbumin hydrolysate and 4 served as the oral controls. The average assay value, orally, was 0.322 and, intravenously, 0.308. These values must be regarded as identical, and within the range of values observed for casein hydrolysate. Six of the 9 dogs had been used for the previous studies with casein hydrolysate, and a comparison of individual performance on different supplements showed closely similar performance on both.

Serum protein hydrolysate intravenously gave an assay value of 0.287 (Table V), but orally, an assay value of 0.660. The intravenous value is not significantly different from 0.308 value for lactalbumin hydrolysate. But an assay value of 0.660 for the protein hydrolysate given by mouth is difficult to interpret. It is unfortunate that our supply of this material was exhausted and that further data could not be obtained. Aside from the fact that there are only 4 observations on oral feedings, it should be noted that the first 2 dogs were penned together and got into a fight on the day before the depletion value was recorded. The dog that showed the greatest assay value was badly beaten in the fight. This may or may not be a pertinent factor. In spite of this, a value of 0.660 is in agreement with that reported (11) for dried beef serum (assay value 0.739) but the limited number of observations impels acceptance of the value as provisional.

DISCUSSION

As a result of the work of others (7, 11 to 16), it is recognized that ingested proteins differ widely in their ability to regenerate plasma albumin. Our work has been entirely with protein hydroly-

TABLE V

Serum albumin regeneration effected by beef serum protein hydrolysate given intravenously and orally (0.334 gram N per kgm.)

Values expressed in grams per 100 ml. of plasma.

Dog No.	Initial plasma albumin	Decline during depletion	Increase during supplementation	Assay value
		Intravenously		
9	2.91	−0.93	+0.53	+0.68
31	2.92	−0.96	+0.09	+0.24
32	4.05	−1.51	+0.19	+0.34
2	3.32	−0.48	−0.32	−0.17
35	3.05	−0.86	+0.19	+0.34
44	3.71	−1.00	+0.14	+0.29
	Average			+0.287±0.075
		Orally		
2	3.04	−0.66	+0.55	+0.70
35	2.71	−1.25	+0.79	+0.94
9	3.38	−0.72	+0.43	+0.58
31	2.97	−0.59	+0.27	+0.42
	Average			+0.660±0.074

sates rather than with whole protein. We have determined the effectiveness of hydrolysates of different proteins and the efficiency of the same hydrolysate given by vein and by mouth in albumin regeneration. All the hydrolysates given by vein resulted in plasma albumin synthesis. In 6 dogs, casein hydrolysate gave an assay value of 0.43 gram per cent; lactalbumin hydrolysate, in 5 dogs, gave 0.308 gram per cent; and serum protein hydrolysate in 6 dogs gave 0.287 gram per cent. Statistically, there is no difference between the high and low values and we must regard the 3 hydrolysates as equivalent in their ability to regenerate albumin.

When the assay values for the same hydrolysate given by mouth and by vein are compared, we can again elicit no statistically significant difference. The greatest difference was recorded for serum protein hydrolysate given by these two routes. The P value for the difference is 0.053, which strongly suggests a significant difference. However, the limited number of dogs fed by mouth, the fact that the animals were penned together, and the finding that neither lactalbumin hydrolysate nor casein hydrolysate showed any difference in regeneration, whether given by mouth or by vein, makes us feel that the same is likewise true for the serum protein hydrolysate.

The finding that intravenously administered hydrolysates of good proteins are equally effective in serum albumin regeneration is clearly different from the conclusions of others that intact proteins fed by mouth are quite widely different in effectiveness. Our hydrolysates were the equivalent of the intact protein as measured by the growth of experimental animals (Table II). In explanation of this difference, we suggest the following hypothesis.

It has been shown that for the establishment of nitrogen balance, all essential amino acids must be present in the circulation at the same time (17). If this is true, it is not unlikely that differences in the rate of protein hydrolysis and in the rate of absorption of the amino acids from the gastrointestinal tract may greatly influence the effectiveness of a particular protein in serum albumin regeneration. That proteins are hydrolyzed at different rates and to different extents, and that amino acids are readily freed from some protein linkages but that some form resistant groupings, are well-recognized facts (18, 19). Further, it was shown (20) that there are differences in the rate of absorption of the different amino acids.

It follows that the composition of the amino acid mixture which may eventually appear in the blood may differ widely from the actual composition of the ingested protein. Therefore, in measuring the effect of an ingested protein by means of plasma albumin regeneration, one may be measuring not an intrinsic difference in proteins based on ultimate composition but the net resultant of varying rates of enzymic hydrolysis and absorption from the intestine.

When a hydrolysate instead of intact protein is given orally, one eliminates the necessity for intestinal hydrolysis and all the amino acids are immediately available for absorption. By intravenous administration, the further factor of differing rates of absorption is eliminated. Since we observed that plasma albumin is regenerated to an equal extent whether the protein hydrolysates were given by mouth or by vein, it would seem that the rate of intestinal hydrolysis of proteins is the factor which has determined recorded differences in the ability of intact protein of good nutritive quality to regenerate plasma albumin.

CONCLUSIONS

Enzymic hydrolysates of casein, lactalbumin, and beef serum protein, which are nutritively equivalent to the original proteins, are equally effective in the regeneration of plasma albumin in hypoproteinemic dogs, whether given orally or intravenously. The significance of these findings is discussed.

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