PARTIAL ACID HYDROLYSATES OF PROTEINS

VI. ASSAY OF LIQUID PROTEIN HYDROLYSATES IN PROTEIN-DEPLETED RATS*

BY DOUGLAS V. FROST AND HARRY H. SANDY

(From the Abbott Laboratories, North Chicago)

(Received for publication, April 21, 1948)

Experiments to determine the comparative nutritive value of partial acid hydrolysates of various proteins have been carried out for several years in this laboratory. Most of this work has involved studies of nitrogen balance in dogs wherein the hydrolysates were administered intravenously. Short term experiments, i.e. 4 to 5 days, were used to determine minimum nitrogen levels at which the N balance could be maintained (1, 2). In these experiments 2, 4, and 6 hour hydrolysates of casein and fibrin (3) all appeared to be well utilized and capable of maintaining the balance at levels of 120 to 150 mg. of N per kilo per day. The casein hydrolysates did not support the balance at this low level unless fortified with sulfur amino acids. In subsequent longer term experiments (4) fibrin hydrolysates proved adequate to support dogs in nitrogen balance at a level of 120 mg. of N per kilo per day for 15 weeks, whereas dogs on somewhat higher levels of casein hydrolysate failed completely in a few weeks. On the basis of these and other experiments it was concluded that short term nitrogen balance studies in normal adult dogs may be misleading and that long term experiments at low levels of nitrogen are more significant.

Another method of approach tested the ability of hydrolysates to bring about rapid recovery of severely depleted dogs when given in massive dosage (5). In these studies partial acid hydrolysates of fibrin were again better utilized than those of casein. Because the casein and fibrin hydrolysates did not appear to differ markedly in essential amino acid value (5), it was deduced that the better utilization of fibrin hydrolysate might be due to better utilization of the bound (peptide) amino acids of fibrin hydrolysate. Christensen, Lynch, Decker, and Powers (6) have reported a somewhat lower excretion of bound amino acids in humans following infusion of partial acid hydrolysates of fibrin than after infusion of an enzymatic digest of casein. These findings point to the need for intravenous studies in the final assessment of intravenous hydrolysates which contain a significant proportion of their total amino acids in bound form.

* Presented in part before the Division of Medicinal Chemistry of the American Chemical Society, New York, September 17, 1947.
Although there is an obvious advantage in the intravenous method, certain practical difficulties arise in the use of dogs as assay animals. Dogs available for use in most laboratories vary as to age, breed, and nutritional history and do not compare in degree of standardization as test agents with the rat. Also there are large quantitative differences between requirements for growth and maintenance. Hegsted, Hay, and Stare (7) have estimated that no more than 1.6 mg. of tryptophan and 15 mg. of isoleucine per kilo of body weight per day are required for nitrogen balance in the adult dog, whereas the similar requirements for maximum growth in rats were estimated to be 94 mg. and 880 mg. respectively. Furthermore the amino acid requirements of the rat are better known than are those of the dog. We were thus influenced to seek a rapid and fairly precise method for screening and control purposes with rats. A method which would clearly reveal the amino acid adequacy of essential amino acids in liquid protein hydrolysates, and would not necessitate drying the hydrolysates, appeared most desirable.

The method described herein is based on the rat repletion method of Wissler, Steffee, Woolridge, Benditt, and Cannon (8) and Frazier, Wissler, Steffee, Woolridge, and Cannon (9). These authors have established that lack of any one of nine amino acids essential for the adult rat results in prompt deficiency symptoms, which are as promptly corrected by return of the missing amino acid to the diet. Their results are qualitatively similar to those reported extensively by Rose (10). The repletion method is more rapid than the rat growth method and is particularly adapted to the problem in hand because of the ability of adult rats to consume large volumes of liquid nutrients, as previously described (8, 11). Several applications of the method are described.

EXPERIMENTAL

All rats used were from our own colony, which has been inbred for many years. Vigorous, rapidly growing, young adult male rats were raised on breeder stock diet until they weighed 160 to 220 gm. They were then placed on Diet NP4 for 12 days, during which they uniformly lost about one-fourth of their initial body weight. Shorter depletion periods did not give as satisfactory results as the 12 day depletion. The highly purified protein-free diet, Diet NP4, which contains only 0.02 to 0.05 per cent nitrogen, is made up as follows: sucrose 83, Salt Mixture 1 (U. S. P.) 4, agar 1.4, Primex 4.2, corn oil 4.2, cod liver oil 1.4, choline chloride 0.15, and inositol 0.14 gm.; thiamine hydrochloride 0.6, riboflavin 1.2, pyridoxine hydrochloride 0.6, calcium pantothenate 5.0, nicotinic acid 3.7, mixed tocopherols 2.5, and ascorbic acid 14 mg. per 100 gm. of diet.

The rats were supplied the 5 per cent hydrolysates in 60 cc. test-tubes
attached to the cages. Aluminum drinking fountains with rather broad planed rims were used. No loss of the solutions occurred from siphoning with this type of drinking tip. Unless otherwise indicated, no water was given. The non-protein diet was fed \textit{ad libitum}. Final weightings were made 24 hours after the last hydrolysate feeding. In order to orient the rats equally the following procedure was adopted. After a 12 day depletion all rats are offered 40 to 50 cc. daily of a standard 5 per cent hydrolysate for 3 days. During this period the rats learn to drink at nearly the maximum rate. They are returned to the non-protein diet with water for 3 more days, during which they again lose weight. Rats thus prepared usually consume the entire allotment of hydrolysate subsequently offered to them, and continue to do so provided no amino acid deficiency is present. Groups of fifteen to thirty rats were prepared at a time. Groups for individual assays numbered four to seven rats. Because of the small size of the groups conclusions are based only on experiments which have been repeated two or three times with the same general results. In experiments in which the intake was limited to 0.12 to 0.32 gm. of N per rat per day, a single measured volume of solution was fed daily. In all instances in which there was good balance of the essential amino acids this volume was taken before bacterial growth became visible. During feeding \textit{ad libitum} the drinking tubes were filled twice daily. An advantage of the method is the ease and accuracy of measuring the liquid and nitrogen intake each day.

The essential amino acids were determined in most of the hydrolysates studied. Tryptophan was determined colorimetrically directly on the unchanged hydrolysates by the method of Graham, Smith, Hier, and Klein (12). This modification of the method, which depends on the color formed by the reaction of tryptophan with \textit{p}-dimethylaminobenzaldehyde, gives somewhat lower values for tryptophan in fibrin hydrolysates than does that of Horn and Jones (13), previously used in our laboratory. Cystine was determined by the method of Folin and Marenzi (14) and methionine as described by White and Koch (15). The remaining essential amino acids were determined microbiologically by the method of Stokes, Gunness, Dwyer, and Caswell (16). Free amino acid nitrogen determinations were made by the ninhydrin method of Van Slyke, Dillon, MacFadyen, and Hamilton (17).

\textbf{Assay of Dried Proteins}—For purposes of comparison a number of dried powdered proteins were fed in small cups attached to the cage wall by metal clips. Water was offered \textit{ad libitum}. All proteins were fed at a level to supply 0.24 gm. of N per rat per day. The fibrin, casein, and wheat gluten supplements were completely, or nearly completely, consumed by all of the rats in these groups. The beef muscle, which had been extracted
with benzene, and the defatted whole egg were refused by some of the rats in these groups. The results are shown in Table I.

Repeated Use of Rats—The adaptability and economy of the method could be extended if rats could be used for repeated experiments. This procedure was tried through three regular runs of similar fibrin hydrolysates, starting with rats of original weights of 160 to 210 gm. In the second and third assays, we dispensed with the drinking trial because the rats were already trained to drink at the maximum rate. Results of the first two assays were quite similar.

Because the general condition of the rats appeared to deteriorate somewhat through three consecutive assays, further experiments were tried in which the rats were returned to the stock breeder diet for 4 days prior to the third depletion. When this was done, the condition of the rats for the third assay was greatly improved, and responses very close to those of the prior assays were obtained. The variation in average weight gain in repeated assay of the same preparation in the same rats was less than 10 per cent. When minimum responses have been obtained on any assay, a transfer to the stock diet for 4 to 5 days has served well to prepare rats for further work.

In general the results reported herein involved only one use of the animals; however, the results at hand suggest that repeated use of the animals is feasible with proper conditioning between experiments.

Nitrogen Feeding Levels—Volumetric feeding of the liquid hydrolysates provided a good opportunity to study the response to different nitrogen levels. The observation was made that liquid feeding ad libitum could be used advantageously to reveal gross differences in amino acid adequacy of

### Table I

**Assay of Dry Protein Supplements Fed Separate from Non-Protein Diet at Level of 0.24 Gm. of N per Day**

<table>
<thead>
<tr>
<th>Protein*</th>
<th>N content</th>
<th>No. of rats</th>
<th>Average intake of allotted N</th>
<th>Weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td></td>
<td>per cent</td>
<td>Range</td>
</tr>
<tr>
<td>Fibrin</td>
<td>15.1</td>
<td>4</td>
<td>100</td>
<td>58-60</td>
</tr>
<tr>
<td>Casein</td>
<td>13.22</td>
<td>6</td>
<td>96</td>
<td>27-52</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>12.65</td>
<td>6</td>
<td>98</td>
<td>11-26</td>
</tr>
<tr>
<td>Beef muscle (defatted)</td>
<td>15.65</td>
<td>4</td>
<td>100</td>
<td>39-53</td>
</tr>
<tr>
<td>Whole egg (defatted)</td>
<td>12.16</td>
<td>3</td>
<td>91</td>
<td>52-57</td>
</tr>
</tbody>
</table>

* The casein, defatted beef muscle, defatted whole egg, and wheat gluten were kindly supplied by the Bureau of Biological Research of Rutgers University as part of a collaborative study. Two of six rats started on beef muscle and three of six rats on whole egg failed to take the supplements and are not included in the averages.
hydrolysates, but that minor differences were sharpened by controlled isonitrogenous feeding. On ad libitum feeding, rats consumed as much as 80 cc. per day of certain hydrolysate solutions, equal to 0.5 gm. of N. The general intake, however, of well balanced hydrolysates was 55 to 60 cc. per day, equal to about 0.35 to 0.4 gm. of N. Ratios of nitrogen efficiency calculated from ad libitum feeding of different hydrolysates showed close correlation with weight gain. The present study was undertaken to determine the levels at which the nitrogen efficiency would be highest for a representative fibrin hydrolysate. This level was then chosen for general assay purposes.

For the study herein reported we used a 5 per cent partial acid hydrolysate of fibrin with 5 per cent dextrose which contained about one-third of its amino acids in peptide form. The hydrolysate solids contained the following percentages of amino acids, calculated to 16 per cent N: isoleucine 5.1, leucine 7.5, valine 4.2, lysine 8.8, histidine 3.4, arginine 7.5, tryptophan 1.0, methionine 3.4, cystine 2.3, threonine 6.6, phenylalanine 3.4, tyrosine 1.5, glutamic acid 13.9. Four groups of rats were oriented to liquid feeding in the usual way and were fed the liquid hydrolysate at levels of 0.12, 0.18, 0.24, and 0.3 gm. of N per rat per day. The amounts of liquid hydrolysate fed daily to the rats in each group were 17.2, 25.8, 34.4, and 43 cc., respectively. All rats were allowed water and the non-protein diet ad libitum. Very little water was taken, whereas the hydrolysate allotments were consumed by all rats throughout. The rats on the lowest level generally drank their daily allotment within an hour after it was offered.

The results are shown in Fig. 1. The nitrogen efficiency ratios on the four groups were 12.1, 12.5, 10, and 9.3, respectively.

Time of Assay—Although a 12 day recovery period appears advantageous for most assays, extension of the time to 15 to 18 days may be needed to develop significant differences between materials of rather close nutritive value. For instance, partial acid hydrolysates made from fibrin which contained a considerable amount of plasma protein appeared to contain a limiting level of isoleucine, i.e. about 3.0 per cent on a dry basis. In order to determine the effect of added isoleucine, two equal groups of four rats each were formed. After a 12 day depletion the separate groups were fed ad libitum the 5 per cent hydrolysate and the hydrolysate to which was added 0.2 per cent of a leucine-isoleucine concentrate. This addition was equal to about 1 per cent of each amino acid on a dry basis. The isoleucine concentrate contained 43 per cent isoleucine and 45 per cent leucine as determined by microbiological assay.

1 Partial acid hydrolysate of purified fibrin for intravenous administration is sold under the trade name, Aminosol.
The results are shown in Fig. 2 together with a record of the 12 day weight recovery of six rats on a hydrolysate of purified fibrin which contained 4.6 per cent isoleucine. All of the rats which received the extra isoleucine had gained more than any of the rats on the unfortified hydrolysate after the 18th day of the assay and the difference appeared significant. Repetition of this experiment in a 12 day assay again revealed a small average increase in growth response on addition of synthetic Dl-isoleucine to the crude fibrin hydrolysate. These results indicate that a longer assay period than 12 days may be desirable, depending on the purpose for which it is used. For purposes of routine testing, a somewhat shorter period than 12 days may prove adequate, particularly when the nitrogen intake is limited to a critical level.

The isoleucine requirement for a maximum rate of repletion was calculated to be somewhat in excess of 75 mg. per rat per day.

Requirement for Tryptophan—Frazier et al. (9) indicated that in complete amino acid mixtures an intake of 18 mg. per day of DL-tryptophan supplied the need for this amino acid for good weight recovery in adult protein-depleted rats, but that 9 mg. of DL-tryptophan were distinctly limiting and
gave only a very small response. As a result of many experiments the fact became clear that partial acid hydrolysates of fibrin which contained upwards of 0.9 per cent natural tryptophan on a dry basis gave uniformly rapid weight recovery in depleted rats. When fed ad libitum, such hydrolysates supplied about 24 mg. of tryptophan per rat per day, with a 12 day weight recovery of about 60 to 65 gm. Hydrolysates which contained 0.6 per cent tryptophan under similar conditions supported an average weight gain of only about 40 gm., with a tryptophan intake of about 14 mg. per rat per day.

In order to make the effect of a limiting deficiency of tryptophan more critical, experiments were run in which the hydrolysate intake was limited to supply 0.24 gm. of N per rat day. Previous work had shown that the maximum weight recovery on various hydrolysates fed at this level is 50 to 55 gm. in 12 days. For the experiments a 5 per cent fibrin hydrolysate was selected which was known to contain a limiting level of tryptophan; i.e., 0.68 per cent on the basis of the hydrolysate solids. To part of this
was added an amount of DL-tryptophan to give 0.92 per cent on a dry basis. The experiment was carried out with two groups of six rats each, as shown in Fig. 3.

The considerable difference in weight gain for the two groups of rats is shown in Fig. 3. The 12 mg. daily level of tryptophan is clearly too low for best performance. The level of 16.5 mg. is thought to be about optimum at the level of nitrogen intake studied. A somewhat higher level,

![Graph showing weight response of protein-depleted rats](http://www.jbc.org/)

**Fig. 3.** Weight response of protein-depleted rats (males) to controlled feeding of a partial acid hydrolysate of purified fibrin alone and with 0.24 per cent DL-tryptophan added on the hydrolysate solids. The solutions fed contained 0.24 gm. of N per day. The assay includes a 3 day orientation period during which the liquid hydrolysate was fed ad libitum, followed by a 3 day depletion before the assay.

i.e. 18 to 20 mg. per rat per day, is required for the maximum response of about 60 to 65 gm. under conditions of ad libitum feeding.

**Effect of Degree of Hydrolysis**—The availability of partial acid hydrolysates of fibrin of varying degrees of hydrolysis offered the opportunity to study the effect of the progressive destruction of strepogenin on nutritive value. Five separate hydrolysates varying widely in the degree of hydrolysis were compared in the repletion test. The hydrolysis conditions used were similar in each instance except that the degree of hydrolysis was varied by the time of heating in acid. The ratio of free amino acid nitrogen to total nitrogen ranged from 42 to 74 per cent in the hydrolysates studied. Complete hydrolysates of fibrin have a ratio of 75 to 76 per cent.
The tryptophan was completely destroyed in Lot 705K308, in which the hydrolysis was nearly complete. When necessary, L-tryptophan was added so that the content of all hydrolysates would be similar, i.e., 1.0 per cent tryptophan on the basis of 16 per cent nitrogen. Also all hydrolysates were similarly fortified to supply 3.2 per cent methionine and 2.7 per cent cystine.

The strepogenin content of the hydrolysates was determined microbiologically with *Lactobacillus casei*, as described by Sprince and Woolley (18). According to their practice Wilson liver fraction L was arbitrarily assigned a value of unity and the potency of the hydrolysate solids was expressed in relation to this standard.

The five hydrolysates made to 5 per cent solids were assayed by the rat repletion method with feeding *ad libitum*. From six to eleven rats were used in each group. Records of liquid intake were kept throughout the 12 day period so that nitrogen efficiency ratios could be calculated. All of the hydrolysates were well taken, the intake averaging about 55 to 60 cc. per day.

The degree of hydrolysis, the average 12 day weight gains, and the relative strepogenin content of the five hydrolysates are shown in Table II. The data indicate that there is no failure in nutritive value as measured by this test following destruction of almost all of the strepogenin originally present in the protein. Nitrogen efficiency values in the narrow range of 12.6 to 13.8 were obtained for the different hydrolysates.

### Table II

*Effect of Degree of Hydrolysis and Strepogenin Content of Acid Hydrolysates of Fibrin on Repletion Response to Feeding *ad libitum*

<table>
<thead>
<tr>
<th>Fibrin hydrolysate,* lot No.</th>
<th>Hydrolysis†</th>
<th>COOH-N to total N</th>
<th>12 day repletion response</th>
<th>Strepogenin content <em>(Lactobacillus casei assay)‖</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kzs. per cent</td>
<td>gm.</td>
<td>gm.</td>
<td>No. of rats</td>
</tr>
<tr>
<td>702K300..................</td>
<td>6</td>
<td>42</td>
<td>9</td>
<td>47-74</td>
</tr>
<tr>
<td>704K300..................</td>
<td>7</td>
<td>44</td>
<td>9</td>
<td>40-56</td>
</tr>
<tr>
<td>702K302..................</td>
<td>8</td>
<td>48</td>
<td>6</td>
<td>47-58</td>
</tr>
<tr>
<td>706K312..................</td>
<td>11</td>
<td>55</td>
<td>6</td>
<td>47-65</td>
</tr>
<tr>
<td>705K308..................</td>
<td>18</td>
<td>74</td>
<td>7</td>
<td>44-50</td>
</tr>
</tbody>
</table>

* All lots contained 5 per cent fibrin hydrolysate and 5 per cent dextrose, and were made to contain equal levels of tryptophan, methionine, and cystine.
† Hydrolysis was carried out at 10 to 20 per cent protein concentration with a ratio of H₂SO₄ to fibrin of 1.2:1. The complete hydrolysate was further hydrolyzed with 8 N HCl for 8 hours.
‖ Liver fraction L (Wilson) = 1.
The lot of fibrin hydrolysate in which hydrolysis was nearly complete, Lot 705K308, was also tested at the level of 0.24 gm. of N. The average weight gain of six rats under these conditions was 52 gm. (range, 41 to 60 gm.), which is about the maximum obtained with partial hydrolysates of fibrin.

Partial Acid Hydrolysates of Casein—Experiments with partial acid hydrolysates of casein are particularly interesting with regard to the utilization of the sulfur amino acids. Experiments were carried out with a casein hydrolysate which contained about 75 per cent bound amino acids. The essential amino acid content, calculated to 16 per cent N, is as follows: isoleucine 4.5, leucine 8.4, valine 6.4, threonine 4.2, methionine 2.8, cystine 1.5, phenylalanine 3.7, tyrosine 4.2, tryptophan 0.4, lysine 8.1, histidine 3.2, and arginine 3.4. The hydrolysate was fortified during manufacture with 0.8 per cent cystine above the normal level of 0.7 per cent. Assay results on this hydrolysate at a level of 0.24 gm. of N per day are shown in

![Graph showing weight response of protein-depleted rats (males) to the controlled feeding of partial acid hydrolysates of casein alone and fortified with DL-tryptophan and DL-methionine.](http://www.jbc.org/)

**Fig. 4.** Weight response of protein-depleted rats (males) to the controlled feeding of partial acid hydrolysates of casein alone and fortified with DL-tryptophan and DL-methionine. New rats after a 3 day orientation period were used in the first instance; rats twice depleted in the latter case. The solutions contained 5 per cent hydrolysate only.
Fig. 4. Only 47 per cent of the liquid allotment was taken and a weight loss occurred in all rats.

The tryptophan deficiency was corrected by addition of DL-tryptophan to a level of 1.6 per cent on 16 per cent N. DL-Methionine was added to supply a total of 6.29 per cent. The hydrolysate was fed at a level of 0.24 gm. of N per day and the average intake was 88 per cent of the allotment. Growth responses for the hydrolysate fortified in this way are shown in Fig. 4.

Further experiments were conducted in which the original hydrolysate was fortified with DL-tryptophan at the same level as above. The casein hydrolysate fortified with tryptophan supported an average weight recovery of 30 gm. (range 20 to 41 gm.) in 12 days in six rats. The rats took 93 per cent of the allotment of 0.24 gm. of N per rat per day.

**DISCUSSION**

The primary rôle played by the “indispensable” amino acids, lysine, histidine, isoleucine, leucine, valine, threonine, tryptophan, and phenylalanine, has been clarified by Rose and his coworkers for growth in rats and maintenance in adult humans (10), and by Cannon’s group (9) for repletion in adult protein-depleted rats. The present method imposes highly critical conditions in the use of still growing, nearly adult rats for depletion. The stimuli for growth and repletion occur simultaneously and deficiencies in assay materials promptly become manifest.

Frazier et al. (9) have reported that amino acid mixtures patterned after casein support as great or greater repletion responses as an isonitrogenous amount of casein. Our experience (11) has been that amino acid mixtures produce a somewhat greater response than casein. This has been true, despite the unavailability of the D forms of valine, isoleucine, and threonine used in the mixtures. Direct comparisons are further complicated by the fact that casein contains about 10 per cent of its nitrogen in the amide groups of asparagine and glutamine. The finding that progressive destruction of strepogenin does not reduce the repletion response to ad libitum feeding strongly supports the thesis that adult protein-depleted rats do not require strepogenin for maximum recovery.

Somewhat opposed to the hypothesis that proteins and protein hydrolysates contain amino acids combined in a way which provides a nutritional advantage, such as that ascribed to strepogenin for the growth of young rats (19), are the repeated findings in this laboratory that complete acid hydrolysates of proteins fortified with tryptophan are not generally inferior, and may even be superior, to the original proteins. Risser (20) reported that complete hydrolysates of casein fortified with tryptophan
and cysteine are slightly more effective in maintaining nitrogen balance orally in dogs at minimum levels than is whole casein fortified with the same amount of cysteine. As previously discussed, partial acid hydrolysates of casein appear to be less well utilized on injection than partial acid hydrolysates of fibrin (4, 5). In the present experiments there is further evidence that the failure in utilization of casein and casein hydrolysates involves the sulfur amino acids, particularly methionine.

The following average weight gains for 12 days, from the above data and Table II, are illustrative: casein 44 gm., casein hydrolysate fortified with tryptophan and cysteine 30 gm., casein hydrolysate fortified with tryptophan, cysteine, and methionine 53 gm., and fibrin 58 gm. The total sulfur amino acid content of these preparations was determined as 3.6, 4.3, 7.8, and 3.8 per cent respectively. The methionine contents of casein and fibrin were found by analysis, and are generally reported to be about 3 and 2.2 per cent respectively. In these experiments, as in the previous work with dogs (5), fortification of partial acid hydrolysates of casein with cysteine to a level of total sulfur amino acids in excess of that in fibrin is insufficient to correct the sulfur amino acid deficiency. Fortification with adequate methionine, however, appears to improve the nutritive character of this type of casein hydrolysate greatly. It is of further interest to note that pure amino acid mixtures, patterned after casein and made to contain about 3.8 per cent total sulfur amino acids, supported nearly maximum repletion responses and no evidence of a sulfur amino acid deficit was apparent (11). Experiments are under way to determine the minimum sulfur amino acid requirements for repletion. This appears from the data in hand to be no more than 70 mg. per rat per day.

The ratio of sulfuric acid to fibrin used in these experiments was 1.2:1, about 50 per cent greater than the ratio used previously (3-5). Experience in this laboratory with the hydrolysis of proteins, particularly recent work by Dr. G. F. Lambert, has indicated that fibrin is unusual with regard to the ease with which it undergoes acid hydrolysis. Although complete destruction of tryptophan, as well as strepogenin, occurred under the conditions used for complete hydrolysis, no significant destruction of other essential amino acids was thought to occur. Casein is considerably more resistant to acid hydrolysis than fibrin and requires rather drastic conditions for completion of hydrolysis. Because of the many interactions which take place during the hydrolysis of proteins it is difficult to assess the resulting hydrolysates in relation to the whole protein. The use of fibrin may reduce these uncertainties to some extent; however, the final answer must be based on comparisons between purified proteins and their counterpart mixtures of pure L-amino acids.
SUMMARY

A relatively rapid method for assaying the nutritive value of liquid protein hydrolysates, based on weight regeneration in protein-depleted rats, is described. A feeding level of 0.24 gm. of N per rat per day was found to give the highest nitrogen efficiency ratio in the case of a partial acid hydrolysate of fibrin, and was adopted as a standard feeding level. Several whole proteins were assayed as dry supplements separate from the diet at the 0.24 gm. of N level.

Partial acid hydrolysates of fibrin were assayed with regard to the optimum level of tryptophan and isoleucine. The requirement for tryptophan for maximum weight gain was estimated at about 18 to 20 mg. per day. The similar requirement for isoleucine was somewhat greater than 75 mg. per day. Experiments with partial acid hydrolysates of casein indicated that the sulfur amino acids contained therein are not completely utilized when taken orally by the rat.

The degree of hydrolysis of partial acid hydrolysates of fibrin did not appear to alter the repletion responses up to the hydrolysis point at which 97 per cent of the amino acids were in free form and no strepogenin remained.

Thanks are expressed to P. N. E. Naidu, in charge of Aminosol manufacture, for supplies of many of the materials studied, to Elsa Proehl for COOH-N determinations, to Eleanor Willerton for microbiological amino acid analyses, and to E. O. Krueger for chemical analyses of amino acids.

BIBLIOGRAPHY

PARTIAL ACID HYDROLYSATES OF PROTEINS: VI. ASSAY OF LIQUID PROTEIN HYDROLYSATES IN PROTEIN-DEPLETED RATS
Douglas V. Frost and Harry R. Sandy


Access the most updated version of this article at http://www.jbc.org/content/175/2/635.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/175/2/635.citation.full.html#ref-list-1