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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION. XXIV. STUDIES ON THE NUTRITIVE VALUE OF HUMAN PLASMA FRACTIONS [1]

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Dried human blood plasma was previously found (1) to be a protein of very poor nutritive value as measured by its ability to support growth in young rats when it served as the only source of protein. Isoleucine was shown to be the chief amino acid deficiency under these conditions. These results with human plasma and young growing rats may be contrasted with the results obtained by some workers (2) and by others using bovine plasma and hypoproteinemic dogs. They found that bovine serum proteins serve well for the regeneration of serum proteins in such dogs. Whether these differences with respect to the nutritional qualities of the plasma proteins represent (*a*) differences in the requirement of the 2 species, (*b*) differences in the requirement of animals of different ages, (*c*) differences in the requirement for growth as compared to serum regeneration, or (*d*) a variation in the amino acid composition of the proteins used is as yet unknown.

The nutritive value of plasma or of the various plasma proteins may be of importance since certain individuals may receive them as the sole source of protein at a time when protein requirements are especially high. Albumin is the protein most abundant in plasma and the protein which may be used in relatively large amounts in intravenous therapy. It was of interest, therefore, to determine the nutritive value of this protein as well as the other plasma proteins available in sufficient amounts for study and possible therapeutic use. In addition to the rat growth studies, we report observations on the nutritive value of human albumin as measured by another technique, namely, nitrogen balance in the adult dog.

EXPERIMENTAL

Rat studies. As in the previous work with plasma using young growing rats (1), the plasma fractions replaced sucrose in the purified basal ration composed of salt mixture 4 per cent, corn oil 4 per cent, and sucrose to 92 per cent. Sufficient amounts of the protein supplements including amino acids, if these were added, were used to give a protein content (N × 6.25) of 20 per cent. Thiamine chloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, nicotinic acid, and choline chloride were added in excess of requirements as the crystalline compounds, and haliver oil fortified with viosterol was fed by dropper twice weekly.

In Experiment I, groups consisting of 3 rats each, average weight 52 grams, were fed rations in which the only source of protein was gamma-globulin, fibrin or albumin

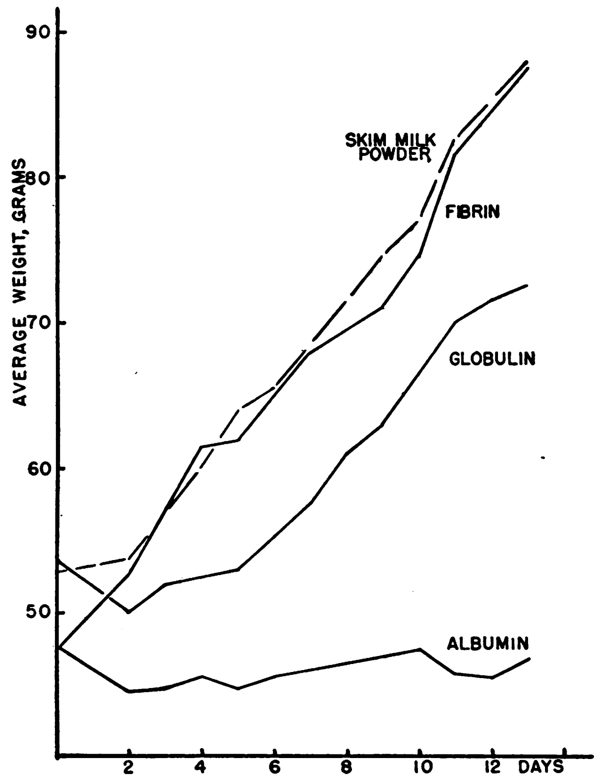

FIG. 1. GROWTH CURVES OF RATS RECEIVING VARIOUS PROTEINS

[1] The products of plasma fractionation employed in this work were developed by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

This paper is Number 34 in the series "Studies on Plasma Protein" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

from human plasma. Skim milk powder was the protein used for the control animals. The growth curves are shown in Figure 1. Albumin supported no growth at all over this period while fibrin compared favorably with skim milk powder and globulin was intermediate between these 2 extremes. As the previous experiments with whole plasma had shown it to be a relatively poor protein (1), it was evident that the amounts of fibrin and globulin present in plasma are too low to supplement albumin adequately.

Since isoleucine is the limiting amino acid in plasma, it was assumed that it was also limiting in albumin. However, the composition of the plasma proteins as given by another group of workers (3) shows a very low content of tryptophane for albumin. These 2 amino acids were investigated in Experiment II, the growth curves of which are shown in Figure 2. Four groups of young rats were fed rations containing 20 per cent albumin with the following additions: Group 1, none; Group 2, 0.3 per cent *l*(–)-tryptophane; Group 3, 1.2 per cent *dl*-isoleucine; and Group 4, 0.3 per cent *l*(–)-tryptophane and 1.2 per cent *dl*-isoleucine. Group 4 was discontinued after a few days when it became apparent that growth was rapid upon this ration. The other 3 groups were continued upon their respective rations until the tenth day when both amino acids were added to each ration. The growth response was immediate in all cases showing that albumin is so low in both of these amino acids as to completely prevent growth. Isoleucine and tryptophane are certainly the chief amino acid deficiencies for the growing rat when albumin is the sole source of protein.

The approximate amount of each of these amino acids that is necessary to give optimum growth in young rats was determined in Experiments III and IV. In Experiment III, 28 rats weighing approximately 60 grams each were equally divided into 7 groups. Each group received the basal ration containing 1 per cent *dl*-isoleucine and sufficient albumin to give a total of 20 per cent protein (N × 6.25) in the ration. *l*(–)-Tryptophane was added at the following levels: 0.0, 0.01, 0.02, 0.03, 0.05, and 0.075 per cent of the ration. In Figure 3, the gain in weight of these groups during a 15-day period is plotted against the

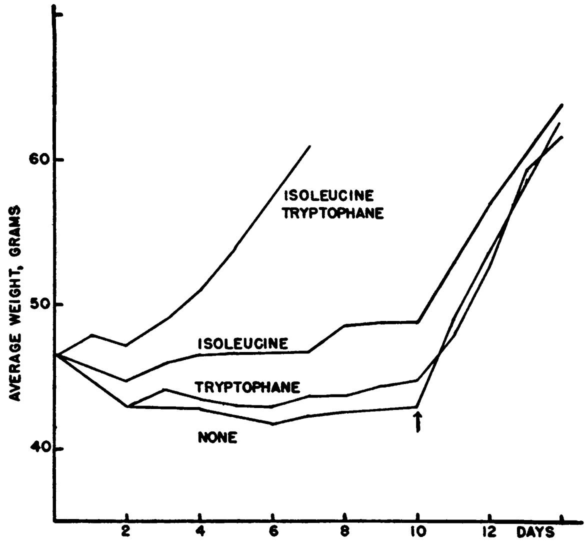

FIG. 2. THE EFFECT OF ISOLEUCINE AND TRYPTOPHANE SUPPLEMENTS ON THE GROWTH OF RATS RECEIVING ALBUMIN AS THE SOLE SOURCE OF PROTEIN (BOTH AMINO ACIDS ADDED ON THE TENTH DAY)

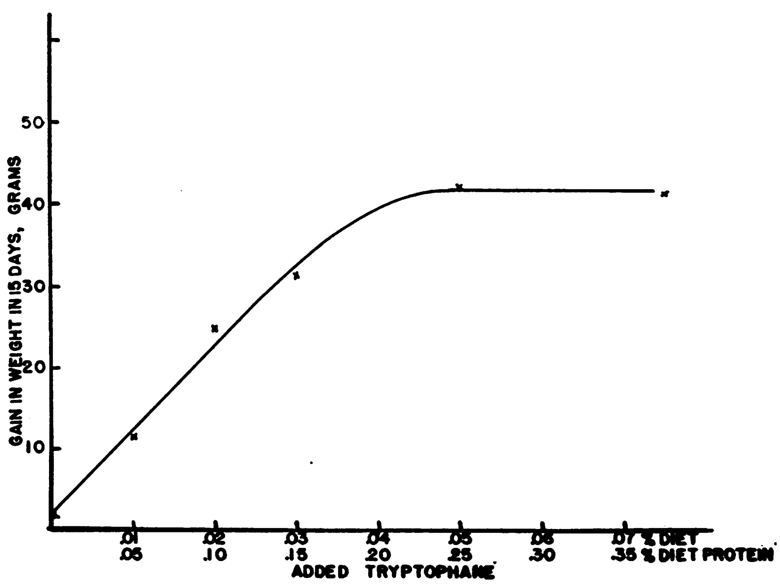

FIG. 3. GAIN IN WEIGHT ON VARIOUS LEVELS OF TRYPTOPHANE ADDED TO ALBUMIN SUPPLEMENTED WITH 1 PER CENT *dl*-ISOLEUCINE

TABLE I

Rat growth in relation to tryptophane intake and protein efficiency

Group	Albumin in diet*	$l(-)$-Tryptophane added		Total tryptophane content†		Protein consumed per rat per day	Tryptophane consumed per rat per day	Gain in weight per rat per day	Protein‡ efficiency
		To diet	To dietary protein	Diet	Dietary protein				
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*grams*	*mgm.*	*grams*	
1	19.34	0.0	0.0	0.035	0.175	1.00	1.75	0.116	0.12
2	19.34	0.01	0.05	0.045	0.225	1.44	3.24	1.44	1.00
3	19.34	0.02	0.10	0.055	0.275	1.49	4.10	1.67	1.12
4	19.34	0.03	0.15	0.065	0.325	1.53	4.97	2.07	1.35
5	19.34	0.05	0.25	0.085	0.425	1.81	7.70	2.81	1.55
6	19.34	0.075	0.375	0.110	0.550	1.72	9.40	2.74	1.59

* One per cent isoleucine supplies nitrogen equivalent to 0.66 per cent protein. This amount was added to all diets.
† Calculated from the tryptophane content of albumin as determined by Dr. Brand.
‡ Grams—gain per gram of protein eaten.

level of tryptophane added to the ration. Maximum growth was obtained at approximately 0.05 per cent of added $l(-)$-tryptophane. Since this albumin sample contained 0.18 per cent of tryptophane,[2] the albumin in the ration would add 0.035 of tryptophane to the diets. The diet, which appears optimum, thus contained a total of 0.085 per cent $l(-)$-tryptophane. According to these results, a protein, when fed at a 20 per cent level, would have to contain at least 0.42 per cent $l(-)$-tryptophane in order to support good growth in the young rat.[3] The data on food intake and the calculations shown in Table I indicate a requirement of about 8 mgm. of tryptophane per day for these rats which had an average weight during the experiment of 85 grams. The animals received about 94 mgm. per kgm. of body weight per day. In our experience comparable animals of this strain gain from 3 to 4 grams per day on purified rations containing 18 per cent casein. Thus the maximum growth obtained in this experiment was somewhat less than might have been expected and considerably less than obtained in the next experiment discussed below. It is possible that the optimum level of tryptophane may be somewhat higher for groups of animals which show better growth.

The data on a similar experiment in which tryptophane was added in constant amount, in excess of the requirement found in the previous experiment, and the level of *dl*-isoleucine was varied are shown in Figure 4 and Table II. Maximum growth was obtained when approximately 0.9 per cent of *dl*-isoleucine was added to the diet. The isoleucine content of the albumin was determined by microbiological methods. The results ranged from 1.5 to 1.8 per cent isoleucine, average 1.7 per cent. Some of the assays were made using the original media of Hegsted (5), and others with a medium in which the amounts of the amino acids in the basal medium were raised to approximate those used by other investigators (6). This change did not affect the results obtained. The calcula-

[2] The authors are greatly indebted to Dr. E. Brand for determining the tryptophane content of the albumin used in these studies.

[3] Acetyltryptophane was also tested as a source of tryptophane since this may find use as a stabilizer for albumin solutions (Dr. E. J. Cohn, personal communications). In confirmation of the results of du Vigneaud, Sealock, and Van Etten (4), acetyl-*dl*-tryptophane proved less effective than *dl*-tryptophane, but amounts effective for stabilization (1 to 2 per cent of the albumin) gave good growth.

TABLE II

Rat growth in relation to isoleucine intake and protein efficiency

Group	Albumin in diet*	$l(+)$-Isoleucine added†		Total $l(+)$-isoleucine content‡		Protein consumed per rat per day	Isoleucine consumed per rat per day	Gain in weight per rat per day	Protein efficiency
		To diet	To dietary protein	Diet	Dietary protein				
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*grams*	*mgm.*	*grams*	
1	20.0	0.0	0.0	0.34	1.70	0.93	15.8	0.0	0.0
2	19.6	0.25	1.25	0.58	2.90	1.51	43.8	2.68	1.77
3	19.5	0.35	1.75	0.68	3.40	1.63	55.4	3.23	1.98
4	19.4	0.45	2.25	0.78	3.90	1.77	69.0	4.00	2.26
5	19.3	0.55	2.75	0.88	4.40	1.75	77.0	4.18	2.39

* All diets were supplemented with 0.25 per cent *dl*-tryptophane.
† Added as *dl*-isoleucine.
‡ Calculated on basis of isoleucine content of albumin as 1.70 per cent.

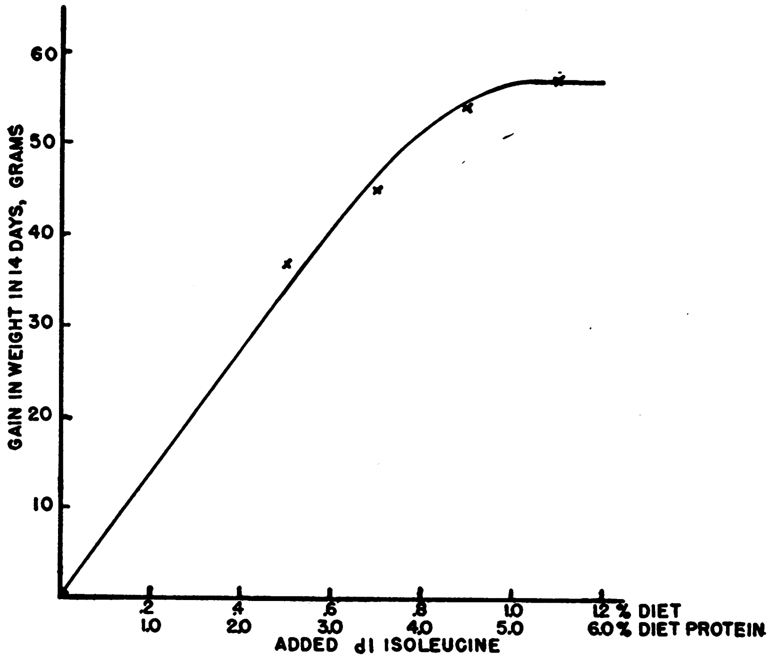

FIG. 4. GAIN IN WEIGHT ON VARIOUS LEVELS OF ISOLEUCINE ADDED TO ALBUMIN SUPPLEMENTED WITH 0.25 PER CENT *dl*-TRYPTOPHANE

tions in Table II are based upon the average figure of 1.70 per cent isoleucine in albumin and on the assumption that only the natural isomer in *dl*-isoleucine is active (7). The isoleucine content of the ration producing maximum growth thus appears to be 0.78 per cent which corresponds to an isoleucine content of the dietary protein of 3.9 per cent. The isoleucine intake of these animals was about 70 mgm. per day, and their average weight during the experiment was 80 grams. On a body weight basis, the intake is thus about 8 or 10 times the tryptophane requirement, approximately 880 mgm. per kgm. per day. In this and in the tryptophane experiment, calculations made at various intervals during the experiment, when both food intake and weight were somewhat less, do not change these results.

Nitrogen balance studies with the dog. The details of this study will be reported later in connection with other similar studies. We have determined the minimum amount of protein required to maintain nitrogen balance in the adult dog fed a purified ration similar to that used in the rat growth experiments, although somewhat higher in fat. With albumin as a sole source of nitrogen, approximately 0.14 gram of nitrogen per kgm. per day was required to maintain positive balance. This corresponds to a protein requirement of 0.875 gram per kgm. per day. If one assumes that tryptophane and isoleucine are the limiting amino acids for the dog, as they definitely are for the growing rat, then the tryptophane requirement would be about 1.6 mgm. per kgm. per day, and the isoleucine requirement about 15 mgm. per kgm. per day. These figures represent studies made over 6-day periods on each level of albumin studied, previous to which the animal had received the "nitrogen-free" basal ration for 10 days.

DISCUSSION

The striking difference obtained with the growing rat and the adult dog when albumin is the sole source of nitrogen is worthy of consideration. On a caloric basis, it was found that when 19 per cent of the calories were derived from albumin by the young rat, the protein was completely inadequate for growth, while approximately 6 per cent of the caloric intake of the adult dog from this source sufficed for maintenance of nitrogen balance. It is well known that other proteins of high biological value will prove adequate for either species when fed at these respective levels. Calculations upon the tryptophane and isoleucine requirements on the body weight basis for these 2 species further emphasize the great difference in requirement. It is realized that such differences may be due to the physiologic state, *i.e.*, growth *versus* maintenance, but until there is adequate evidence, one should not apply data obtained with 1 animal to others, including man.

It is thus readily apparent that before significance can be assigned to the low tryptophane and isoleucine content of albumin with regard to intravenous feeding of man, direct studies upon the human must be obtained. Studies are also needed to determine whether the reestablishment of nitrogen equilibrium and the restoration of the losses of body nitrogen after injury demand protein supplies similar to those of growth or of maintenance.

The requirement of tryptophane for the growing rat as determined in these studies is about 0.08 per cent of the diet compared to Rose's (8) figure of 0.2 per cent. The requirement of isoleucine, on the other hand, of approximately 0.8 per cent is somewhat higher than that given by Rose which is 0.5 per cent. The above figure of 0.8 per cent compares to a figure of 0.7 per cent calculated in a previous study (1). Block (9) has calculated the amino acid requirements of the human upon the basis of the requirements of the young rat as determined by Rose (8). These studies point out the futility of such calculations.

SUMMARY

1. For the growth of rats, human fibrin is a high quality protein, while human albumin supports no growth at all when fed as the sole source of protein. Globulin is intermediate between the two.

2. For the growth of rats, human albumin is relatively deficient in tryptophane and isoleucine.

3. Maximum growth was obtained in rats with rations containing approximately 0.08 per cent *l*(−)-tryptophane and 0.8 per cent *l*(+)-isoleucine. These amounts correspond to intakes of 94 mgm. and 880 mgm. per kgm. of body weight per day, respectively.

4. In contrast to the poor nutritional quality of albumin as found in rat growth studies, nitrogen balance in the adult dog was maintained when this protein supplied only 6 per cent of the calories. It is estimated that no more than 1.6 mgm. of tryptophane and 15 mgm. of isoleucine per kgm. of body weight per day are required by the adult dog.

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