Nutritive Inadequacy of Whole Blood to Support Protein Repletion. (21219)

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The nutritive inadequacy of human hemoglobin, due to a primary deficiency of isoleucine, has been convincingly shown in studies with rats(1-3). Human plasma is also primarily deficient in isoleucine(4). Only fibrin, among the blood proteins, proved of highest value(5). As previously shown(6,7) protein-depleted rats avoid amino acid solutions which are devoid of one or more of the essential amino acids. Similarly an acid hydrolysate of whole hog blood was accepted only after fortification with the missing amino acids(8). The objectives of this study were to determine whether protein-depleted rats would accept whole human blood unfortified and fortified with the limiting amino acids (isoleucine and methionine). A fibrin hydrolysate of known nutritive value(9,10) was used as a standard.

Experimental. Whole blood. Whole human blood used was regular bank blood collected sterilely under vacuum in standard anticoagulant solution (Formula B, U.S.P. and N.I.H.). According to this formula each 100 cc of whole blood is made to contain dextrose 1.47 g, sodium citrate 1.32 g, and citric acid 0.48 g. Phenylmercuric nitrate, 0.002%, was added as preservative after bottle seals were broken. Blood was stored in refrigerator thereafter and removed only for use in feeding. By Kjeldahl analysis blood was found to contain 2.57% nitrogen. For feeding purposes 4.7 cc blood was diluted with water to 18-19 cc to provide 120 mg N/rat day. This dilution was chosen to produce a nitrogen content comparable to that of 5% fibrin hydrolysate. *Repletion assay.* Young adult male rats, 150-200 g were prepared for assay by 12-day depletion on non-protein diet and by orientation to protein solution feeding by the usual method(8). Basal diet contained: sucrose 40, dextrin 30, lard 18, corn oil 3, agar 2.5, haliver oil 0.5, wheat germ oil 0.25, choline chloride 0.3, and Jones and Foster salt mixture 4.0 g, thiamin • HCl 0.6, riboflavin 1.2, pyridoxine • HCl 0.6, calcium pantothenate 5.0, nicotinic acid 3.7, mixed tocopherols 2.5, folic acid 0.1, inositol 5 mg, and vit. B_{12} 3 μg/100 g of diet. Water was supplied by drinking fountain, except during assay or drink trial when the test solution, or fibrin hydrolysate, were offered in place of water.

Acceptance trial. In the first trial 10 male young adult rats were selected which had been used for one 12-day assay of an experimental fibrin hydrolysate. These rats consumed the complete allotment of 5% fibrin hydrolysate, 0.24 g N/day, throughout the assay period and all gained weight at a rate greater than 4 g/day. Following this assay they were redepleted for 12 days on non-protein diet. Rats prepared in this way show an inordinate interest in their drinking fountains and immediately drink some of any new solution offered them. When the diluted whole human blood was offered to 5 of these depleted rats, fairly good initial acceptance was shown. An average of 15.4 cc (range 9-18.4 cc) was consumed the first day. This was 83% of the allotment of 18.4 cc, equivalent to 120 mg N/rat day. Thenceforth, however, very little interest was shown in the blood. Fresh aliquots of diluted blood were offered daily, but the rats showed little interest in the solution. An average of only 2.6 cc (0-5 cc)/day was consumed for the next 8 days and the rats continued to lose weight at nearly the same rate as they had on non-protein diet. As positive control, a 5% fibrin hydrolysate was offered to the remaining 5 rats at 120 mg N/rat day. This was consumed completely by all rats within a few hours, and all 5 rats gained weight rapidly. This simple experiment clearly indicated that whole blood is not acceptable to the protein depleted rat. It remained to determine whether taste aversion or amino acid inadequacy of whole blood accounted for this failure.

Response to fortified blood. The 5 depleted rats which did not accept the whole human

blood, as described above, were repleted on stock diet for 10 days and again redepleted on non-protein diet for 12 days. They were then offered whole human blood to which had been added 1.5% *DL*-isoleucine and 0.32% *DL*-methionine. The latter were dissolved by cautious warming and agitation. By calculation these simple additions should correct the nutritive imbalance in the amino acid content of whole blood. Response of all 5 rats to this fortified whole blood was actually more prompt and dramatic than we had expected. Beginning with the initial offering all rats consumed the full daily allotment of 18.4 cc/day equal to 120 mg N. Intake of allotment was 100% for the 12 days feeding. Average weight gain was 15 g, range 7-20 g. These data indicate that nutritive inadequacy of whole blood is sufficient to account for its apparent distaste to protein-depleted rats.

A further test was made to compare fortified whole blood with 5% fibrin hydrolysate. Unfortified blood was included in this assay as a negative control. Fifteen male rats, 140-190 g, depleted for 12 days on non-protein diet, were prepared by the standard drink trial and divided into 3 equal groups. The 3 groups respectively were offered equal amounts of 5% fibrin hydrolysate, whole human blood, and whole blood plus 1.5% *DL*-isoleucine and 0.32% *DL*-methionine. All solutions were adjusted with water so that 18 cc contained 120 mg N, the daily allotment.

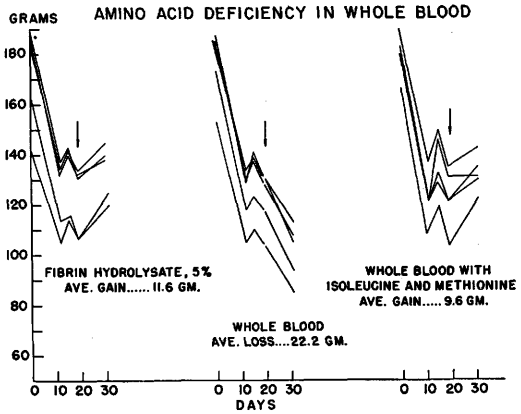

FIG. 1. Response of protein depleted rats to daily supplements of 3 test solutions. In each case the rats were depleted 12 days on non-protein diet and prepared for assay by 3-day drink trial on a standard hydrolysate followed by 3-day redepletion. During assay rats were offered 18 cc equivalent to 120 mg N per day of respective solutions as shown.

Fig. 1 shows the weight changes for the 12-day feeding test. Acceptance of the whole blood alone was incomplete, as before. An average of only 5.6 cc/rat day was taken by this group. Again the rate of weight loss was only slightly less than when no protein was fed. Conversely, intake of allotment was 100% for the group which received the fortified whole blood and weight gain was almost as great as that for the fibrin hydrolysate.

Discussion. The severity of isoleucine deficiency was described by Rose *et al.*(11), who state, "no other type of amino acid deficiency produces in man so prompt and so profound a nutritive failure. On the 4th day of isoleucine deprivation the young volunteer subjects 'complained bitterly of a complete loss of appetite' and showed symptoms of nervousness, exhaustion, and dizziness which were exaggerated to a degree not observed before or since in other types of dietary deficiencies." In view of this one wonders about the effect of long continued injections of whole blood to patients unable to ingest isoleucine-containing foods in any form.

In spite of the lack of clear supporting evidence it is not uncommon to ascribe high nutritive value to both human plasma and whole blood. Traditional belief in the intrinsic nutritional value of whole blood and plasma received support from the extensive early work of Whipple *et al.*(12), which was carried out chiefly with dog blood, or blood components, in dogs. On the other hand, Robscheit-Robbins *et al.*(13) reported that human globin failed to maintain weight and nitrogen balance in hypoproteinemic dogs, whereas dog globin did maintain weight and nitrogen balance. Miller(14) further demonstrated that only methionine supplementation to dog blood is needed for maintenance of N balance in dogs. By way of explaining these differences in nutritive value between human and dog hemoglobin, Brand and Grantham (15) then found that human hemoglobin contains 1.32% methionine, but no isoleucine. Conversely, dog hemoglobin contains by their analysis 0.42% methionine and 1.36% isoleucine. Both isoleucine and methionine would

obviously be needed to supplement human blood as the sole source of protein.

The value of plasma and whole blood for treatment of shock and for blood replacement is beyond question. On the other hand, they are poorly balanced nutritionally, and this fact should be clearly recognized. In a recent review Pollack and Halpern(16) describe some of the limitations of whole blood and plasma from the nutritive standpoint. These authors emphasize the fact that neither plasma nor blood are immediately available for nutritional purposes, but do not discuss the fundamental question of the adequacy of their amino acid make-up. Actually, however, we have been unable to find any report in the literature of the essential amino acid composition of whole human blood. The available data on individual amino acid content of certain of the constituent proteins of whole human blood are reviewed by Tristram(17). Amino acid analysis of whole human blood will be undertaken.

Summary. The inadequacy of whole human blood to serve as the sole dietary source of amino acids for repletion in the protein-depleted rat is demonstrated. Fortification of blood with isoleucine and methionine, the 2 indispensable amino acids in which blood is most deficient, largely corrects this nutritive inadequacy.

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