Effects of Mineral Deficiencies on Amino Acid Utilization. Critical Role of Potassium and Phosphorus. (20278)

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The acute metabolic disturbance which attends the simultaneous deficiencies of protein and potassium has been strikingly demonstrated by Cannon, Frazier and Hughes(1,2). Their studies show that potassium deficiency limits protein regeneration and that the rate of amino acid utilization under certain dietary circumstances may be proportional to the dietary adequacy of potassium. Part of the University of Chicago study was carried out with Aminosol,* a commercial fibrin hydrolysate, known to be almost devoid of potassium. Fibrin itself is low in all inorganic elements and has been used to advantage in designing a low phosphorus diet(3). In preparation of Aminosol, the fibrin is well washed prior to hydrolysis. Particular attention is paid to avoidance of sodium throughout manufacture. The nutritive balance of essential amino acids in this tryptophan fortified hydrolysate of fibrin is favorable, as demonstrated by the low nitrogen input required to bring about rapid protein regeneration in dogs(4), and rats(5,6). Daily requirements of the rat for some of the important mineral elements have been reported by Farris and Griffith(7) as follows: potassium 8-15 mg, calcium 40-50 mg, phosphorus 35-45 mg, and magnesium 1 mg. Twenty ml of the 5% fibrin hydrolysate, an amount which supplies amino acids for rapid recovery in protein-depleted rats, provided only about 0.1 mg of potassium, and not more than 0.02 mg of calcium, phosphorus or magnesium per rat day.

The present studies were designed to determine the influence of deficiencies of the above four mineral elements and of sodium on the response of protein-depleted rats to the fibrin hydrolysate.

Experimental. The non-protein diet, NP 11, used in these studies was essentially as before(5), except that it contained a higher proportion of fat. Also a change was made to Jones and Foster(3) salt mixture, which is well adapted to this type study. The composition is: 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 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 and inositol 5 mg per 100 g of diet.

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TABLE I. Complete Salt Mixture and Mixtures Minus K, P, Ca, and Mg.

Salt mixture*	KH_2PO_4, %	$MgSO_4$, %	$CaCO_3$, %
Complete	38.9	5.7	38.1
Minus Ca	38.9	5.7	—
" Mg	38.9	—	38.1
" K	($NaH_2PO_4H_2O$—38.9)	5.7	38.1
" P	(KCl—21.2)	5.7	38.1

* Each salt mixture also contained NaCl 13.9, $FeSO_4 \cdot 7H_2O$ 2.7, $MnSO_4 \cdot 2H_2O$ 0.45, KI 0.071 $ZnSO_4$ 0.055, $CuSO_4 \cdot 5H_2O$ 0.048, and $CoCl_2$ $6H_2O$ 0.002%. Sucrose replaced the $CaCO_3$ and $MgSO_4$ when these were omitted.

1) *Deficiencies of K, P, Ca and Mg.* Male rats, 170-190 g, raised on the colony stock diet were depleted on non-protein diet in the usual way(5). Following drink trial and redepletion, five uniform groups of 6 rats each were formed. All rats were allotted 18.5 ml of 5% fibrin hydrolysate daily to supply 120 mg N. As a positive control, one group was continued on the non-protein diet adequate in all nutrients except protein. The test groups were fed non-protein diet in which the salt mixture was altered to impose a different mineral deficiency on each of the 4 groups. Table I shows the composition of the salt mixture(3) used in the non-protein diet, together with the changes devised to eliminate potassium, phosphorus, calcium or magnesium individually. The salt mixtures were all used at 4% of the non-protein diet. The 5 groups were fed the 5 different diets *ad libitum* for 11 weeks. The results are summarized in Fig. 1.

Deficiency of calcium or magnesium is surprisingly well tolerated in the protein-depleted

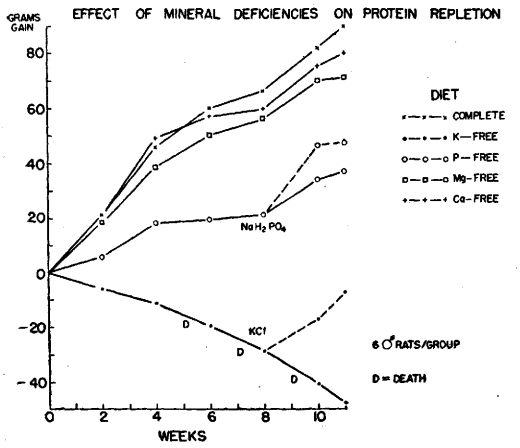

FIG. 1. Response of protein depleted rats to an intravenous fibrin hydrolysate (Aminosol) when fed *ad libitum* a non-protein diet complete in all mineral requirements, or deficient in the single elements potassium, phosphorus, magnesium or calcium.

rat, whereas lack of potassium becomes manifest very soon. Total deficiency of phosphorus places a less critical immediate stress on the organism than deficiency of potassium, but nevertheless manifests itself promptly in the decreased ability to build new tissue. A prompt weight response was observed when deficiencies of either potassium or phosphorus were corrected in individual rats. All of the potassium deficient rats became moribund and showed signs of hemorrhage.

2) *Quantitative requirement for K.* According to Cannon, *et al.*(2), 20 mg of KCl (10.5 mg K) per rat day is about the critical level of potassium needed to support full protein repletion. The Chicago studies were made using a solution of synthetic and purified amino acids practically devoid of potassium. We were interested to obtain the critical requirement for potassium in the 5% hydrolysate of fibrin which contained naturally only 2-3 μg K per cc. Five groups of 6 male rats each, which had been used twice previously for routine repletion assays, were fed stock colony diet for one week for equilibration and then depleted 17 days on non-protein diet. The weight loss during the depletion period was 20-25%. At the start of assay, the rats were transferred to the non-protein diet containing salt mixture minus potassium phosphate (Table I). One group was offered 36.5 ml of the fibrin hydrolysate equal to 0.24 g N per day with no added potassium. Other groups were offered the same volume of hydrolysate with graded amounts of KH_2PO_4 added to supply 1.5, 3.7, 7.5 and 15 mg potassium and 1.1, 2.7, 5.5 and 11 mg phosphorus respectively per day. The assay was carried for 12 days. Table II shows the average weight gain and per cent intake of allotment for each group.

The data, as regards potassium requirement, are in accord with those of Cannon, *et al.*(2) indicating that a full repletion response is not obtained with 7-8 mg K per rat day. The Chicago data suggest that 10.5 mg K per day as KCl is adequate. The 45.6 g response of our rats which received 14 mg K per rat day is considered about maximal at the 0.24 g N feeding level(5). The separate requirement for P will be considered in another report. Ten milliequivalents per liter of KH_2PO_4 in Aminosol appears to supply both K and P in adequate amount. Apparently 11 mg P per rat day is above the critical requirement level.

3) *Effect of sodium deficiency.* Comparison was made of the effects of sodium versus potassium deprivation. Non-protein diet was made up as free as possible of sodium. $MgCl_2 \cdot 6H_2O$ was used in the salt mixture in place of $MgSO_4$ and NaCl. The amount used provided twice as much Mg and 85% as much Cl as the regular salt mixture (Table I). KI was used to replace NaI. Potassium free salt mixture was prepared for this experiment by substituting calcium glycerophosphate for KH_2PO_4. This avoided the excess of sodium which had prevailed in the K-free diet in the first experiment (Table I). The amount of calcium glycerophosphate used provided 65% as much P and 150% as much Ca as the normal salt mixture. These changes did not significantly alter the concentrations of other ions in the diet. Three groups of 5 standard protein-depleted male rats were formed. Aminosol 5% with dextrose 5%, U.S.P. (Lot 617-5907R) was fed at a level of 18 ml to provide 120 mg N per rat day. This supplied only 3.2 mg of sodium per rat day, whereas Farris and Griffith(7) cited 40 mg Na per day as optimal. Intake of the

TABLE II. Effect of Adequacy of Potassium and Phosphorus, as KH_2PO_4, on Amino Acid Utilization by Protein-Depleted Rats.

KH_2PO_4, mQv, added/liter Aminosol*	K supplied/ rat day, mg	Intake of allotment, %	Avg 12 day wt gain and stand. error, g
0	.07	81	4.3 ± 3.3
1	1.4	90	3.6 ± 2.7
2.5	3.5	93	16 ± 2.7
5.0	7	100	27.6 ± 4.4
10.0	14	100	45.6 ± 3.0

* 36.5 ml of Aminosol, 5%, with dextrose, 5%, (serial 615-5914) fed/rat day with KH_2PO_4 additions as shown. Six male rats/group.

TABLE III. Comparison of Sodium and Potassium Deprivation on Ability of Protein-Depleted Rats to Use Amino Acids.

Deficiency in non-protein diet	Avg 12 day wt gain and stand. error, g
Control-complete	19.4 ± 1.5
Na	24.2 ± 3.1
K	4.0 ± 2.6

18.3 ml Aminosol, 5% in dextrose, 5% supplied 3.2 mg sodium and .04 mg potassium/rat day to all groups. The full supplement was consumed by all rats throughout.

daily allotment was complete for all rats throughout the 12-day assay. Results of the test shown in Table III indicated that sodium withdrawal does not immediately alter utilization of amino acids.

Discussion. A histological study of the heart lesions in protein depleted potassium deficient rats was made by French(8) as a corollary to the nutritional studies of Cannon, *et al.*(2). Myocardial lesions appeared in less than 12 days in the protein depleted animal when a diet deficient only in potassium was supplied. Potassium, supplied following initial deprivation, induced a rapid arrest of the progress of the heart lesions. The least severely damaged muscle fibers appeared to recover in part with compensatory hypertrophy and condensation of the surviving tissue.

The clinical syndrome characterized by potassium deficit and the strategic use of potassium salts to correct potassium imbalance has attracted much recent attention. Eliel, Pearson and White(9) have recently described the use of potassium phosphate supplementation in conjunction with complete parenteral feeding in postoperative patients. Such therapy receives strong theoretical support in our studies. Indeed, judging from the results of our studies, one would need to supply among the major mineral elements only potassium and phosphorus to meet short-term metabolic needs of the surgical patient. Phosphorus plays a less critical role, but its practical and theoretical significance is clearly apparent. Requirement for sodium, magnesium and calcium were apparently met by body stores of the rat for as long as 12 days under the conditions of our experiments.

The recent report of Menaker and Kleiner (10), which appeared after the completion of our studies, attaches more significance to magnesium deprivation than was apparent in our results. These authors indicated that magnesium deficiency did not result in any anatomical or physiological signs of deficiency, other than decrease in appetite and weight gain. Nevertheless they concluded "the unfavorable effect of magnesium deficiency on the synthesis of protein appears to be approximately as great as that reported for potassium deficiency." Although it is not our intention to minimize the relative importance of magnesium, it would seem from both our results and those of Cannon, *et al.*(2) that potassium is more critical. The point is an important one requiring considerable study. It would be impractical to incorporate many mineral elements into amino acid mixtures for intravenous use. Incorporation of potassium in commercial protein hydrolysates is quite practical and is, we believe, well founded nutritionally.

Various considerations suggested that a level of 10 mQv of potassium per liter of 5% protein hydrolysate would supply just enough of this element to support full utilization of amino acids. On the other hand, this level of potassium would offer no danger of inducing hyperkalemia. During fasting N and K are excreted in the ratio 10 to 1, the approximate ratio of these elements in muscle. The addition of 10 mQv K per liter provides a ratio of N to K in Aminosol of approximately 17 to 1, clearly a safe ratio. This allows for separate therapy with balanced electrolyte solutions, including additional K if desired.

Much more work is needed to establish how

best to supply total nutriment parenterally. Part of the problem involves how best to combine amino acids, minerals and calories in the relatively small volumes of liquid which can be given parenterally. At the present stage of development it is generally possible to provide only those nutrients for recuperation to the point where full oral intake is resumed. Potassium appears to fit into this special group of nutrients.

Summary. 1. Results of this study point to the critical role of potassium in amino acid utilization following protein depletion. Rats depleted only of protein failed completely, even when offered a complete source of amino acids, if at the same time potassium was withheld. If phosphorus was withheld during the repletion period, the rate of recovery was lessened, but complete failure and deaths did not result as with potassium deprivation. Deprivation of sodium, calcium or magnesium did not appear to have immediate limiting effects on the appetite of protein depleted rats for a complete amino acid source, or on their ability to recover lost weight. 2. Fourteen mg of potassium per rat day appeared to meet the requirement for this element. Addition of 10 mQv of potassium per liter to an intravenous protein hydrolysate appears to supply an effective, safe ratio of potassium to amino acid nitrogen.

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Isolation and Purification of Plasma Thromboplastin: Its Physico-Chemical and Biologic Properties.* (20279)

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In the course of studies on the isolation of various platelet and plasma factors active in the process of blood coagulation the observation was made that prothrombin and stable factor cannot be adsorbed on $BaSO_4$ from citrated plasma(1,2). A saline eluate from the adsorbent, however, contained a factor able to shorten considerably the clotting time of recalcified human plasma and increase markedly the prothrombin utilization of native platelet-poor human plasma. This article describes some of the properties and the biologic activity of this factor which is tentatively identified as "plasma thromboplastin" (PT).

Materials and methods. (a) *Separation of PT from other coagulation factors.* Blood was collected from healthy individuals in Silicone† coated syringes and through Arquad 2-C‡ coated 19-gauge needles using one-tenth volume sodium citrate 0.2 M as the anticoagulant. Platelet-rich plasma was separated

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† General Electric Dri-Film 9987.

‡ Available from Armour Co., North Chicago, Ill.