# THE EFFECT OF PROTEIN ON THE NICOTINIC ACID AND TRYPTOPHANE REQUIREMENT OF THE GROWING RAT*

By W. A. KREHL, P. S. SARMA, and C. A. ELVEHJEM

*(From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison)*

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Previous work (1, 2) has clearly established that the presence of corn in a synthetic low protein diet alters the dietary nicotinic acid and tryptophane requirement of the growing rat. This relationship between nicotinic acid and tryptophane is not peculiar to corn-supplemented rations, since similar effects can be demonstrated (3) with non-corn rations which are low in both tryptophane and nicotinic acid.

Since tryptophane is most directly concerned with this syndrome, the present report deals with a more extensive examination of proteins other than casein which contain different amounts of tryptophane (*i.e.* egg albumin, fibrin, and soy bean globulin) and their influence in counteracting the effect of corn. Further evidence is also presented to show that the interchangeable rôle of nicotinic acid and tryptophane is intimately related to the nature of the total amino acid content of the ration.

## EXPERIMENTAL

Egg albumin and fibrin which were obtained commercially contained practically no nicotinic acid. Soy bean globulin was prepared according to the method of Teresi *et al.* (4), by extracting ground Illini type soy beans with 10 per cent NaCl solution, and dialyzing the extract in cold running water at temperatures not exceeding 15°. The globulin which precipitated after 48 to 72 hours was removed and dried after washing with ethanol and ether. The globulin was autoclaved at 15 pounds pressure for 1 hour. The protein so prepared was practically free from nicotinic acid.

Each of the proteins was analyzed for total protein (N × 6.25) and sufficient quantities were used to supply 10 and 15 per cent of total protein

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in the diet (Table I). The composition of the different basal diets was as follows:

| | Low protein | High protein |
|---|---|---|
| Sucrose | 83 | 78 |
| Protein | 10 | 15 |
| Corn oil | 3 | 3 |
| Salts IV | 4 | 4 |
| *l*(−)-Cystine | 0.15 | 0.15 |

The final ration was prepared by mixing 60 parts of each of the above rations and 40 parts of corn grits. The vitamins in these and ensuing rations were added in the following amounts per 100 gm. of ration: thiamine 0.2 mg., riboflavin 0.3 mg., pyridoxine 0.25 mg., calcium pantothenate 2.0 mg., choline 100 mg., 2-methyl-1,4-naphthoquinone 0.1 mg., inositol 10 mg., and biotin 0.01 mg. Vitamins A and D were supplied as halibut liver oil, 2 drops every week, together with vitamin E, 1 mg. per week per rat. The basal rations in this and the following series contained practically no nicotinic acid unless added as indicated.

Weanling male rats of the Sprague-Dawley strain, 35 to 45 gm. in weight, were used throughout. At least three rats were used in each group in all experiments, and most groups were repeated to confirm the results. The range of growth for all animals is given in parentheses in Tables I and III. The tryptophane content of the rations was calculated from the figures available in the literature (5) (the value for casein of 1.1 to 1.2 per cent has been verified in this laboratory).

When egg albumin, fibrin, and soy bean globulin were used as proteins, the addition of corn grits had no growth-inhibiting effect at either the lower or upper level of protein, and the addition of nicotinic acid did not cause any significant increase in growth. This is in marked contrast to the results obtained with casein at comparable levels (2). This difference is undoubtedly dependent upon the higher tryptophane content in these proteins as compared with casein. Lysine, arginine, glycine, and methionine were tried at levels of 0.5 per cent in corn-supplemented diets with casein as the protein but all were ineffective in promoting growth. This further emphasizes the specificity of tryptophane.

Since previous results (3) indicated that alterations in the intestinal flora might be responsible for the action of corn in retarding growth, further attempts were made to duplicate these observations with non-corn-containing rations. It was further hoped that by this means the rôle of nicotinic acid in the apparent intestinal synthesis of tryptophane could be clarified.

Originally it had been planned to prepare a ration analogous to the corn-supplemented ration with respect to casein (*i.e.* a 9 per cent level of casein) but since this level of casein supplies a suboptimum amount of several of the essential amino acids other than tryptophane it was decided also to prepare a second basal ration with 6 per cent gelatin in addition to 9 per cent casein. Although experiments conducted at about the same time with wheat gluten and gelatin (3) indicated that undesirable growth might occur when nicotinic acid and tryptophane are simultaneously low, it seemed warranted to suppose that a ration composed of 9 per cent casein and 6 per cent gelatin would allow better growth than a ration which contained only 9 per cent protein as casein. It seemed that this ration had

Table I

*Influence of Different Proteins on Growth-Retarding Effect of Corn Grits*

| Sucrose basal diet | | | Protein content (N × 6.25) | Niacin content | Trypto-phane content | Gain per wk. (range) |
|---|---|---|---|---|---|---|
| Protein | Corn grits | Niacin* | | | | |
| | | | *per cent* | *mg. per cent* | *mg. per cent* | *gm.* |
| 10% fibrin | − | − | 10.0 | <0.01 | 340 | 17 (12–21) |
| 10% " | + | − | 9.6 | 0.25 | 226 | 19 (17–23) |
| 10% " | + | + | 9.6 | 1.25 | 226 | 19 (18–20) |
| 15% " | − | − | 15.0 | <0.01 | 510 | 27 (21–32) |
| 15% " | + | − | 12.6 | 0.25 | 338 | 23 (20–28) |
| 15% " | + | + | 12.6 | 1.25 | 338 | 23 (21–24) |
| 10% egg albumin | − | − | 10.0 | <0.01 | 140 | 15 (13–18) |
| 10% " " | + | − | 9.6 | 0.25 | 106 | 13 (11–14) |
| 10% " " | + | + | 9.6 | 1.25 | 106 | 14 (12–15) |
| 15% " " | − | − | 15.0 | <0.01 | 210 | 19 (18–19) |
| 15% " " | + | − | 12.6 | 0.25 | 148 | 17 (14–21) |
| 15% " " | + | + | 12.6 | 1.25 | 148 | 18 (18–19) |
| 15% soy bean globulin | − | − | 15.0 | <0.01 | 240 | 12 (10–16) |
| 15% " " " | + | − | 12.6 | 0.25 | 166 | 12 (11–13) |
| 15% " " " | + | + | 12.6 | 1.25 | 166 | 12 (7–16) |

* Niacin was added at a level of 1 mg. per 100 gm. of ration.

the further advantage of increased total protein without disturbance of the tryptophane content of the ration, which remained at 108 mg. per cent.

However, the growth results obtained with the rations supplying the two levels of protein were quite different than what had been anticipated. Growth on the 9 per cent casein diet was surprisingly good regardless of the addition of either nicotinic acid or tryptophane, whereas growth on the ration containing 6 per cent gelatin in addition to the 9 per cent casein was extremely poor. More important, however, is the fact that nicotinic acid at a level of 1.5 mg. per cent or tryptophane added at a level of 50 mg. per cent counteracted this poor growth in a manner exactly analogous to that experienced with the corn rations. The composition of these rations and the others used in the rest of the experiments is shown in Table II. The growth results described above are summarized in Table III, Groups 1 through 16.

Inasmuch as the kind of carbohydrate employed in the corn rations had markedly influenced the extent of the growth depression on the corn rations, attempts were made to see whether the same carbohydrate effects could be observed when non-corn rations were employed. It is evident (Table III, Groups 8, 11, and 14) that dextrin, glucose, or corn-starch did not have as marked an influence in counteracting the growth depression resulting from the casein-gelatin diet as they had in the previous corn-supplemented rations (3). This might be due to the slightly lower tryptophane content of the present rations.

Previous results with a wheat gluten-gelatin ration (3) had been thought to be due at least in part to a concomitant lysine deficiency. However, analytical data indicated that the ration also supplied inadequate amounts of other amino acids. To overcome these possible deficiencies, acid-hydrolyzed fibrin prepared according to the method of Berg and Rose (6) was added to the wheat gluten basal ration at a level of 2 per cent. It will be noted that this preparation did not increase the tryptophane content of the ration because of the destruction of this amino acid during acid hydrolysis. Lysine was also added as indicated to bring the level of this amino acid to 1 per cent of the ration. As was the case with casein and gelatin, the addition of 2 per cent acid-hydrolyzed fibrin resulted in growth retardation, which was in this case partially corrected by lysine but completely so by either nicotinic acid or tryptophane (see Table III, Groups 17 through 22). When gelatin at levels of 6 or 10 per cent was added to the wheat gluten ration in addition to 2 per cent acid-hydrolyzed fibrin, the growth depression was additive and was corrected by tryptophane or nicotinic acid, whereas lysine was ineffective (Table III, Groups 23 through 28). It appears that somewhat higher levels of tryptophane would be required to overcome the combined deleterious effect of 10 per cent gelatin and 2 per cent fibrin hydrolysate.

In a simultaneous experiment acid-hydrolyzed fibrin was added at a level of 2 per cent to the 9 per cent casein ration in an attempt to supply more nearly adequate amounts of the essential amino acids lysine, histidine, and threonine, but again very poor growth was obtained. The addition of more histidine and lysine did not improve growth and not until either nicotinic acid or tryptophane was added did good growth result (see Table III, Groups 29 through 33). The further addition of either 3 or 6 per cent gelatin to this basal ration gave evidence of an additive growth-retarding action inasmuch as nicotinic acid did not correct

TABLE II

*Composition of Basal Rations*

| Ration constituent | Ration No. | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| Sucrose | 84 | | | | 78 | | | | 76 | 74 | 68 | 64 | 82 | 79 | 76 | 81 | |
| Dextrin | | 84 | | | | 78 | | | | | | | | | | | 81 |
| Glucose | | | 84 | | | | 78 | | | | | | | | | | |
| Corn-starch | | | | 84 | | | | 78 | | | | | | | | | |
| Casein* | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | | | | | 9 | 9 | 9 | 9 | 9 |
| Wheat gluten† | | | | | | | | | 17 | 17 | 17 | 17 | | | | | |
| Fibrin hydrolysate | | | | | | | | | | 2 | 2 | 2 | 2 | 2 | 2 | | |
| Gelatin | | | | | 6 | 6 | 6 | 6 | | | 6 | 10 | | 3 | 6 | | |
| Zein | | | | | | | | | | | | | | | | 3 | 3 |
| Corn oil | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Salts IV‡ | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| *l*(−)-Cystine | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | | | | | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |

Vitamins were added to all rations at adequate levels; see the text.

* Casein three times extracted with 95 per cent ethanol.

† 17 per cent wheat gluten provided 15 per cent protein.

‡ Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, **109,** 657 (1935).

### Table III

*Growth Results on Various Diets*

| Group No. | Ration No. (Table II) | Diet | Added niacin* | Added tryptophane† | Gain per wk. for 4 wks. (range) |
|---|---|---|---|---|---|
| | | | | | *gm.* |
| 1 | 1 | Sucrose, casein | | | 15 (12–16) |
| 2 | 2 | Dextrin, " | | | 23 (20–28) |
| 3 | 3 | Glucose, " | | | 21 (19–23) |
| 4 | 4 | Corn-starch, casein | | | 26 (24–29) |
| 5 | 5 | Sucrose, casein, gelatin | | | 3 (2– 5) |
| 6 | 5 | " " " | + | | 18 (16–21) |
| 7 | 5 | " " " | | + | 24 (21–28) |
| 8 | 6 | Dextrin, " " | | | 10 (8–14) |
| 9 | 6 | " " " | + | | 24 (21–29) |
| 10 | 6 | " " " | | + | 21 (20–30) |
| 11 | 7 | Glucose, " " | | | 11 (7–14) |
| 12 | 7 | " " " | + | | 21 (19–23) |
| 13 | 7 | " " " | | + | 20 (19–20) |
| 14 | 8 | Corn-starch, casein, gelatin | | | 10 (8–11) |
| 15 | 8 | " " " | + | | 20 (18–22) |
| 16 | 8 | " " " | | + | 22 (20–24) |
| 17 | 9 | Sucrose, wheat gluten, lysine | | | 17 (15–18) |
| 18 | 9 | " " " " | + | | 16 (15–17) |
| 19 | 10 | " " " fibrin | | | 4 (3– 5) |
| 20 | 10 | " " " " lysine | | | 14 (13–15) |
| 21 | 10 | " " " " " | + | | 20 (17–24) |
| 22 | 10 | " " " " " | | + | 21 (19–23) |
| 23 | 11 | " " " " gelatin, lysine | | | 6 (5– 7) |
| 24 | 11 | " " | + | | 18 (16–20) |
| 25 | 11 | " " | | + | 23 (20–25) |
| 26 | 12 | " " | | | 5 (4– 5) |
| 27 | 12 | " " | + | | 20 (14–24) |
| 28 | 12 | " " | | + | 14 (12–16) |
| 29 | 1 | Sucrose, casein, lysine, histidine | | | 20 (16–24) |
| 30 | 13 | " " fibrin | | | 2 (2– 3) |
| 31 | 13 | " " " lysine, histidine | | | 4 (3– 6) |
| 32 | 13 | " " " " " | + | | 20 (13–26) |
| 33 | 13 | " " " " " | | + | 22 (20–24) |
| 34 | 14 | " " " gelatin, lysine, histidine | | | 3 (2– 5) |
| 35 | 14 | " " | + | | 18 (16–19) |
| 36 | 14 | " " | | + | 26 (22–29) |
| 37 | 15 | " " | | | 5 (3– 5) |
| 38 | 15 | " " | + | | 15 (14–17) |
| 39 | 15 | " " | | + | 22 (21–23) |
| 40 | 15 | " " | | + | 24 (22–27) |
| 41 | 16 | Sucrose, casein, zein, lysine, histidine | | | 3 (2– 5) |
| 42 | 16 | " " " " " | + | | 22 (21–24) |
| 43 | 16 | " " " " " | | + | 23 (20–25) |
| 44 | 17 | Dextrin, " " " " | | | 12 (8–16) |

TABLE III—*Concluded*

Lysine and histidine refer to $l(+)$-lysine and $l(-)$-histidine respectively, which were added as the hydrochlorides in such amounts as to supply the minimum requirement of each; *i.e.*, 1.0 and 0.4 per cent respectively. Fibrin refers to fibrin hydrolysate.

* Niacin added at a level of 1.5 mg. per cent.

† Tryptophane added at a level of 50 mg. per cent except for Group 40 for which the level was 100 mg. per cent.

this growth depression as adequately as before and since larger amounts of tryptophane seemed to give somewhat better growth (see Table III, Groups 34 through 40).

Although the 17 per cent wheat gluten rations contain a little more tryptophane than the 9 per cent casein ration, *i.e.* 120 and 108 mg. per cent respectively, results obtained with these two proteins plus tryptophane-free proteins were remarkably comparable.

From the above results it is quite clear that rations which are deficient in nicotinic acid and marginal in their tryptophane content are inadequate for good rat growth.

Since this is the condition that prevails in corn-supplemented rations, the effect of zein, the principal protein in corn, was tested by adding it at a level of 3 per cent to a 9 per cent casein ration at the expense of carbohydrate. (This level of zein is about that contained in the corn-supplemented ration.) The very poor growth obtained with zein (Table III, Group 41) unless nicotinic acid or tryptophane is added (Table III, Groups 42 and 43) extends the observations made with gelatin and acid-hydrolyzed fibrin and indicates quite strongly that the deleterious action of corn in creating a nicotinic acid deficiency or increased tryptophane requirement in the rat is intimately related to the protein or more correctly to the distribution of the $\alpha$-amino acids in the protein of this cereal.

## DISCUSSION

The addition of corn grits to diets containing fibrin and egg albumin reduced the protein level from 15 to 12.6 per cent and caused a very slight decrease in the growth of rats. This decrease, however, was not prevented by nicotinic acid, and cannot be compared to the drastic growth retardation which prevails when casein is used at comparable levels. The protective action of fibrin and 15 per cent egg albumin can be attributed to their respective tryptophane contents. Growth on the 10 per cent egg albumin ration was quite poor and consequently little can be said concerning the effect of corn grits.

The present observations confirm the findings of Hegsted *et al.* (7) that the tryptophane requirement of the growing rat under normal conditions is considerably less than the level of 0.2 per cent set by Rose (8). It should be noted, however, that the diet used by Rose in establishing this level was a relatively high fat, high calorie diet, while the diets employed here are low in fat. That the definition of normal conditions can be somewhat ambiguous is exemplified by the above results in which the tryptophane requirement of the rat on a nicotinic acid-free diet was increased from about 0.1 per cent or less of the ration to about 0.15 per cent simply by increasing the concentration of total protein. The use of dextrin in place of sucrose apparently decreases the tryptophane requirement. Since the rat does not require a dietary source of nicotinic acid, the use of the ration free of this vitamin could not be considered abnormal.

Although the growth-inhibiting effect of corn can be attributed to the character of the protein which it contains, the solution of the problem of why an increased concentration of certain amino acids on a nicotinic acid-low diet gives poor growth and precipitates an increased need for tryptophane remains to be answered.

Of considerable interest is the finding by Martin (9) that 2 per cent succinylsulfathiazole in diets containing casein, enzymatic casein digest, or a mixture of ten essential amino acids respectively resulted in great weight loss and death when the ten essential amino acids were the source of nitrogen. Growth on the other two diets was not markedly affected. Martin interprets these results by suggesting that intestinal bacteria must synthesize amino acids essential to the host which are not present in the mixture of the ten known essential amino acids.

If it is true that intestinal bacteria play a rôle in the synthesis of tryptophane, then it is reasonable to think that the intestinal flora could be so altered in its synthetic capacity as to be reflected in an increased dietary requirement. The presence or absence of nicotinic acid could be influential in such an alteration. It is possible too that the bacterial flora under certain conditions destroys or prevents the utilization of tryptophane, thereby increasing the dietary requirement from about 0.1 per cent to about 0.15 per cent.

Hier *et al.* (10) reported a failure to realize optimum growth with gelatin supplemented with essential amino acids in which gelatin is lacking. This growth failure was attributed to the toxicity of certain specific amino acids. Gelatin as a substitute for plasma proteins has been shown to have a limited ability to contribute to the synthesis of plasma protein and hemoglobin in dogs and toxicities are evident on prolonged administration (11).

A direct toxicity would hardly explain the present results, since good growth can be obtained when relatively small amounts of tryptophane and nicotinic acid are added to the ration.

The present findings suggest that the effectiveness of synthetic mixtures of amino acids or protein digests might be impaired if precautions are not taken to supply adequate amounts of tryptophane and nicotinic acid. It is also suggested that the usually effective supplemental action of certain proteins may be thwarted under conditions of nicotinic acid deficiency.

## SUMMARY

The deleterious action of corn grits in a synthetic diet was prevented when fibrin, egg albumin, or soy bean globulin was used in place of casein. This protection is attributed directly to the more adequate amount of tryptophane supplied by these proteins.

A syndrome analogous to that produced by corn was duplicated with non-corn-containing rations by adding tryptophane-free proteins or an acid-hydrolyzed protein to nicotinic acid-low rations which also contained marginal amounts of tryptophane. In a similar manner the growth-inhibiting effect of corn was shown to be related to the nature of its protein.

Possible explanations of these observations are discussed.

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