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Does "Animal Protein Factor" Occur in Green Plants?

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A situation of more general interest than was at first anticipated developed in the poultry nutrition field when attempts were made to raise poultry on all-plant rations for the purpose of conserving animal protein sources. The attempts failed and since amino acid supplements did not remedy the flaw, it was postulated that animal proteins have associated with them a factor necessary for chick nutrition. The designation "animal protein factor" came into use. Further work with deficient diets and especially attempts at microbiological assay have raised questions as to its multiplicity.

Very significant progress was made when Bird and associates[1] found that there is a factor in cow manure which has the full effect of the so-called animal protein factor as it is represented by fish meal. From dried cow manure concentrates were made (Bird)[2] having approximately 1,000 times the potency of the original material. The deficient rations were of the natural ration type (not purified) in which soybean meal was used as a protein source and led to low hatchability and poor growth of the young.

In rats, we have carried out studies[3] on all-plant rations, based on either cottonseed meal or soybean meal as a protein source and containing yeast or yeast extract. The deficiency state was best observed in following the growth of offspring from weaning time on. As in the case of the chick studies, mammalian liver, a component of fish (the so-called "fish solubles"), and a 1 to 100 cow manure concentrate relieved the deficiency. Crude casein also contains the missing factor.

Cary and associates[4] have exhaustively extracted crude casein and thereby removed material essential to growth and well-being of the offspring of rats. Their dams were fed a diet containing such casein and 10% dried yeast together with the known nutritional factors. Data are reported on the growth of the young from 4 weeks on. Among the supplements which led to improvement in growth were milk, cheese, meat, crude casein and liver, as well as dried grasses and alfalfa.

The question arises whether in these 3 types of experiments it is reasonable to consider the deficiency as of the same nature. One obstacle is to be found in the fact that the diet of Bird and associates[1,2] and that of Zucker and Zucker[3] contain 5% of alfalfa and on the diet of Cary and associates alfalfa is an effective supplement.[4a,b,c] The experiments given below were undertaken to throw some light on this question.

Experimental. Three sources of leafy material have been tried—alfalfa leaf meal, dried young cereal grass (Cerophyl Laboratories) and fresh cut spring lawn grass. The last source was included since in drying grasses may lose some of their nutritional properties. The fresh moist grass was easily made into a homogeneous mixture with the diet by means of a meat grinder. A 2 days' supply was made up each time.

In the first series of experiments, weanling rats from mothers on basal diet Pr 60, previously described by us, were given supple-

[1] Rubin, M., and Bird, H. R., *J. Biol. Chem.*, 1946, **163**, 387, 393.

[2] Bird, H. R., *Yearbook of Agriculture*, U. S. Dept. Agriculture, 1947, p. 235.

[3] Zucker, L. M., and Zucker, T. F., *Arch. Biochem.*, 1948, **16**, 115.

[4] (a) Cary, C. A., and Hartman, A. M., *Yearbook of Agriculture*, U. S. Dept. Agriculture, 1947, p. 779; (b) Hartman, A. M., and Cary, C. A., U. S. Dept. Agriculture, Bur. of Dairy Ind. BDIM-Inf. 53, Sept., 1947; (c) Hartman, A. M., *Fed. Proc.*, 1946, **5**, 137; (d) Cary, C. A., Hartman, A. M., Dryden, L. P., and Likely, G. D., *Fed. Proc.*, 1946, **5**, 128; (e) Hartman, A. M., Dryden, L. P., and Cary, C. A., *J. Biol. Chem.*, 1941, **140**, liv.

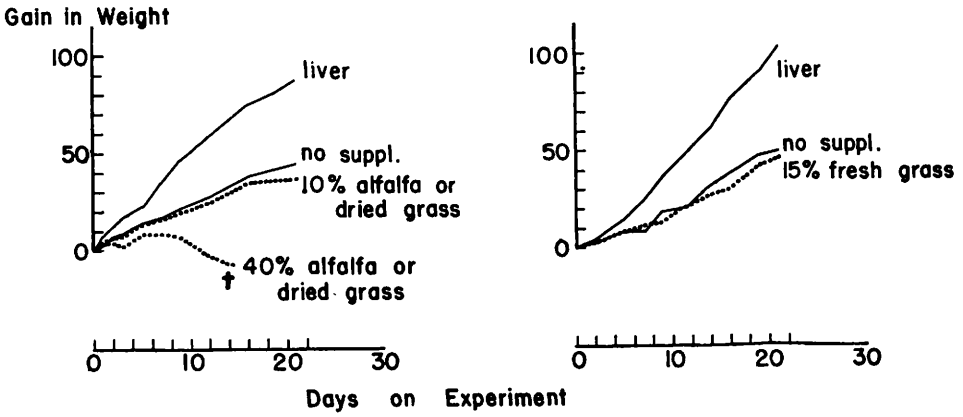

FIG. 1.

The effect of additional leafy feed and of liver supplements on rats raised to weaning on a zoopherin-deficient diet containing 5% alfalfa. There are 4 animals in each experimental group.

ments of 10% and 40% alfalfa leaf meal or dried grass or 15% fresh grass in addition to the 5% alfalfa already in the diet, to determine whether the zoopherin* requirement can be supplied by very high levels of such leafy materials. Fig. 1 compares the growth on the various supplements with the growth on the unsupplemented diet. Controls from the same litters with 5% of 1:20 Wilson liver powder are also shown. The results with alfalfa and with dried grass are averaged because they are indistinguishable. The deaths on the 40% level are perhaps ascribable to the excess of fiber and the resulting low caloric density superimposed on the deficiency. The fact that in spite of dilution of the diet with large amounts of fiber Cary and associates[4a,b,c] obtained positive results emphasizes the potency of the leaf material under their experimental conditions. The difference in the results on Cary's diet and ours is therefore more striking than appears on the surface.

In a second type of experiment, we have tried the effect of omitting the alfalfa entirely.

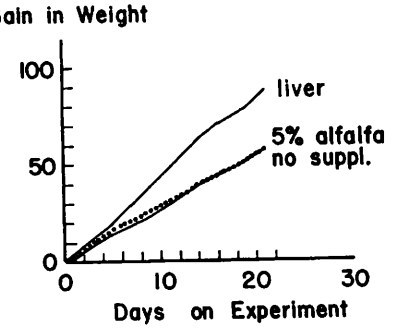

FIG. 2.

The effect of alfalfa and of liver supplements on rats raised to weaning on a zoopherin-deficient diet containing no alfalfa or hay. There are 3 animals per group.

Two stock females were placed at the time of impregnation on basal diet Pr 60, alfalfa being omitted; the young at weaning were divided into 3 groups, one continuing on the same regime, and the other 2 getting supplements of 5% alfalfa leaf meal or 5% 1:20 liver powder. As seen in Fig. 2, alfalfa added at weaning to a diet previously free of it produces no improvement in growth, while liver is a very effective supplement. Neither did the absence of the alfalfa in the diet interfere with good lactation as measured by the 3 weeks' weights of the young (averaging 41 g) or the dams' gain during lactation (averaging 20 g). The observation that zoopherin deficiency itself does not disturb lactation has been described[4,5] and comparable data under

* Zoopherin is defined experimentally as the water soluble factor which restores post-lactation growth to normal in the offspring of dams on all-plant diets. The term is used for convenience and to distinguish it from other substances which have been described as "animal protein factor." This paper also inquires into its distinction from unknown growth factors occurring in leafy material which may be stored in the liver or transferred to the young through the milk.

[5] Zucker, T. F., and Zucker, L. M., in press.

various conditions have been given.

The results indicate that the alfalfa effect in the experiments of Cary and associates[4] is not due to the same factor for which our procedure tests. It appears likely that, aside from the principal factor under consideration the yeast and pure vitamin preparations which Cary and associates have in their diet do not satisfy the conditions for best growth of the young. Whatever significant nutritional factors occur in alfalfa are in our case adequately supplied by other diet components, probably the large amount of cottonseed flour.

Discussion. The data constitute evidence on two points: 1) that the amounts of alfalfa, dried grass or fresh grass fed do not contain effective amounts of zoopherin and 2) that whatever unknown factors affecting growth of young rats are contained in alfalfa are also otherwise provided by the all-plant ration. The first of these points stands on its own feet. The second becomes more significant if there is other evidence for a factor in alfalfa which can play the role we attribute to it in the experiments of Cary and associates.[4] Ball and Barnes[6] have reported that purified diets containing 8% of yeast are not adequate for lactation of mice (measured by the preweaning growth). Both dehydrated cereal grass and wheat bran effectively improved the situation but these two substances when added to the basal diet alone or combined did not give as good results as the stock commercial diet. Bowland, Ensminger and Cunha[7] in observations with purified diets (alcohol extracted casein, pure B factors, etc.) on rats have found that when 15% of dried young alfalfa was used as a supplement, 43% of the young given to the female to raise, were weaned. This contrasts with 13% weaned when the same 10 B factors, including folacin, were given without alfalfa. Spitzer and Phillips[8] have also shown evidence for a marked effect of alfalfa on lactation in rats. Their later brief communication,[9] however, indicates that at least a part of this is attributable to known factors.

Summary. A postweaning growth inhibition in the offspring of rats on a well fortified all-plant ration has been described. While this is corrected by a factor in liver, neither alfalfa leaf meal, dried grass nor young fresh grass have such an effect.

6 Ball, Z. B., and Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 692.

7 Bowland, J. P., Ensminger, M. E., and Cunha, T. J., *Arch. Biochem.*, 1948, **16**, 257.

8 Spitzer, R. R., and Phillips, P. H., *J. Nutrition*, 1946, **32**, 631.

9 Spitzer, R. R., and Phillips, P. H., *Fed. Proc.*, 1947, **6**, 422.

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Inhibition of Growth of the Vaccinia Virus by β-2-Thienylalanine and its Reversal by Phenylalanine.*†

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The effect of β-2-thienylalanine upon the growth of *Saccharomyces cerevisiae, Escherichia coli* and certain other microorganisms has been reported by du Vigneaud and coworkers.[1,2] Thienylalanine was observed to inhibit the growth of these organisms, and the inhibi-

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† Presented in part at the meeting of the Society of American Bacteriologists at Minneapolis, Minn., in May, 1948.

1 du Vigneaud, V., McKennis, H., Jr., Simmonds, Sofia, Dittmer, K., and Brown, G. B., *J. Biol. Chem.*, 1945, **159**, 385.

2 Dittmer, K., Ellis, G., McKennis, H., Jr., and du Vigneaud, V., *J. Biol. Chem.*, 1946, **164**, 761.