SPECIAL ARTICLES

THE EFFECT OF ADRENAL CORTICAL AND PITUITARY ADRENOTROPIC HORMONES ON TRANSPLANTED LEUKEMIA IN RATS

IT has recently been reported that removal of the adrenals greatly increases the susceptibility of rats to a transplanted lymphatic leukemia.[1] This result suggested that adrenal secretions have some inhibitory action on the development of the disease. The present report is based on preliminary tests of adrenal cortical extracts and of pituitary adrenotropic hormone which stimulates the secretion of the adrenal cortex. The experiments were as follows.

Rats from a highly susceptible strain were inoculated intraperitoneally with leukemic cells and some hours later intramuscular injections of a hormone preparation were started.

Desoxycorticosterone acetate.[2] This preparation in oil was given daily in .1 cc doses to 33 rats inoculated with leukemia and to 41 rats in the same dosage 3 times a week. Approximately 20 per cent. of these animals did not develop leukemia, while the rate of survival in the 70 control rats was 5.7 per cent.

Eschatin, a cortical extract[3] administered in .1 to .2 cc amounts twice a day gave from 20 to 37 per cent. protection against the development of leukemia with the controls showing no survivals.

Adrenal cortex hormones in oil (Upjohn[4]). Various doses of this preparation have been tested on 87 inoculated rats. In 3 groups given .05 cc doses (2 rat units) daily the survival rates were 44.4 per cent., 50 per cent. and 60 per cent., while the survival among the controls was less than 5 per cent. There was definite protection when .075 cc was given daily, but larger or smaller doses did not give so definite a result.

Pituitary adrenotropic hormone.[5] These results varied somewhat with different lots of the material. Among the 67 treated rats, one group showed as high as 75 per cent. survival. Over 40 per cent. of the whole treated group remained free of the disease, which is a definite effect when compared with the 10 per cent. survival among the 68 controls.

The above studies were made entirely with transplanted leukemia and the action of the hormone was to prevent the development of the disease. It would be entirely unjustifiable on the basis of these observations to predict that the adrenal hormone would be of value in treating either the transplanted or the spontaneous disease. The results have a certain scientific interest in presenting further evidence of hormonal control of the lymphoid system.

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[1] J. B. Murphy and E. Sturm, SCIENCE, 98: 568–569, 1943.
[2] Schering Corporation.
[3] Parke, Davis and Company.
[4] The Upjohn Company.
[5] We are indebted to Dr. H. O. Singher, of the Memorial Hospital, New York, for this preparation.

ADEQUACY OF THE ESSENTIAL AMINO ACIDS FOR GROWTH OF THE RAT

IN a recent article, Albanese and Irby[1] have reported that rats lost weight rapidly on a diet which contained as the chief source of nitrogen the ten essential amino acids plus cystine. The relative amounts of amino acids were the same as in casein and were fed in quantity equal approximately to 14 per cent. of the total diet. When double the proportion of amino acids was fed, three out of six animals died and the remaining three ate poorly until they were sacrificed three weeks later. Despite the losses in weight, the animals on the 14 per cent. level amino acid diet maintained a positive nitrogen balance. These writers suggested that "the nutritive inadequacy of the essential amino acid diet may be due in part to toxic effects of unnatural forms of certain amino acids that cannot be utilized."

The above findings are not in agreement with results obtained by the present authors who, incidental to some other work, have regularly observed growth on diets containing the ten essential amino acids as the chief source of nitrogen. Also, Rose,[2] without giving the composition of the diets used or growth curves, states that animals fed a simplified diet containing the active amino acids (11.2 per cent.) "gained in weight just as rapidly as when all the protein components were supplied preformed." In view of the desirability of having the issue clarified, we have repeated and amplified our earlier experiments.

It will be noted that Albanese and Irby supplied the B vitamins in the form of yeast. Since the preformed protein components of yeast might alter the amount of growth, we have supplied all the necessary B vitamins in a pure form.

Young, inbred, Sherman strain albino rats were used in all the studies. The diets consisted of the same amino acid-free base[3] and a nitrogenous mixture which was varied in the following manner:

[1] A. H. Albanese and V. Irby, SCIENCE, 98: 286, 1943.
[2] W. C. Rose, *Physiol. Rev.*, 18: 109, 1938.
[3] Amino acid-free base.

Cod liver oil	2 grams
Corn oil	10 grams
Salt mixture	4 grams
Thiamin hydrochloride	224 micrograms
Riboflavin	400 micrograms
Pyridoxine hydrochloride	243 micrograms
Calcium pantothenate	1.5 milligrams
Nicotinic acid	2.5 milligrams
Choline chloride	134 milligrams
Para-aminobenzoic acid	1.0 milligram

Starch varied to make 100 grams of diet in conjunction with the nitrogenous components.

Diet	*Nitrogenous constituents*
1	10 essential amino acids at 5.8 per cent. level[4]
2	10 essential amino acids at 11.6 per cent. level
3	18 per cent. casein—paired feeding with diet 2
4	10 essential amino acids at 5.8 per cent. level plus 9 per cent. glycine in addition
5	18 per cent. casein—paired feeding with diet 4
6	18 per cent. casein—*ad libitum*

RESULTS

The lower three curves of Fig. 1 (diet 1) show that rats will grow when the sole source of amino acid nitrogen consists of the ten essential amino acids at a level of 5.8 per cent.; however, as would be expected, they grow slowly. The middle curves (diet 2) show the results of feeding amino acids at double this level, *i.e.*, 11.6 per cent. It will be observed that the additional amount of amino acids considerably increases the growth rate. The nutritive value of this ration appears to be slightly over one half that of an 18 per cent. casein ration. This may be seen by comparing the curves representing animals on diets 2 and 3. The growth on the 11.6 per cent. amino acid mixture was nearly identical with that we have observed in other experiments when rats were fed *ad libitum* diets containing 9 per cent. casein.

The result of supplying additional nitrogen in the form of glycine, in addition to the ten essential amino acids at the 5.8 per cent. level, may be seen from the two lower curves of Fig. 2. The growth rates in these two animals does not appear to differ significantly from those in which the glycine was omitted. The absence of growth in this experiment may be accounted for only partly on the basis of food consumed. The latter may be seen from the growth of rats (diet 5) which were fed a diet containing 18 per cent. casein but in quantities identical with those consumed by the animals on diet 4.

The upper curves of Fig. 2 show the growth rates of rats fed *ad libitum* a diet in which the nitrogen was supplied in the form of casein at an 18 per cent. level. The latter experiment illustrates the adequacy of the base ration and shows that the amino acid content of the other rations represented the limiting factor for growth.

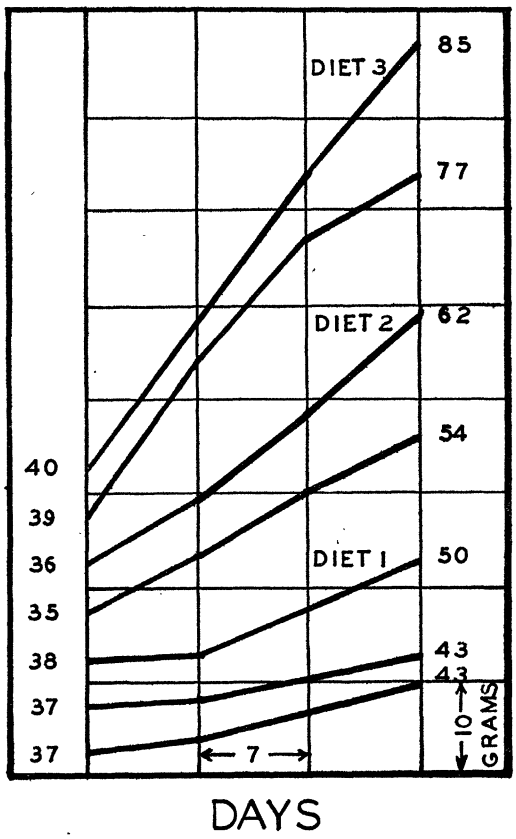

FIG. 1. Growth of rats on diets containing the following as the sole source of nitrogen: Diet 1, 10 essential amino acids at 5.8 per cent. level; Diet 2, 10 essential amino acids at 11.6 per cent. level; Diet 3, 18 per cent. casein—paired feeding with diet 2.

DISCUSSION

The failure of glycine to augment the growth rate when added to the diet suggests that more than the mere addition of a source of nitrogen is required for good growth, even though the required amounts of the essential amino acids are provided. However, the possibility that glycine in these quantities is toxic has not been eliminated.

In the absence of additional data it is not possible

[4] Proportions of amino acids fed are those given by Rose as the minimal amount of each necessary to support normal growth when the non-essentials are included.

*Amino acid	Required level of the active form per cent.	Grams per 100 grams of diet as fed, corrected for HCl and inactive enantiomorph
l(-)arginine mono HCl	0.2	0.242
l(-)histidine mono HCl	0.4	0.494
dl isoleucine	0.5	1.000
l(-)leucine	0.9	0.900
l(-)lysine mono HCl	1.0	1.250
dl methionine	0.6	0.600
dl phenylalanine	0.7	0.700
dl threonine	0.6	1.200
dl tryptophane	0.2	0.200
dl valine	0.7	1.400

* All the amino acids used in these experiments were obtained from Merck and Company, Rahway, N. J.

to make a quantitative comparison with Rose's work. In general we have found that at the levels fed, the essential amino acids will give rise to almost as much growth as will an equivalent weight of casein.

We found no evidence of toxicity in spite of using racemic mixtures of six of the amino acids. Without knowing the source of the amino acids used by Albanese and Irby we cannot account for the widely different results obtained. Our findings for rats appear

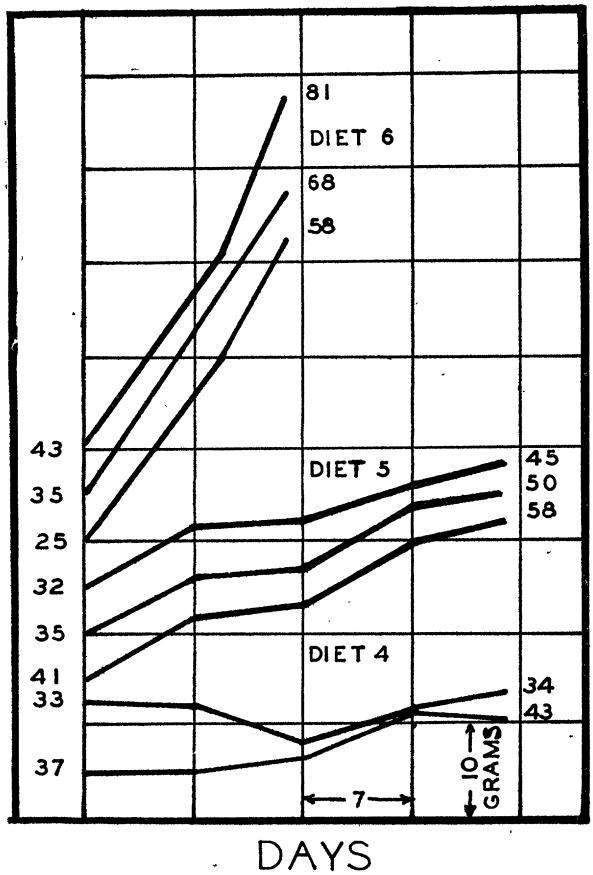

FIG. 2. Growth of rats on diets containing the following as the sole source of nitrogen: Diet 4, 10 essential amino acids at 5.8 per cent. level plus 9 per cent. glycine; Diet 5, 18 per cent. casein—paired feeding with diet 4; Diet 6, 18 per cent. casein—*ad libitum*.

compatible with those obtained in experiments on other animals. For example, Bauer and Berg[5] found slow growth when mice were fed the ten essential amino acids at a 15.6 per cent. level. Furthermore, Madden *et al.*,[6] using dogs, found that plasma protein production after feeding or injecting the essential amino acids was as good as when most proteins were fed in the diet. Moreover, they found no apparent clinical disturbance, even from the rapid injection of the amino acids intravenously.

[5] C. D. Bauer and C. P. Berg, *Jour. Nutr.*, 26: 51, 1943.
[6] S. C. Madden, J. R. Carter, A. A. Kattus, Jr., L. L. Miller and G. H. Whipple, *Jour. Exp. Med.*, 77: 277, 1943.

SUMMARY

Growth was obtained in rats on synthetic diets in which the ten essential amino acids were the sole source of amino acid nitrogen. The growth rate was dependent upon the quantity of amino acids fed and appeared to compare favorably with that obtained when a similar quantity of nitrogen was fed in the form of casein. No increase in growth was observed when nitrogen in addition to that given in the form of the essential amino acids was supplied as glycine. Our experiments would not support the contention that the unnatural forms of the amino acids are toxic.

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METABOLIC EFFECTS OF THIOURACIL IN GRAVES' DISEASE[1, 2]

EARLY in 1943, Astwood[3] introduced a new type of therapy for Graves' disease. This was based on the goitrogenic effects of sulfonamides and certain derivatives of thiourea as demonstrated in animals by studies of the MacKenzies and McCollum[4, 5] and of Astwood and co-workers.[6] A systematic investigation of such compounds[7] showed thiouracil to be the most potent and least toxic and led to its selection for clinical trial by Astwood[3] and by Williams and Bissell[8] and others.[9] The mode of action of these drugs appears to be an interference with the enzymatic synthesis of thyroid hormone. The exact point of block is still not known, but it is definitely established that the thyroid gland is rendered incapable of utilizing iodine for this process. The resultant thyroid insufficiency leads, via anterior pituitary stimulation, to an ineffectual hyperplasia of the thyroid acinar cells. The peripheral action of administered thyroid hormone, however, remains unimpaired.

[1] From the Russell Sage Institute of Pathology in affiliation with the New York Hospital and the Department of Medicine, Cornell University Medical College.
[2] With the technical assistance of Vincent A. Toscani.
[3] Astwood, *Jour. Am. Med. Asn.*, 122: 78, 1943.
[4] MacKenzie, MacKenzie and McCollum, SCIENCE, 94: 518, 1941.
[5] MacKenzie and MacKenzie, *Endocrinol.*, 32: 185, 1943.
[6] Astwood, Sullivan, Bissell and Tyslowitz, *ibid.*, 32: 210, 1943.
[7] Astwood, *Jour. Pharmacol. and Exp. Therap.*, 78: 79, 1943.
[8] Williams and Bissell, SCIENCE, 98: 156, 1943; *New Eng. Jour. Med.*, 229: 3, July, 1943.
[9] Himsworth, *Lancet*, 245: 483, 1943.