11. Counce, S., Smith, P., Barth, R., Snell, G. D., *Ann. Surgery,* 1956, v144, 198.

12. Werder, A. A., Hardin, C. A., Morgan, P., *Ann. N. Y. Acad. Sci.,* 1958, v73, 722.

13. Fox, A. S., *ibid.,* 1958, v73, 611.

Received March 2, 1959. P.S.E.B.M., 1959, v101.

Reduced Growth Hormone Content in Anterior Pituitaries of Rats on Protein-Free Diets.*† (24844)

H. H. SREBNIK, M. M. NELSON AND M. E. SIMPSON

Inst. of Experimental Biology and Dept. of Anatomy, University of California, Berkeley

Diets inadequate in protein or lacking specific essential amino acids retard skeletal growth. Several investigators(2-10) have measured tibia length and width of epiphyseal cartilage plates to assess growth retardation during such deficiencies in the rat. Frandsen *et al.*(8) found that in complete absence of dietary protein, the tibial proximal epiphyses were sealed from shaft by bone, known to occur after hypophysectomy(11). To determine whether growth arrest in absence of dietary protein was due to decreased synthesis of growth hormone, anterior pituitaries of protein-deficient rats were bioassayed for growth hormone activity and studied histologically. An attempt was also made to measure growth-promoting potency of blood from such animals to gain additional information on pituitary function.

Methods. Adult female rats (Long-Evans strain) weighing approximately 200 g were maintained 5 weeks on purified diet free of protein, though complete in all other known dietary essentials.‡ The animals had lost an average of 65 g. Groups of control rats of similar age and body weight were maintained either on purified diet containing 24% casein‡ or on stock diet XIV(13) and during same period gained 40-50 g. Bioassay results for control groups were in such close agreement that they have been combined in the Tables. Anterior pituitaries from protein-deficient and control rats were dissected, weighed, ground, suspended in isotonic saline with 2% butanol, and stored at 4°C. Total doses ranging from 1/32 to ½ anterior lobe were injected subcutaneously into immature female hypophysectomized rats. Injections were given once daily for 4 days, followed by autopsy 24 hours after last injection. The right tibia was stained with silver nitrate(14). Width of uncalcified portion of proximal epiphyseal cartilage was measured with ocular micrometer and cartilage widths of 200 μ or more, *i.e.*, increase of at least 40 μ over uninjected control widths, were considered evidence for growth-stimulating activity. Plasma from protein-deficient and control rats was bioassayed by same method(14). Blood was collected for 4 consecutive days from the aorta in heparinized syringes and centrifuged under mineral oil 30 minutes at 3200 rpm. Plasma for each day was pooled and injected subcutaneously

* Preliminary report presented before Am. Assn. Anat., Buffalo, N. Y., April, 1958(1).

† Aided by grant from U.S.P.H.S. We are greatly indebted to Dr. Randolph Major, Merck and Co., for generous supplies of 2-methyl-1,4-naphthoquinone and crystalline B vit.; to Dr. Elmer L. Severinghaus, Hoffmann-LaRoche, for crystalline *d*-biotin, *d*-calcium pantothenate, and *dl*-alpha-tocopherol; and to Dr. T. H. Jukes, Lederle Labs., Pearl River, N. Y. for synthetic pteroylglutamic acid.

‡ The protein-free diet contained sucrose 88%, hydrogenated cottonseed oil 8%, modified salts No. 4 (12) 4%. Crystalline vit./kg of diet were: vit. B_{12} 0.05 mg, *d*-biotin 0.6 mg, 2-methyl-1,4-naphthoquinone, 10 mg, thiamine HCl 10 mg, pyridoxine HCl 10 mg, pteroylglutamic acid 11 mg, riboflavin 20 mg, p-aminobenzoic acid 20 mg, niacin 40 mg, *d*-calcium pantothenate 100 mg, inositol 800 mg, choline chloride 1000 mg. The control diet contained sucrose 64%, alcohol-extracted casein 24%, hydrogenated cottonseed oil 8%, modified salts No. 4(12) 4% and vit./kg of diet listed above with B_{12} omitted. All rats received weekly a fat-soluble vit. supplement of 800 U.S.P. units vit. A, 115 chick units vit. D, 6 mg synthetic alpha-tocopherol and 650 mg corn oil (Mazola).

TABLE I. Growth Promoting Activity of Rat Anterior Pituitaries.

Total fraction of gland	Normal donors: Wt of gland, mg	Normal donors: No. of recipients*	Normal donors: Body wt change, g	Normal donors: Tibial cartilage width, μ	Protein-deficient donors: Wt of gland, mg	Protein-deficient donors: No. of recipients	Protein-deficient donors: Body wt change, g	Protein-deficient donors: Tibial cartilage width, μ
0	.0	33	−1	158 ± 4†	.0	29	−1	152 ± 4
1/32	.3	24	1	181 ± 5	.2	10	−1	166 ± 5
1/16	.6	23	3	220 ± 6	.3	10	1	163 ± 4
1/8	1.1	22	5	236 ± 8	.6	31	1	190 ± 6
1/4	2.3	21	6	269 ± 9	1.2	31	3	208 ± 7
1/2	4.5	22	7	283 ± 9	2.4	32	3	243 ± 8

* Immature female rats, hypophysectomized 26-28 days of age, 16-33 days post-operative and maintained on stock diet I plus lettuce(13).

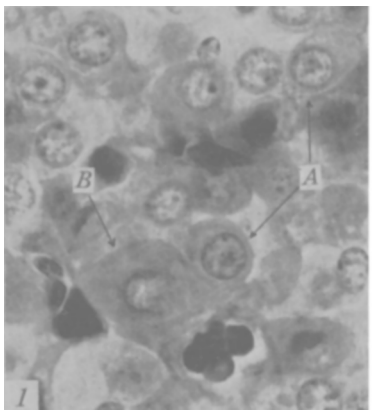
† Stand. error of mean.

into immature hypophysectomized rats in doses of 2 to 8 ml. The larger doses were administered in 2 or 3 ml at 1-2 hour intervals. Autopsy was performed 24 hours after last injection. Groups of 2 to 4 rats were used for each dose level and every level was repeated at least twice. Pituitary glands for histological analysis were collected from additional donor animals and fixed in Zenker-Formol, embedded in nitro-cellulose, sectioned at 4 μ, and stained with Mallory-Azan(15).

Results. Anterior lobes of control rats proved consistently more potent in increasing width of tibial epiphyseal cartilages in recipient animals than glands of protein-deficient rats (Table I). Growth-promoting activity could be detected in 1/32 to 1/16 total dose of anterior lobe from control rats, whereas 1/8 to 1/4 of gland was the minimal effective dose for such activity in pituitaries of protein-deficient rats, *i.e.*, in protein-depleted rats the pituitary contained approximately 1/4 normal amount/gland or 1/2 amount/mg tissue.

Cells of anterior pituitary of protein-deficient rats were reduced in size as judged by increased number of nuclei in any given field (Figs. 1 and 2). There were fewer acidophils in anterior lobes of protein-depleted group than in comparable areas of control pituitaries. The acidophils present were small and stained less intensely, presumably due to degranulation.

Plasma from protein-deficient donors appeared less effective in stimulating epiphyseal cartilages of recipient rats than plasma from control rats (Table II). However, evaluation of data was difficult due to marked variability in response of test animals. Results obtained with higher dosage levels (6 to 8 ml plasma daily) could not always be repeated, perhaps



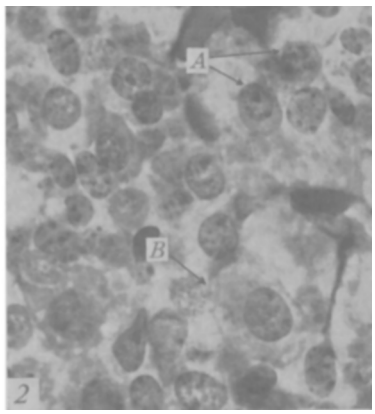


FIG. 1. Photomicrograph of portion of anterior pituitary of female rat on control diet showing general distribution of acidophils (A) and basophils (B). Mallory-Azan (× 890).

FIG. 2. Photomicrograph of portion of anterior pituitary of female rat on protein-free diet. Note increased number of nuclei. Acidophils are reduced in number and size. Mallory-Azan (× 890).

TABLE II. Growth Promoting Activity of Rat Plasma.*

	Normal donors			Protein-deficient donors		
Total dose, ml	No. of recipients	Body wt change, g	Tibial cartilage width, μ	No. of recipients	Body wt change, g	Tibial cartilage width, μ
0	22	−3	162 ± 3	23	1	163 ± 5
8	13	0	182 ± 4			
16	16	2	202 ± 7	9	2	180 ± 8
24	10	2	221 ±10	10	1	206 ± 8
32	6	1	252 ± 9	7	1	210 ± 7

* Abbreviations as in Table I.

due to variable absorption of such volumes. A high mortality rate occurred in groups receiving larger doses, whereas there were no deaths among recipients receiving smaller volumes.

Discussion. Reduced growth hormone content of the hypophysis during protein deprivation may signify decreased synthesis of growth hormone, an assumption supported by reduction in number and size of pituitary acidophils. Similar changes have been described previously in pituitaries of young male rats deprived of certain essential amino acids(6,7, 9,10,16). Decrease in growth-stimulating activity of blood in this study would not be unexpected under those circumstances.

Failure of growth hormone synthesis in absence of dietary protein may not be the only cause of growth retardation. Lack of amino acids as "building blocks" due to protein deprivation is undoubtedly a limiting factor in skeletal growth. Inability of skeleton to respond to growth hormone may also be a factor in growth arrest in protein-deficient rats, for it is known that the response of hypophysectomized rats to injected growth hormone, as judged by gain in body weight, varied with quality and quantity of protein fed(17). Administration of pituitary growth hormone has been reported to stimulate inactive epiphyseal cartilage plates in intact rats fed diets deficient in tryptophane(18) or in phenylalanine and tyrosine(19); no such effect has so far been demonstrated in complete absence of dietary protein.

Summary. Anterior pituitaries of protein-depleted and of control female rats were bioassayed for growth-stimulating activity. Absence of dietary protein for 5 weeks reduced growth hormone content of hypophysis to ¼ that found in normal glands or ½ normal amount/mg tissue. There was a parallel decrease in number and size of pituitary acidophils. Growth-promoting potency could be detected in plasma of protein-depleted animals but appeared to be reduced below normal levels.

1. Srebnik, H. H., Nelson, M. M., *Anat. Rec.,* 1958, v130, 376.
2. Maun, M. E., Cahill, W. M., Davis, R. M., *Arch. Path.,* 1945, v39, 294.
3. ———, *ibid.,* 1945, v40, 173.
4. ———, *ibid.,* 1946, v41, 25.
5. Lussier, J. P., *Rev. Canad. Biol.,* 1951, v10, 33.
6. Schwartz, C., Scott, E. B., Ferguson, R. L., *Anat. Rec.,* 1951, v110, 313.
7. Scott, E. B., Schwartz, C., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 271.
8. Frandsen, A. M., Nelson, M. M., Sulon, E., Becks, H., Evans, H. M., *Anat. Rec.,* 1954, v119, 247.
9. Scott, E. B., *Arch. Path.,* 1954, v58, 129.
10. Scott, E. B., *Am. J. Path.,* 1955, v31, 1111.
11. Becks, H., Simpson, M. E., Evans, H. M., *Anat. Rec.,* 1945, v92, 121.
12. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., Hart, E. B., *J. Biol. Chem.,* 1941, v138, 459.
13. Srebnik, H. H., Nelson, M. M., Simpson, M. E., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 57.
14. Greenspan, F. S., Li, C. H., Simpson, M. E., Evans, H. M., *Endocrin.,* 1949, v45, 455.
15. Koneff, A. A., *Stain Tech.,* 1938, v13, 49.
16. Scott, E. B., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 134.
17. Chow, B. F., Greep, R. O., *ibid.,* 1948, v69, 191.
18. Bavetta, L. A., Bernick, S., Ershoff, B. H., *Endocrin.,* 1956, v58, 701.
19. Scott, E. B., Dynes, T. F., *Growth,* 1957, v21, 115.

Received March 3, 1959. P.S.E.B.M., 1959, v101.