clonic seizures occurred particularly with the larger doses.

Electroencephalographic changes accompanied motor seizures, and in epileptic monkeys sometimes briefly (up to ½ minute) preceded the onset of clinical seizures (Fig. 1). Significant detectable changes were otherwise not evident; increased muscle artefact potentials became more prominent after injection when the animals appeared to become more restless. Clinical seizures were accompanied by diffuse spike convulsive patterns.

Summary. (1) Threshold convulsant dosages to rapid intravenous injection of semicarbazide were significantly lower in chronically epileptic monkeys than in normal unoperated controls. (2) Clinical seizures following intravenous injection of threshold convulsant doses of semicarbazide occurred after a latent period of 2 to 3½ hours. With suprathreshold doses the latent period was diminished greatly. (3) Use of a test dose of 30 to 40 mg/kg of semicarbazide by rapid intravenous injection is suggested to distinguish epileptic from non-epileptic monkeys since clinical convulsions should be produced within 3 to 4 hours in epileptic monkeys and not in non-epileptic normals.

1. Lowell, D. J., Ross-Duggan, J. K., and Piazza, S., *J. Pharm. & Exp. Ther.*, 1952, v106, 404.

2. Kopeloff, L. M., Chusid, J. G., and Kopeloff, N., *Neurology*, 1954, v4, 218.

Received March 1, 1956. P.S.E.B.M., 1956, v92.

Histopathology of Amino Acid Deficiencies. V. Isoleucine.* (22410)

EARL B. SCOTT.

Department of Anatomy, School of Medicine, Universtiy of S. Dakota, Vermillion.

The indispensability of isoleucine for growth in rats was demonstrated by Womack and Rose(1). Later, in experiments with dogs, several investigators showed that isoleucine was necessary for the maintenance of nitrogen balance(2), the formation of hemoglobin(3) and of plasma protein(4). The essential nature of this amino acid for human nutrition was established by Rose and his coworkers(5). Hegsted, McKibben and Stare (6) and Albanese(7) reported that the isoleucine content of human plasma protein and hemoglobin was insufficient to support normal growth in rats. Histological studies of isoleucine deficiency have not been reported for any species. The present report is the fifth in a series dealing with the histopathological effects of the total omission of an essential amino acid from the diet of rats.

Method. Young male Sprague-Dawley rats were divided into 3 groups. One group of 5 rats (normal-control) received a purified synthetic diet consisting of a mixture of 19 crystalline amino acids, vitamins, sucrose, cottonseed oil and the necessary minerals and salts as described by Rose, Oesterling and Womack (8). A second group of 15 rats (deficient) was fed the same diet, complete in every respect except for the total omission of isoleucine. The caloric value of the missing amino acid was supplied by additional sucrose. A third group of rats was pair-fed the complete diet so that the food consumption of each was no greater than that of its isoleucine-deficient mate. All rats were kept in individual cages and, with the exception of the pair-fed group, fed *ad libitum*. At the end of a 30-day experimental period, all rats, with the exception of 2 deficients, were sacrificed by decapitation without anesthesia. As rapidly as possible, the liver of each rat was removed, a small portion fixed for histological procedures, and the remainder immersed in liquid nitrogen for total glycogen determination by the method

* This research was supported by grant A-289 from National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

TABLE I. Comparison of Body Weights and Organ Weights of Isoleucine-Deficient, Pair-Fed Control, Normal-Control and Recovered Rats.

Body wt (g)			Organ wt (mg)		
Initial	Final	Change	Adrenals (2)	Testes (2)	Pituitary
		Isoleucine-deficient			
46	31	−15	18.0	116.8	2.4
45	27	−18	18.2	137.8	2.4
48	31	−17	17.4	135.2	1.6
54	34	−20	17.2	151.2	2.6
50	34	−16	19.2	142.4	2.6
47	28	−19	13.2	115.2	1.4
56	34	−22	18.8	185.6	2.0
55	35	−20	15.6	148.8	1.0
51	32	−19	15.2	188.4	1.4
53	32	−21	17.4	150.4	1.8
60	38	−22	17.6	217.2	2.8
64	32	−32	17.0	173.2	1.2
55	33	−22	18.2	141.8	0.8
Mean	32.4 ± .78*		17.2 ± .44	154.2 ± 7.85	1.9 ± .18
Avg % of body wt			.053	.048	.006
		Pair-fed control			
47	45	− 2	20.4	268.4	1.4
52	41	−11	20.4	258.4	2.0
53	43	−10	24.4	297.4	2.4
49	42	− 7	20.0	516.8	2.8
50	45	− 5	22.8	546.6	2.4
55	46	− 9	18.4	373.2	3.2
52	45	− 7	18.8	546.0	—
49	40	− 9	17.4	324.2	2.4
50	43	− 7	20.6	299.2	2.6
52	42	−10	19.2	373.4	2.2
43	42	− 1	19.8	532.8	2.2
46	38	− 8	21.8	239.0	2.0
51	38	−13	18.6	288.8	2.4
Mean	42.3 ± .67		20.2 ± .50	374.2 ± 31.6	2.3 ± .12
Avg % BW			.048	.089	.006
		Normal-control			
51	99	+ 48	33.2	2012.6	5.6
86	188	+102	37.5	1597.0	8.3
70	184	+114	36.3	2721.5	8.0
65	184	+119	29.4	2595.5	7.0
72	191	+119	37.6	2639.3	7.4
Mean	169.2 ± 15.7		34.8 ± 1.38	2313.2 ± 195.2	7.3 ± .42
Avg % BW			.021	1.4	.004
		Recovered			
67	210 (33)†		37.3	2673.9	7.3
68	210 (33)†		25.9	2712.9	8.0
Avg % BW			.015	1.3	.004

* Stand. error of the mean.
† Wt of these rats at end of deficient period when they were placed on recovery diet.

of Good, Kramer and Somogyi(9). Small pieces of liver, fixed in Gendre's fluid, were sectioned and stained by the Best's carmine and periodic acid-Schiff (PAS) methods for glycogen. All other tissues, except the pituitaries, were fixed in Bouin's solution and stained with hematoxylin and eosin. The pituitary bodies were fixed in Zenker-formol and stained by the aldehyde-fuchsin (AF) and periodic acid-Schiff (PAS) methods. These technics were developed by Halmi(10) and Purves and Griesbach(11,12) to demonstrate and differentiate the thyrotrophic and gonadotrophic basophils of the anterior pituitary. Employment of these methods has been described in our earlier reports(13,14). Pituitary sections were also stained with acid fuchsin for examination of the acidophils.

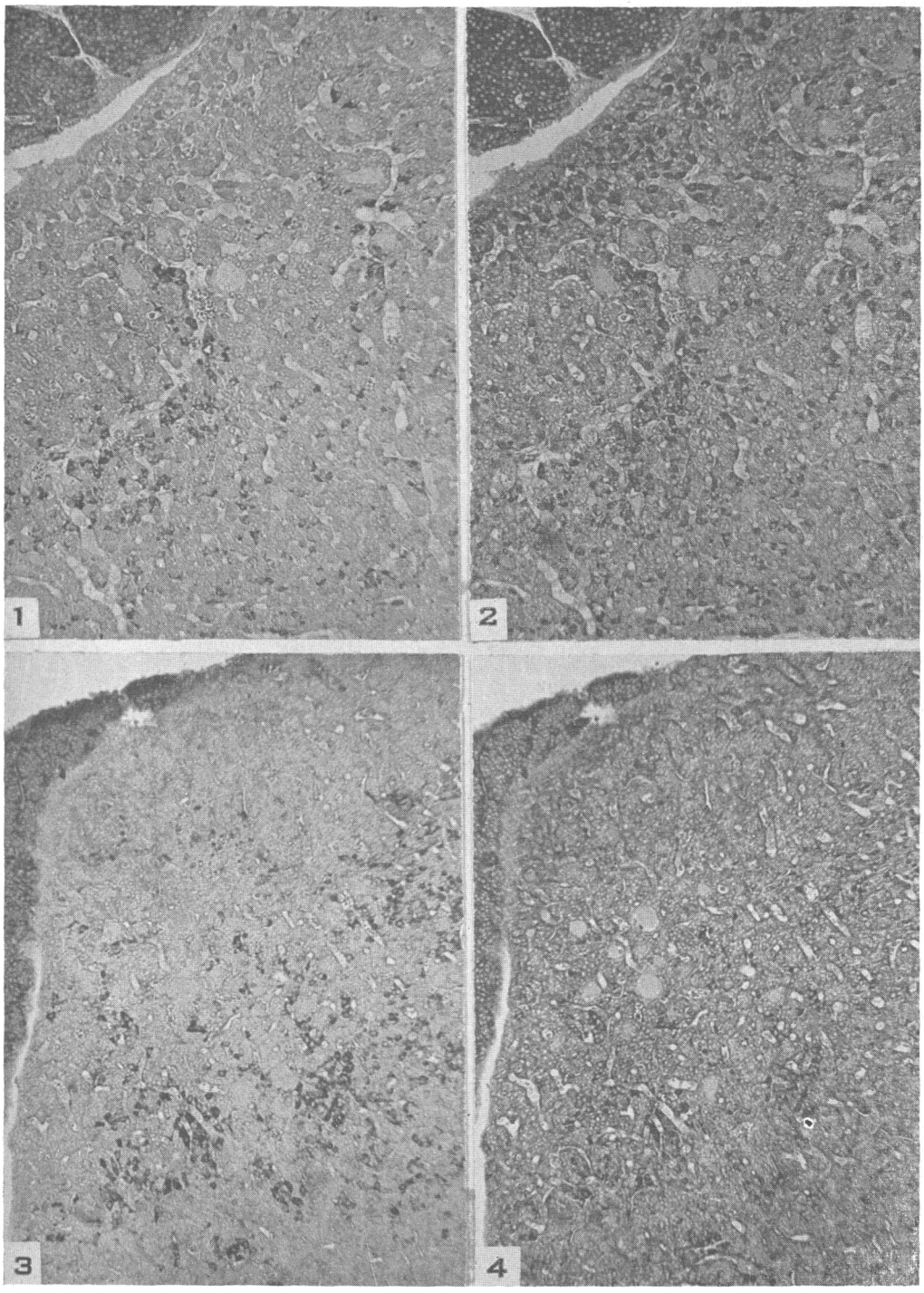

FIG. 1. Anterior pituitary of a normal-control rat stained by aldehyde-fuchsin (AF) method for identification of thyrotrophic basophils (dark cells). ×100.

FIG. 2. Same section and field shown in Fig. 1 restained by the periodic acid-Schiff (PAS) method. All basophils are PAS-positive, but those cells which are apparent here, but not in Fig. 1, are gonadotrophic basophils. ×100.

FIG. 3. Anterior pituitary of an isoleucine-deficient rat stained by AF method. Thyrotrophic cells are prominent. ×100.

FIG. 4. Same section and field shown in Fig. 3 restained by the PAS method. Only those cells which are AF-positive in Fig. 3 are PAS-positive here, illustrating absence of active gonadotrophic cells. ×100.

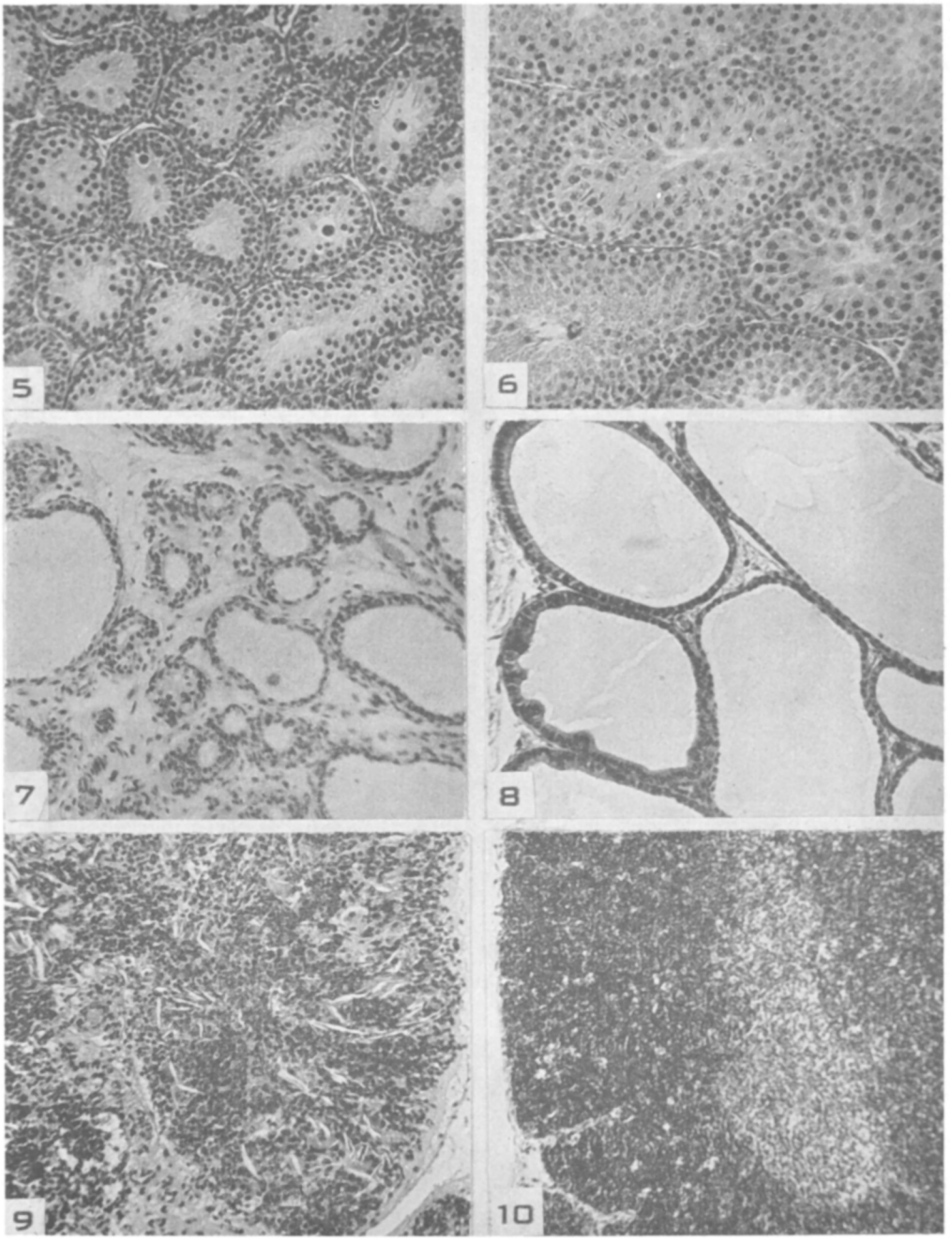

FIG. 5. Testis of an isoleucine-deficient rat. ×100.
FIG. 6. Testis of a pair-fed control rat. ×100.
FIG. 7. Prostate of an isoleucine-deficient rat. ×100.
FIG. 8. Prostate of a pair-fed control rat. ×100.
FIG. 9. Thymus of an isoleucine-deficient rat. ×100.
FIG. 10. Thymus of a pair-fed control rat. ×100.

Two isoleucine-deficient rats were not sacrificed with the others, but were placed on the complete diet (including adequate isoleucine) for a 30-day recovery period and then sacrificed.

Observations. Loss in body weight was consistently greater in the isoleucine-deficient rats than in their pair-fed mates. Table I lists the initial and final body weights as well as the weights of the adrenals, testes and pituitary of each rat. Comparison of the adrenals and pituitaries on an average percentage of body weight basis shows no significant difference between the deficient and pair-fed groups.

Histological examination of the pituitary bodies, testes and accessory sex glands showed that the total lack of isoleucine resulted in alterations which were similar to the changes observed by us in rats deprived of dietary threonine(13), histidine(14) or tryptophan (15). The pituitary thyrotrophic cells were not affected (Fig. 3), the gonadotrophic cells were completely deleted (Fig. 4) and the pituitary acidophils greatly reduced in size. The lack of gonadotrophic stimulation from the pituitary resulted in atrophy of the seminiferous tubules (Fig. 5) and testicular interstitial cells. Spermatogenesis was completely inhibited. The accessory sex glands suffered, in turn, from the lack of Leydig cell hormonal support. The prostatic epithelium was flat, atrophic and non-secretory (Fig. 7). The amount of interstitial stroma was increased; edema was slight.

In the pair-fed control rats the pituitary gonadotrophic cells, while not giving the intensely positive PAS reaction of the normal-controls, were demonstrable, although functioning at a reduced rate. This was evident by the histological appearance of the prostates and testes of these animals. The seminiferous tubules were smaller in diameter than normal but not so seriously reduced as in the deficients. Spermatogenesis lagged, but spermatozoa were being formed (Fig. 6). The prostatic epithelium was receiving some, but not total, stimulation from the testicular interstitial cells. Much of the prostatic epithelium was normal, but some acini were composed of low inactive cells (Fig. 8). The pituitary acidophils, although smaller than normal, were larger than in the deficient rats.

Thymic involution resulting from protein inadequacy is a well known reaction. The response of the thymus to isoleucine deficiency followed a pattern similar to that observed in our earlier studies. Formation of giant cells, loss of normal architecture and depletion of thymocytes were observed (Fig. 9). Needle-shaped clefts, which are usually associated with cholesterol deposits, were present. Such clefts were seen also in tryptophan-deficient rats but not in rats deprived of threonine or histidine.

The thymuses of the pair-fed rats were smaller than normal, but the cortical and medullary zones were intact (Fig. 10). Giant macrophages were not present and no evidence of crystalline deposition was noted.

Degenerative changes in skeletal muscle were observed in the deficient animals. The areas of injury did not involve all fibers of the muscle which was examined (sternomastoideus). In all instances the damaged muscle fibers were losing, or had lost, their cross striations. Some damaged fibers were swollen and hyalinized (Fig. 11); in other fibers, hyalinization and fragmentation were most apparent (Fig. 12).

Discussion. In the light of the effect of isoleucine deficiency upon skeletal muscle, the recent study of Blahd, Bloom and Drell (16) is of interest. These investigators reported that one of the amino acid groups which appeared most frequently in the urine of male muscular dystrophy patients was isoleucine-leucine. Cole and Scott(17) noted that liver glycogen accumulation was high in tryptophan-deficient rats and that the glycogen content of the livers of pair-fed rats was low. Application of the glycogen staining method to liver sections of isoleucine-deficient rats showed that a large amount of glycogen was present (Fig. 13), while the hepatic cells of the pair-fed rats had little or none (Fig. 14). Quantitatively, the total liver glycogen content of the deficient group was $2.96 \pm .15$ mg/g and that of the pair-fed group was $0.57 \pm .06$ mg/g. The possible causes of

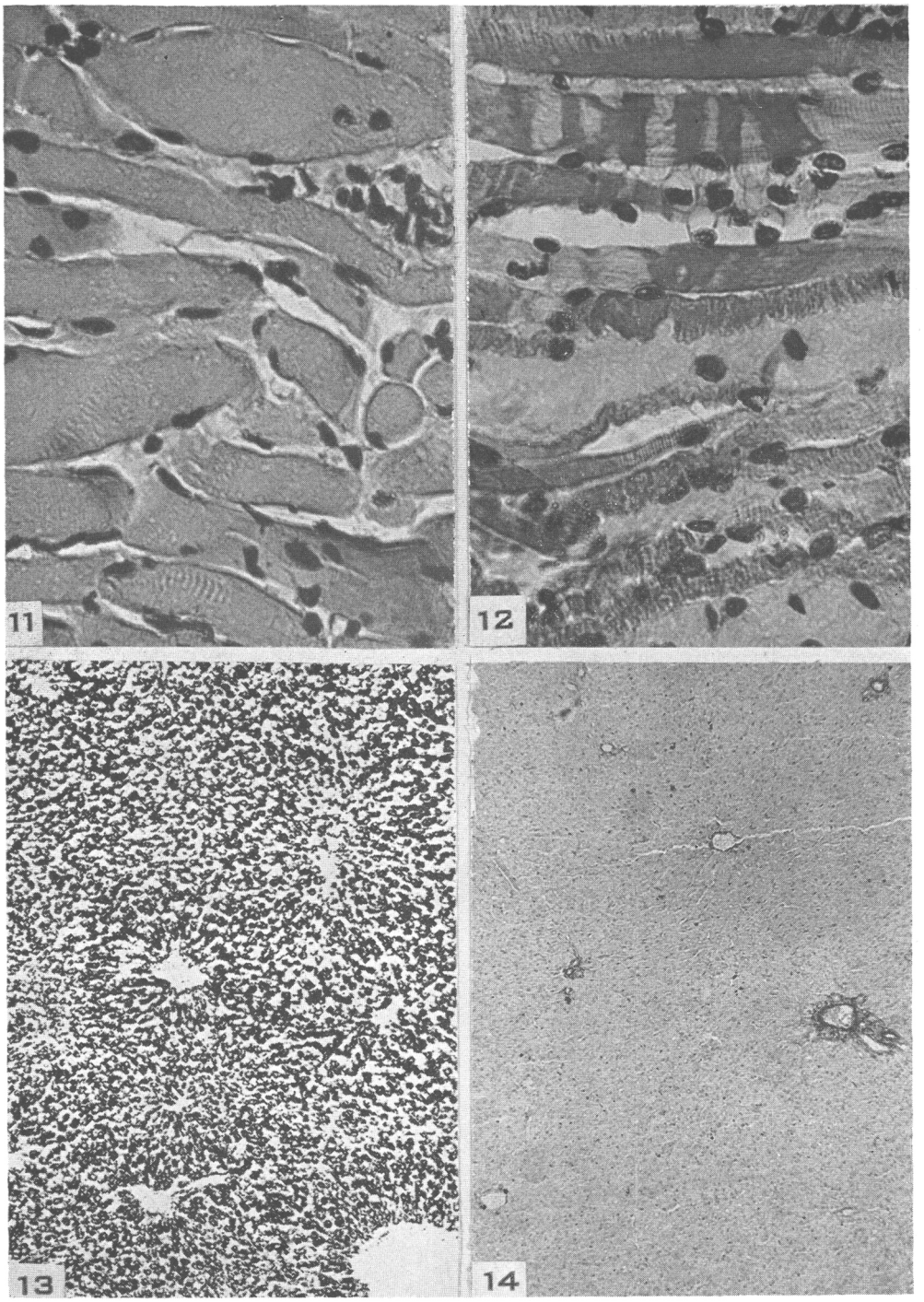

FIG. 11 and 12. Degenerating skeletal muscle fibers in isoleucine-deficient rats. ×430.

FIG. 13. Liver section of an isoleucine-deficient rat, stained by PAS method, illustrating presence of large amounts of intracellular glycogen. ×100.

FIG. 14. Liver section of a pair-fed control rat, stained by PAS method, showing absence of glycogen. ×100.

hepatic glycogen retention in amino acid deficient rats poses many problems for which there is no satisfactory explanation at this time. It is believed that the tissue alterations observed in the deficient rats were not the result of changes in water content.

The tissues of the 2 isoleucine-deficient rats which were placed on the recovery diet showed no abnormalities. Thus, the effects of the total lack of isoleucine were not permanent and were correctable by the addition of adequate dietary isoleucine.

Summary. The total lack of isoleucine in the diet of rats resulted in regression in the size of the pituitary acidophils, deletion of the pituitary gonadotrophic cells, atrophy of the testes and accessory sex glands, thymic involution, interference with somatic growth, liver glycogen accumulation and degenerative changes in skeletal muscle. Replacement of adequate isoleucine to the diet of isoleucine-deficient rats resulted in complete recovery.

The author is grateful to Dr. W. O. Read, Department of Physiology, for his assistance in making the total liver-glycogen determinations.

1. Womack, M., and Rose, W. C., *J. Biol. Chem.*, 1936, v116, 381.

2. Rose, W. C., and Rice, E. E., *Science*, 1939, v90, 186.

3. Whipple, G. H., and Robscheit-Robbins, F. S., *J. Exp. Med.*, 1940, v71, 569.

4. Madden, S. C., Anderson, F. W., Donovan, J. C., and Whipple, G. H., *ibid.*, 1945, v82, 77.

5. Rose, W. C., Haines, W. J., Johnson, J. E., and Warner, D. T., *J. Biol. Chem.*, 1943, v148, 457

6. Hegsted, D. M., McKibben, J. M., and Stare, F. J., *J. Clin. Invest.*, 1944, v23, 705.

7. Albanese, A. A., *J. Biol. Chem.*, 1945, v157, 613.

8. Rose, W. C., Oesterling, M. J., and Womack, M., *ibid.*, 1948, v178, 753.

9. Good, C. A., Kramer, H., and Somogyi, M., *ibid.*, 1933, v100, 485.

10. Halmi, N. S., *Endocrinology*, 1950, v47, 289.

11. Purves, H. D., and Griesbach, W. E., *ibid.*, 1951, v49, 244.

12. ———, *ibid.*, 1951, v49, 652.

13. Scott, E. B., and Schwartz, C., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 271.

14. Scott, E. B., *A. M. A. Arch. Path.*, 1954, v58, 129.

15. ———, *Am. J. Path.*, 1955, v31, 1111.

16. Blahd, W. H., Bloom, A., and Drell, W., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 704.

17. Cole, A. S., and Scott, P. P., *Brit. J. Nutrition*, 1954, v8, 125.

Received March 13, 1956. P.S.E.B.M., 1956, v92.

Vit. B_{12}: Correlation of Serum Concentrations and Pregnancy. (22411)

WILLIAM P. BOGER, LEMUEL D. WRIGHT, GEORGE D. BECK, AND GILBERT M. BAYNE.
Department of Research Therapeutics, Norristown State Hospital, Norristown, Pa., Sharp & Dohme, Division of Merck & Co., West Point, Pa., and Chestnut Hill Hospital, Philadelphia, Pa.

Vit. B_{12} occupies a significant place in the nutrition of microorganisms, lower animals and man. It seems purposeful to seek clinical situations in which a deficiency of vit. B_{12} may be important, either as the sole factor or a contributing factor to the causation of the signs or symptoms which are observed. There is general agreement that a deficiency of vit. B_{12} exists in pernicious anemia (P.A.) and in this clear-cut clinical example of the deficiency state, the serum concentration of vit. B_{12} is a good index of the situation, being less than 100 $\mu\mu$g/ml and approaching zero in frank untreated P.A. It is fully recognized that there is the possibility of a relatively normal serum concentration in the presence of deficiency in the total body stores and conversely the possibility of a lowered serum concentration in the absence of a measurable total body deficiency(1). Caution in interpreting serum vit. B_{12} concentrations is further enjoined on the basis of the accumulating evidence that when patients with P.A. in remission have had their vit. B_{12} serum concentrations assayed, the values may be below 100 $\mu\mu$g/ml despite the fact that clinically the