titers of hemagglutinins in both control and experimental eggs, the increase in yield of virus in cortisone-treated hosts was approximately of the same order of magnitude as that observed with 400 ID_{50} of challenge virus.

The effect produced by injections of cortisone was not ascribable to the action of substances present in the cortone vehicle, for prior treatment of embryos with 0.2 ml of the latter failed to cause any significant increase in final yield of viral hemagglutinins.

In other interference tests in which 11-day embryos were given 2.5, 5 and 7.5 mg of cortisone 30 minutes before interfering virus, it was found that all 3 concentrations of cortisone were equally effective in promoting an increased yield of viral hemagglutinins (results not shown) in the presence of a minimal completely interfering dose of inactivated virus. This was attested by the more or less identical yields of viral hemagglutinins in all three groups of cortisone-treated eggs.

Discussion and summary. The results reported herein have shown that prior injections of cortisone in embryonated eggs subsequently given minimal completely interfering doses of inactivated virus followed by challenge virus resulted in yields of viral hemagglutinins which were 7- to 10-fold greater than that found in untreated eggs. All 3 concentrations of cortisone (2.5, 5 and 7.5 mg) utilized in these experiments appeared to be equally effective in this respect.

An enhanced growth of influenza viruses in cortisone-treated eggs has been described previously(4), and the viral concentration of pooled allantoic fluids from cortisone-treated eggs was shown to be 123 to 155% of the viral concentration of control eggs (for influenza A virus). In the interference experiments reported in this paper, the yield of virus in cortisone-treated eggs was 700 to 1000% of the yield in untreated embryos. In view of this discrepancy in the increased yields of virus in the two reports, it would appear that enhancement of reproduction of challenge virus in residual free cells not blocked by interfering virus was not the sole determining factor in the increased yield of virus reported in this paper.

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Histopathology of Amino Acid Deficiencies. II. Threonine.* (20615)

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The unfavorable effect of protein deficiency on body economy has been frequently described. Whether or not there is a specificity of tissue response to protein inadequacy, or more particularly to specific amino acid deficiency, is not well established. A previous report of phenylalanine deficiency(1) described alteration of the anterior pituitary, gonads, accessory sex glands, bone and adrenal glands. Investigations of threonine reported

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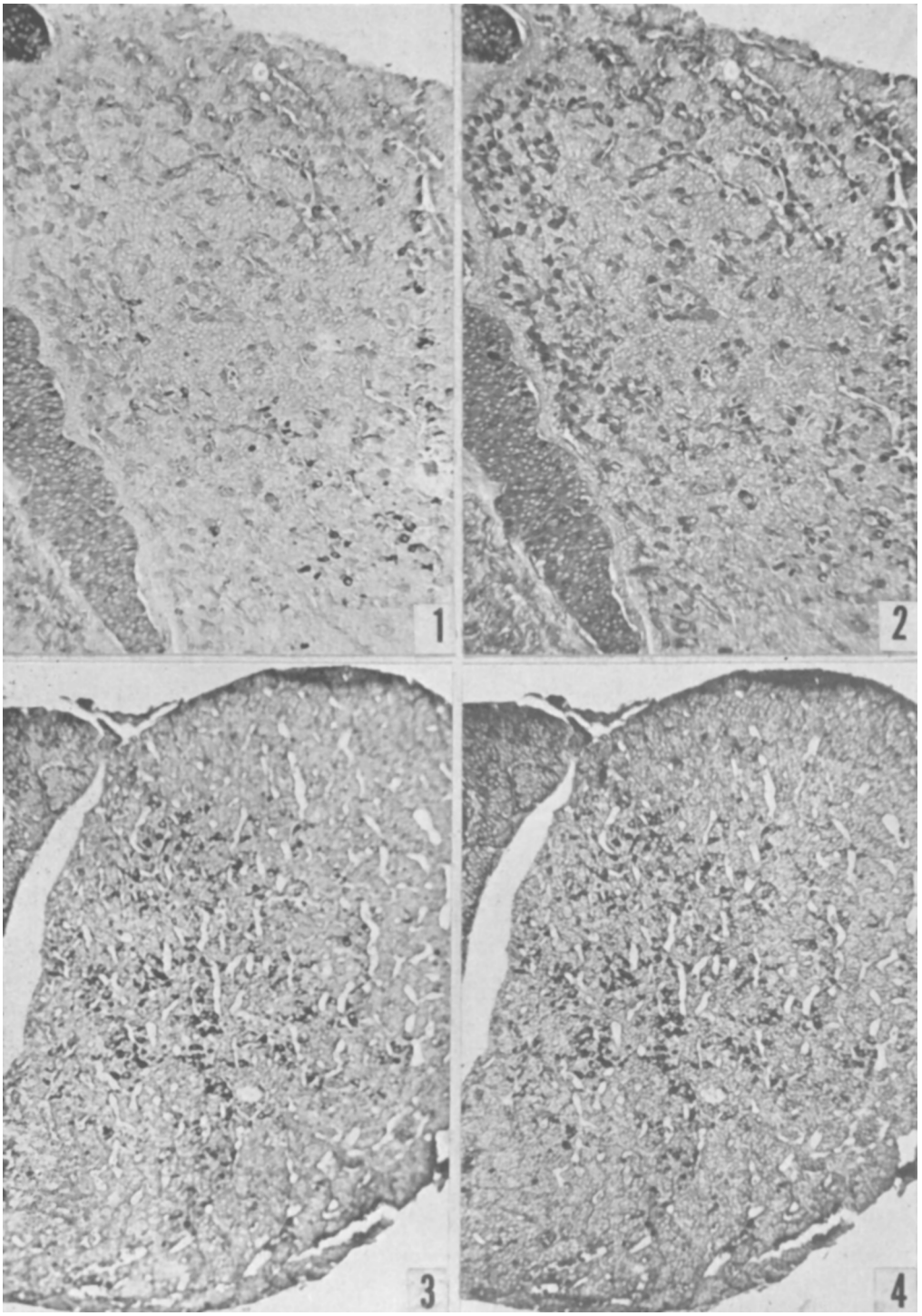

FIG. 1. Ant. pituitary of a control rat stained by Gomori method. Gomori positive cells (dark) are the thyrotropic basophils. ×100.

FIG. 2. Same section and field shown in Fig. 1 restained by periodic acid-Schiff (PAS) method. All basophils are PAS positive. Visual subtraction of Gomori positive thyrotropic cells indicates the gonadotropic basophils. ×100.

FIG. 3. Ant. pituitary of a threonine-deficient rat stained by Gomori method. Gomori positive cells are conspicuous. ×100.

FIG. 4. Same section and field shown in Fig. 3 restained by the PAS method. Only those cells which were Gomori positive in Fig. 3 are PAS positive in this photomicrograph demonstrating the absence of gonadotropic basophils. ×100.

in the literature deal principally with the requirement of this amino acid and not with histological effects of its deficiency. Nasset, Anderson and Siliciano(2), in a study of nitrogen balance in adult rats fed a low threonine diet, reported that death, in many instances, resulted from edema of the esophagus. Bauer and Berg(3) described ascites and generalized edema in growing mice maintained on a threonine deficient diet.

Materials and methods. Sprague-Dawley male rats, averaging 100 g weight, were divided into 3 groups. One group of 5 received a complete "synthetic" diet containing 19 amino acids as described by Rose, Oesterling and Womack(4). Another group of 12 received the same diet except that this diet was completely lacking in threonine. These diets were fed *ad libitum*. A third group of 9, referred to as "starved control", were fed a limited amount of the control diet on alternate days. All rats were weighed 3 times weekly. After 45 days the rats were sacrificed by exsanguination under ether anesthesia. Two of the deficient and one of the "starved control" rats, however, were not sacrificed at this time but were placed on a complete recovery diet for 45 days and then sacrificed. The tissues removed for histological examination, except the pituitaries, were fixed in Bouin's fluid and stained with hematoxylin and eosin. The pituitaries were fixed in Zenker-formol and stained by methods described below. For demonstration of the eosinophilic cells of the anterior pituitary, sections were stained with acid fuchsin. Other sections were stained by methods described by Purves and Griesbach(5,6) to identify and differentiate thyrotropic and gonadotropic basophils of the anterior pituitary. In using these procedures it was necessary first to treat the sections by a method originated by Gomori(7) and developed by Halmi(8) and to photograph these sections. The same sections were then restained by the periodic acid-Schiff (PAS) reaction, which stained all basophils, and the identical fields rephotographed. In this manner records of two different technics could be obtained for the same section and field. By comparing the two photographs it was possible to visually subtract the Gomori positive cells from the PAS positive cells and thus identify the presence or absence of both thyrotropic and gonadotropic basophils (Fig. 1, 2, 3, 4).

Observations. Loss in body weight, an unkempt appearance and other general deficiency symptoms developed in those rats deprived of threonine. The average loss in body weight was 41(±9) g for each of the threonine deficient rats and 11(±6) g for each of the "starved control" rats. Each control rat gained an average of 131(±11) g. The deficient rats which had been placed on the recovery diet gained weight rapidly and, at the termination of the recovery period, weighed as much as the average control rat.

At autopsy the adrenals, testes and pituitaries were weighed and, although these organs in the deficient rats weighed considerably less than normal, the weights were variable and appeared to have no correlation with the loss in body weight. Ascites was present in only one rat but the testes of all deficient rats were soft and flabby to the touch. In general, all organs, when examined grossly, were smaller than normal. Those tissues in which histological change was observed are reported below. In all other tissues examined histologically, alterations, if they occurred, were not of an order which could be detected by the methods employed.

The testes of the threonine-deficient rats were extremely atrophic with no evidence of active spermatogenesis (Fig. 8). Some resting spermatogonia were in evidence, but the tubules were composed principally of Sertoli cells resting on the basement membrane. Single nucleated and multinucleated degenerating cells were commonly observed in the lumen. The testicular interstitial tissue cells were smaller and flatter than normal with little cytoplasm. The epididymides, seminal vesi-

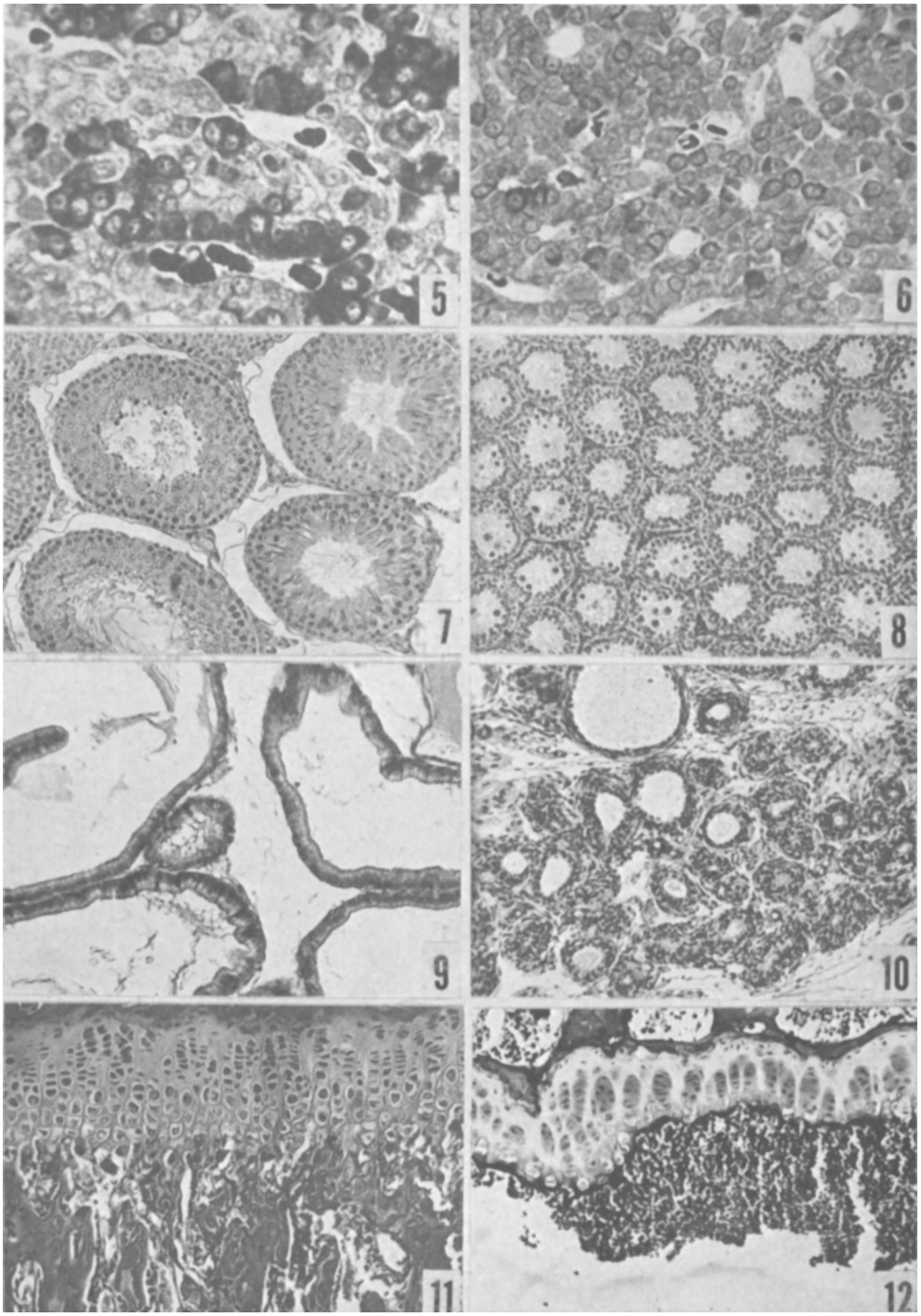

FIG. 5. Ant. pituitary of a control rat stained with acid fuchsin. Eosinophils are prominent. ×470.

FIG. 6. Ant. pituitary of a threonine-deficient rat stained with acid fuchsin. Eosinophils are fewer, smaller and fainter. ×470.

FIG. 7. Testes of a control rat. ×100.

FIG. 8. Testes of a threonine-deficient rat. ×100.
FIG. 9. Ventral prostate of a control rat. ×100.
FIG. 10. Ventral prostate of a threonine-deficient rat. ×100.
FIG. 11. Epiphyseal plate of tibia of a control rat. ×100.
FIG. 12. Epiphyseal plate of a threonine-deficient rat. ×100.

cles and ventral prostates were in an extremely atrophic condition. The epididymal epithelium was considerably lower than normal and the cells crowded. Free cells, sloughed from the seminiferous tubules, had moved into the lumen of the epididymis. The seminal vesicles and prostates were in a state similar to that observed in these organs following castration. The prostatic acini were much smaller than normal and the acinar cells extremely regressed (Fig. 10). The negative image of the Golgi apparatus, which could plainly be observed in the normal prostate (Fig. 9) and is considered a criterion of the prostatic secretory activity, could not be observed in the deficient prostatic epithelium. Coincident with acinar regression, there occurred an increase in prostatic interstitial tissue. The testes of the "starved control" rats were smaller than normal with reduction in the size of the seminiferous tubules and partial interruption of spermatogenesis. The Leydig cells were morphologically normal. That the capacity of the testicular interstitial tissue to secrete its hormone was reduced was made evident by the histological appearance of the ventral prostate. This organ appeared to be partially stimulated, since its acini were large and composed of both tall secretory and flat non-secretory cells.

The thyroid glands of the threonine-deficient rats were reduced in size but the height of the follicular cells did not appreciably differ from the normal. The follicles were variable in size ranging from large peripheral colloid filled follicles to small centrally located follicles, with and without colloid. The thyroids of the "starved control" rats were indistinguishable from the normal in size and histology.

The thymus gland was very prominent in the control rats but not identifiable grossly in the threonine-deficient rats. Partial thymic involution occurred in the "starved control" rats but the cortical and medullary areas remained histologically intact. No extensive increase in the connective tissue of this organ was observed.

The anterior lobe of the hypophysis was the only portion of this gland histologically affected by the lack of threonine. The eosinophilic cells were conspicuously reduced in number, size and staining capacity (Fig. 6). These cells had only a thin ring of lightly staining cytoplasm. The thyrotropic basophils, demonstrated as being Gomori positive, were present in numbers and morphologically unaltered (Fig. 3). Other basophils, which in the normal rat are PAS positive (Fig. 2), were not demonstrable in the deficient rats after use of the PAS method (Fig. 4). The anterior pituitary chromophilic cells of the "starved control" rats compared favorably with those of the control rats in size and distribution.

Bone growth was considerably reduced in the threonine-deficient rats. The tibial epiphyseal cartilage plate was thinner than normal. No bony trabeculae were present in the diaphysis (Fig. 12). The bone shaft was also thinner and its circumference less than normal.

The rats placed on the recovery diet made uneventful complete recoveries from the deficiency. Histological examination of their tissues revealed that they were normal in all respects.

Discussion. Comparison of the effects of partial inanition ("starved control") with those of threonine deficiency demonstrated that tissue regression was more pronounced in rats fed the threonine-deficient diet. Siperstein(9) described testicular damage resulting from chronic and acute inanition. Maun, Cahill and Davis(10,11), Adamstone and Spector(12) and Schwartz, Scott and Ferguson (1) reported similar damage following specific amino acid deficiencies. Jackson(13) and Mulinos and Pomerantz(14) described thymic involution in studies of chronic and complete inanition. Dougherty and White(15) and Frank, Kumagai and Dougherty(16) reported that adrenal cortical hormones diminish lymphatic tissue by destruction of lymphocytes. It is our opinion that the testicular damage and thymic involution of the threonine-de-

ficient rats resulted from the lack of this amino acid superimposed upon the effects of partial inanition. Since the adrenals gave no evidence of being hypersecretory, it was unlikely that thymic degeneration resulted from stimulation of the adrenal glands.

The assumption that a primary effect of the lack of dietary threonine was expressed upon the anterior pituitary appears to be justified in the present study. Purves and Griesbach (5) reported that all basophils of the anterior pituitary react in a positive manner to the PAS reaction. These authors later described (6) the identification of thyrotropic cells by use of the Gomori method. By employment of the methods they described, it was evident that a total lack of threonine, while depressing the gonadotropic basophils, had no effect upon the thyrotropic cells. Comparison of Fig. 1 and 2 demonstrates the presence of the thyrotropic and gonadotropic cells in the normal pituitary. Comparison of Fig. 3 and 4 reveals that in the threonine-deficient rat gonadotropic basophils were not in evidence but the thyrotropic cells were intact. The lack of gonadotropic hormone resulting from this diminution of gonadotropic cells resulted in atrophy of the testicular tubules and interstitial tissue. The nonfunctional state of the Leydig cells was reflected by the atrophy of the accessory sex glands. Further interference with the pituitary gland was evidenced by alteration of the eosinophilic cells. The defective appearance of the tibial shaft and epiphyseal plate are an expression of the impediment of somatic growth.

Since no serious alteration in the chromophilic cells of the anterior pituitary was observed in the "starved control" rats, it is reasonable to assume that the lack of threonine resulted in the pituitary changes described above.

The adrenals of the threonine-deficient rats were small but no change in the regions of the cortex and medulla was apparent. Since the pituitary eosinophils were reduced considerably in size and number and since the PAS positive gonadotropic basophils were completely lacking, is it possible that the adrenal cortical stimulating hormone has its source in those cells referred to as thyrotropic cells?

Despite the marked changes brought about by the complete lack of threonine, all rats placed on the recovery diet made complete recoveries. Thus, any deterioration resulting from threonine deficiency is reversible.

Summary. Young male rats fed a "synthetic" diet, complete in every respect except for the total lack of threonine, showed marked loss in weight, interruption of somatic growth, atrophy of the testes and accessory structures and alterations in the chromophilic cells of the anterior pituitary. The deficiency effects were reversed by adding the missing amino acid to the diet.

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