release of amino acids from the two caseins.

If the greater nutritive value of crude casein is due to an impurity, this growth promoting factor must be fairly heat stable since it is present in crude casein which is heated during its preparation. However, after extraction with alcohol and concentration of the extracts, the activity cannot be recovered. The fact that the extract prevents hair loss indicates that either a partial loss of the factor occurred during concentration or that two factors are involved—one with prevention of hair loss and one with growth.

It is also important to point out that the alcohol treatment used may produce toxic substances which appear in both the extracted casein and the alcohol extracts. The fact that similar results were obtained with sucrose, dextrose and dextrin and the fact that the addition of the concentrates gave further weight retardation, give added weight to this possibility. Food consumption records also showed a decreased food consumption associated with the reduced growth. If the effect is due to the presence of a toxic factor, the addition of fresh liver overcomes this action either by counteracting the toxicity or by supplying factors which cannot be produced by intestinal flora in the presence of the toxic material.

Summary. The nutritive quality of crude casein is reduced by continuous extraction with hot 95% ethanol. These results confirm the observations of Hartman and Cary. Rats fed rations containing such extracted casein grow at a reduced rate and develop a characteristic hair loss. The alcohol extracts obtained from the extraction of crude casein do not increase the rate of growth of rats ingesting extracted casein but do prevent the characteristic hair loss. The growth rate of rats ingesting alcohol extracted casein can be increased to normal by feeding fresh liver. The analysis of the extracted casein and the crude casein for 14 amino acids following enzymatic or acid hydrolysis failed to show any appreciable differences.

16590

Some Histological Manifestations of a Tryptophan Deficiency in the Chick.

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Although many investigations have been conducted on the effect of amino acid deficiencies on the growth rate and on the amino acid composition of tissues and excreta, few studies have been conducted on the histological changes in the various tissues attributable to the ingestion of amino acid deficient diets by the animal. Particular attention however, has been given to changes in blood composition, primarily in the level of hemoglobin and plasma proteins.[1] Other studies have revealed corneal vascularization resulting from various amino acid deficiencies[2,3] and atrophy of the thymus, adrenal cortex, liver and epithelial cells of the seminiferous tubules when leucine deficient diets were fed to rats.[4] Similar studies have been conducted

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with rats fed histidine or phenylalanine deficient diets.[5,6]

In the course of studies on the metabolism and dietary requirement of tryptophan by the chick,[7-9] it was of interest to determine the histology of various tissues from chicks fed diets deficient in tryptophan, adequate in tryptophan, or adequate in tryptophan with the food intake restricted to that of the deficient group.

Experimental and Results. The purified basal diet used has been described previously.[7] The major protein components were supplied by oxidized casein,[10] gelatin, fish solubles, cystine and methionine. The remainder of the ration consisted of starch, corn oil, mineral mixture and adequate quantities of all vitamins. The control chicks used for histological study received the basal diet plus *L*-tryptophan added in excess of the minimum requirement.[8] The food allocated to the restricted group was determined by the average food consumption of the chicks fed the deficient diet.

New Hampshire X White Leghorn crossbred chicks 10 days of age at the start of the experiment were used. They were kept on experiment for 14-18 days and comparable numbers of deficient and control chicks were taken for histological study at the same time.

The effect of feeding the deficient diets on the growth rate was rapid and marked. The deficient chicks lost from 12 to 20 g during the experimental period, showed retarded comb growth and were emaciated. The chicks fed adequate levels of tryptophan gained an average of 124 g while the restricted group made an average gain of 24 g until the last 5 days of the experimental period. The chicks of the latter group either lost weight or failed to gain during the last 5 days of the experiment due to the severe food restriction during this period. The hemoglobin and plasma protein levels for the deficient chicks were essentially normal after 14 days on experiment.

Histological observations were made in the following manner. The tissue was fixed in Bouin's fixative, dehydrated and imbedded in paraffin. Sections were prepared of 7 micron thickness. The slides were stained by each of the following methods: hematoxylin-eosin, Masson's trichrome technic and iron hematoxylin. Photomicrographs of sections from the control and tryptophan deficient chicks are shown in the accompanying figues.‡

Histological examination of the tissues from the group receiving a restricted food intake demonstrated that the tissues were normal with the exception of the liver. The cells of the liver from the tryptophan deficient group appeared normal in size, shape and staining properties. The liver cells of the birds that received the restricted diet, however, showed a very lightly staining cytoplasm with large vacuoles present, which indicated that exhaustion of the cell contents occurred.

The following changes to be described were observed in chicks fed the tryptophan deficient diet. The gall bladder was greatly distended with thick mucous bile. The gall bladder itself revealed an erosion of the epithelium and mucosa with only fragments of these layers remaining intact. The perimuscular connective tissue formed the bulk of the gall bladder with an apparent decrease in the thickness of the smooth muscular layer. Difficulty was experienced in making accurate comparative measurements due to the degree of distention.

The spleen of the deficient chicks macroscopically appeared to be about one-fifth the size of the normal control. It was a grayish color instead of the normal dark red. Histologically the spleen contained very few erythrocytes and the splenocytes were irregular

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‡ We are indebted to Paul Wilhelm for assistance in the preparation of slides and photomicrographs.

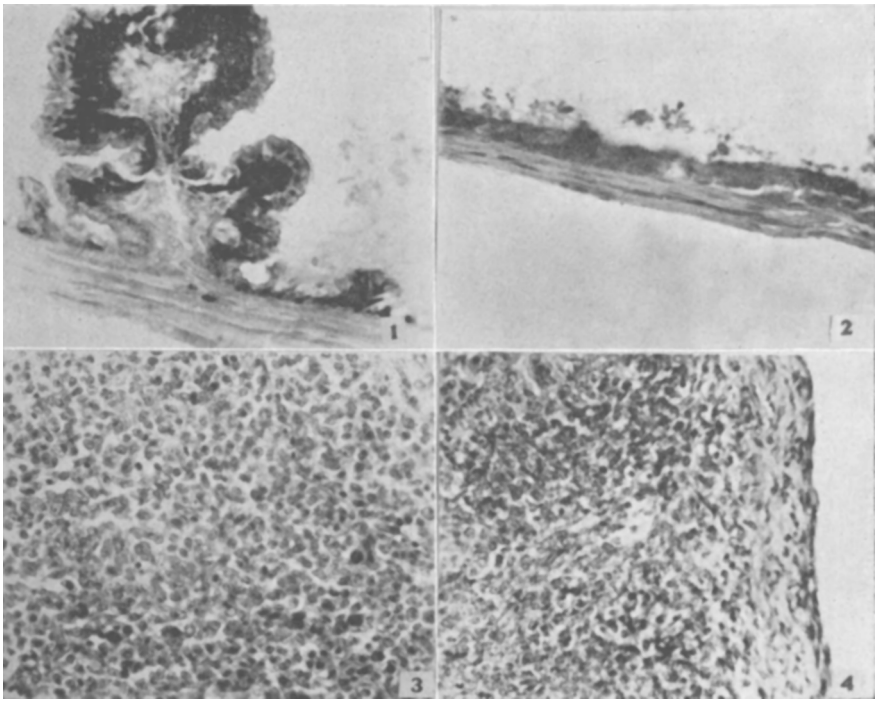

FIG. 1. Section of gall bladder of control chick showing development of the normal mucosa. (× 375.)

FIG. 2. Section of gall bladder of tryptophan-deficient chick showing erosion of the mucosa. (× 375.)

FIG. 3. Section of the normal spleen of the control chick. (× 375.)

FIG. 4. Section of the spleen of a tryptophan-deficient chick showing degeneration and metachromacity of the reticulo-endothelial cells. The absence of erythrocytes may be noted. (× 375.)

in size and showed signs of degeneration. Isolated splenic nodules were present.

The muscle tissue cells of the deficient chicks showed a decrease in diameter and staining property. The intercellular spaces were greatly increased so that most of the fibers remained as distinct entities. The spaces between the cells were evidently filled with a non-staining lymphoid fluid; hence such tissue could be considered as edematous. The average diameter of a muscle fiber from the control chicks was 21 microns as compared to 10.5 microns for muscle fibers from the deficient chicks.

The testes of both the control and the tryptophan-deficient birds were immature. The seminiferous tubules of the deficient chicks were larger in diameter and showed a degeneration of the seminiferous epithelium. The tubules lacked a central lumen and were apparently filled with a lightly staining degenerative edematous mass. The nuclei of the spermatagonial cells were irregular; the interstitial cells were atropic. The ovarian tissue showed a retardation in the development of the oocytes.

Discussion. The histological manifestations of this deficiency indicate what might be considered as 2 types. The first was apparently caused by the failure of the body to synthesize protein adequate for normal growth and was evidenced by a general exhaustion of cellular protoplasm as demonstrated by histological examination. This seems to be the case for such tissues as muscle and spleen. The second manifestation was evidenced by changes in the specific structure of the organ as in the degeneration of seminiferous tubules and the enlargement of the gall bladder and erosion of its layers.

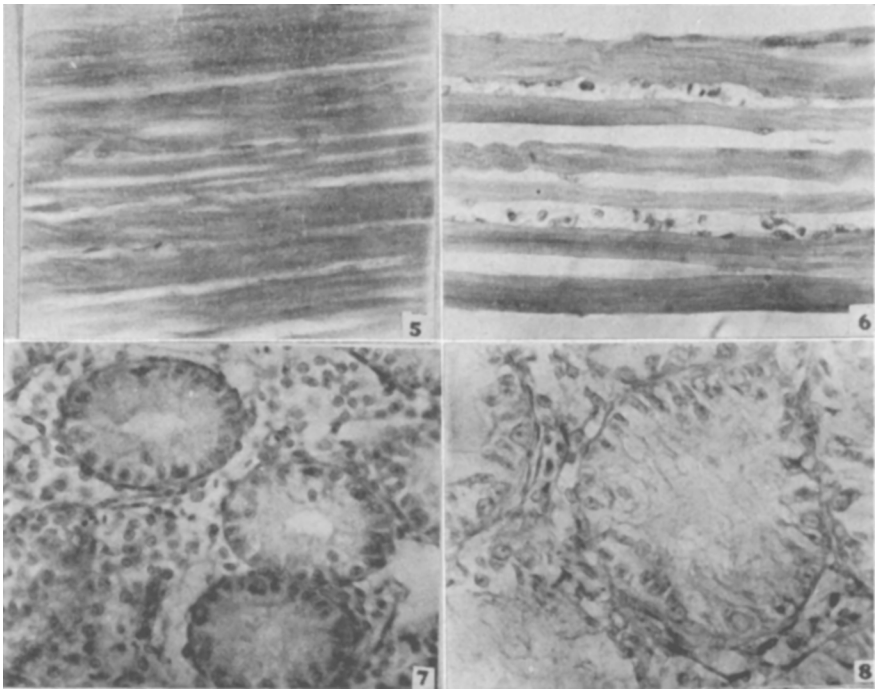

FIG. 5. Section of normal muscle tissue of the control. (× 375.)
FIG. 6. Section of muscle tissue from tryptophan-deficient chick showing a decrease in diameter and separation of the muscle fibers. (× 375.)
FIG. 7. Cross section of the immature seminiferous tubules of a control chick. (× 375.)
FIG. 8. Cross section of the seminiferous tubules of a tryptophan-deficient chick showing degeneration of the tubular epithelium and swelling of the tubules and atrophy of the interstitial cells. (× 375.)

Summary. Chicks fed a tryptophan deficient purified diet exhibited certain abnormalities when examined histologically. These were an enlargement and erosion of the gall bladder, edematous swelling of the seminiferous tubules, degeneration of spleen cells and a decrease in the diameter of the muscle fibers.