Immunophoretic Evaluation of Blood Serum Proteins in Chickens. III. Effects of Nutritional Myopathy-Producing Diets Deficient in Vitamin E and Sulfur Amino Acids.* (31240)

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In a recent report(1) it was demonstrated that the paper electrophoresis pherogram of plasma proteins of chickens showing symptoms of nutritional myopathy is characterized by a pronounced increase in the beta globulin fraction. Since the total proteins of the plasma were decreased and the albumin fraction was considerably lowered, a striking decrease in the albumin/globulin (A/G) ratio was encountered. These findings parallel those previously reported(2,3) in exudative diathesis and may be characteristic of those occurring in chickens affected with a chronic vitamin E avitaminosis. On the other hand, the acute state of vit E deficiency as seen in nutritional encephalomalacia is characterized in addition to the elevated beta globulin fractions and a drop in the A/G ratio, by an elevated total protein and a normal albumin level. The experiments reported here represent an effort to understand better the significance of the elevated beta globulin in the vit E deficiency state by use of immunophoretic techniques previously described(4).

Methods and materials. The same procedures were followed in this study as were employed when immunophoresis of serum was performed in chicks on nutritional encephalomalacia-producing diets, except for modifications in the purified diet required to produce nutritional myopathy(1).

Eighteen one-day-old chicks† were divided into 2 groups.

Group I. Nutritional myopathy. Eleven chicks were placed on a diet deficient in vit E or any synthetic antioxidant, and in sulfur amino acids(1). When these chickens began to show symptoms of leg or breast muscle paralysis, between the eighth and twelfth week, they were sacrificed by bleeding under sodium pentathol (40 mg/kg) anesthesia. Their ages ranged from 43 days to 89 days.

Group II. Normal chicks. Seven chicks were fed stock diets used in the laboratory for normal growth and development(5). These represented normal chickens. They were sacrificed simultaneously with experimental chicks and under identical conditions. Another study(5) on normal chickens includes this group of chickens. Immunophoretic preparations of blood serum were obtained by methods previously described(5).

Results. Clinical findings. Not only were the chickens on the experimental diet stunted in growth, as the weight charts of these and normal chickens demonstrated, but they lost all feathers about the head, and many over other parts of the body. Their legs were swollen and leg veins very prominent. There was progressive weakness of leg muscles; chickens were unwilling to walk about the cage. There was clumsiness in attempting to turn over, though by the time of sacrifice all chickens were still able to do so, more or less. Dissection of the breast muscle showed the white streaks so characteristic of the nutritional myopathy. While such streaks were not observable in the "red" muscles of tibialis anterior and gastrocnemius, these were definitely small in size when compared with the normal chicken muscles. In concurrent studies, analyses of the LHD isoenzyme distribution in homogenates of these muscles and of heart muscles are being prepared, as well as examination of immunophoretic patterns of these homogenates.

Hearts seemed to be dilated, and it is felt

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† Chicks obtained from Nicolas Lohman Co., Cuxhaven, Germany, consisting of Leghorns and red chickens, resembling Rhode Island Reds.

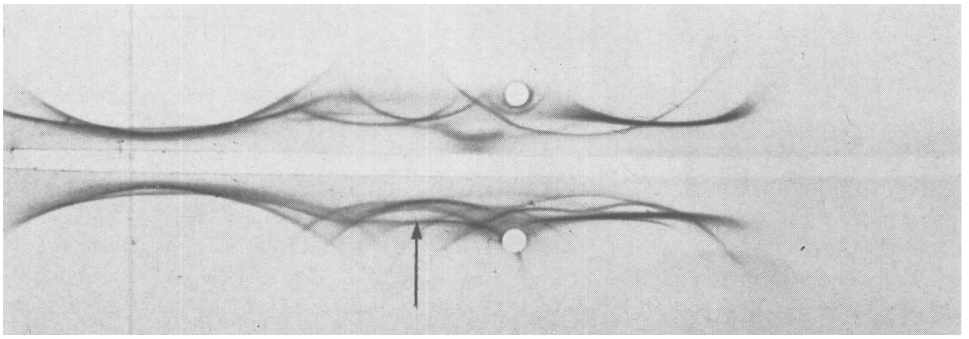

FIG. 1. Immunophoretic patterns of blood serum of normal and nutritional dystrophic chickens. Upper trace, 70-day-old normal chicken. Lower trace, 53-day-old nutritional dystrophic chicken.

that the leg edema represented some degree of congestive heart failure. Blood coagulated very slowly as compared with the very rapid coagulation of blood from the control chicks.

Immunophoretic findings. Prealbumin fraction was absent in practically all preparations of serum from the experimental chickens. In only 4 of the 11 chickens, what could be considered a trace of prealbumin was identified. In some, but not all of the preparations, the albumin precipitate appeared to be less wide than that in the control. In most of the preparations, albumin lines in control and experimental serum looked alike.

The most significant alterations were observed in the region of lipid-bearing fractions; experimental sera uniformly exhibited a strong precipitate with the mobility of beta lipoprotein.

Fig. 1 shows the pronounced alteration in the experimental chicken sera compared with the normal of the same age. In the control preparations, no precipitation line existed in the mobility zone of beta lipoprotein fraction as compared to the constant dense precipitate in the experimental sera. Transferrin, in control and experimental chickens, was faintly represented in the preparation, which is not surprising in view of the older ages of these chickens(5).

Three or four fractions of gamma globulin were found in both experimental and control sera. The 7S gamma globulin fraction of experimental serum was a "short and thick" line in almost half of the samples of experimental sera studied; this resembled those frequently seen in the gamma 7S globulin position of immunophoretic preparation of tissues (brain and muscle).

Discussion. As in the electrophoretic preparations of blood plasma and serum of chickens on antioxidant-deficient diets, we have observed in the immunophoretic studies the presence of a fraction in the globulin mobility range which is heavily precipitated, and not present in serum of normal chickens of the same age. The position of this fraction is that of the beta lipoprotein fraction, but apparently is not beta lipoprotein according to ultracentrifugation flotation tests. That this fraction is related to the disturbed lipid metabolism is a strong possibility.

It is known that cholesterol is increased in serum and tissues in animals on vit E-deficient diet(6,7). Lipoproteins transport most of the cholesterol in serum. One might anticipate that serum lipoproteins would increase as a compensatory mechanism for the transport of the excess of cholesterol in the serum. The role of tocopherols as an antioxidant is well established: in its absence, autoxidation of the unsaturated fatty acids occurs. Nishida and Kummerow(8) have demonstrated the lipoproteins and the hydroperoxide of sodium linoleate *in vitro* have an affinity for each other with the resultant denaturation of the beta lipoprotein. The possibility that linoleic acid is autoxidized in the blood serum in the absence of vit E is to be considered as one method of *in vivo* denaturation of beta lipoprotein. Investigations are currently under way to determine the nature of this abnormal antigen-antibody precipitation in vit E-deficient serum. If indeed it

represents a denatured lipoprotein, the possibility of its precipitation *in vivo* must be considered. Such denatured proteins might alter vascular permeability as well as all membrane permeability.

With the exception of the persistence of the beta lipoprotein positioned curve in the experimental sera, which presumably accounts for the increase in the beta globulin fraction previously reported in this condition, the remainder of the immunophoretic pattern remains like that found in normal sera. The previously observed decrease in albumin in chronic E-deficient chicks is not reflected in these tests, which are qualitative, not quantitative. The unusual appearance of the 7S gamma globulin curve in some experimental preparations is not explained.

Clinical findings of retarded growth, feather development and other skin changes are expression of the lack of vit E in the diet. This vitamin has a definitive growth promotion factor. In addition, it facilitates the use of vit A which, even though present to excess in the diet, is apparently insufficiently utilized, as evidenced by the skin changes.

Summary and conclusions. 1. Eleven one-day-old chickens were placed on diets deficient in vitamin E or any synthetic antioxidant and on sulfur amino acids. Eight chickens of the same hatch were kept on normal stock diets for comparison. All of the experimental chickens developed paralyses of breast muscles, and weakness of leg muscles in from 6 to 8 weeks. Normal chickens were free from symptoms. Both sets of chickens were sacrificed at the same time. Blood sera, obtained by cardiac puncture, were examined by immunophoresis, using anti-chicken serum antibodies in rabbit serum. 2. Significant immunophoretic finding was the persistence of a precipitation curve in the beta lipoprotein mobility range in sera of experimental chickens, which is normally not found in chickens of the same age. The remainder of the immunophoretic preparations pattern was alike in experimental and normal sera. 3. Interpretation of the altered serum protein in chronic vit E-deficient chickens is discussed in terms of altered lipid metabolism.

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Stimulation of DNA Synthesis in Human and Hamster Cells by Human Adenovirus Types 12 and 5.* (31241)

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Human adenovirus type 12 was found to be oncogenic for newborn hamsters(1), and transformation of hamster cells was reported following exposure to this virus *in vitro*(2,3). Transformation *in vitro* has been much more difficult to obtain with adeno-12 virus than with polyoma virus and SV40 virus. The nature of the association between polyoma virus and hamster cells has been studied by several authors. Hamster fibroblasts, including the stable line BHK 21, show little or no degeneration, but a proportion of the cells undergo neoplastic transformation(4). Fraser and Gharpure(5) have shown by immunofluorescence that virus antigens persist in the BHK

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