through the increase in the level of circulating FFA. The latter effect would fit the lipostatic hypothesis for regulation of food intake(12) rather than the glucostatic(13) or thermostatic(14) hypotheses. The present preliminary evidence that, if anything, injection of this substance causes a reduction in oxygen consumption, argues against a thermostatic mechanism and the hypoglycemia against a glucostatic mechanism being involved in this particular inhibition of food intake. It is intriguing, however, to speculate that there may be hormones or hormone metabolites that directly effect the nervous regulation of food intake. The origin, characteristics, actions and factors affecting the secretion of this substance in the rat are being investigated.

Summary. A substance has been extracted from the urine of fasting rats that causes an increase in release of free fatty acids from rat epididymal fat pads *in vitro* and in the serum FFA of the rat *in vivo*. This fat mobilizing substance (FMS) is similar in chemical composition (containing at least 7 amino acids and some carbohydrate) and in biological activity (causing a hypoglycemia and body weight loss in addition to lipolysis) to FMS extracted from urine of fasting man by others. It does, however, cause a significant decrease of food intake in the rat, particularly in the hypothalamic hyperphagic animal.

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Effect of High Linoleic Acid and Antioxidant Deficient Diet on Blood Serum Proteins in Chicks.* (28932)

LOUIS L. TUREEN, JOHN CONOMY AND DANIEL K. LEE
(Introduced by Theodore Cooper)
Max and Anna Tureen Neurology Research Laboratory, and Department of Neurology and Psychiatry, St. Louis University School of Medicine, St. Louis, Mo.

Chickens fed diets high in linoleic acid and deficient in Vit. E develop an encephalomalacia which is accompanied by abnormal electroencephalic tracings(1). The mechanism of the production of lesions in the brain in antioxidant deficiency states in chickens is unknown. Altered capillary transfer due to antioxidant deficiency has been considered a cause of the changes(3,4). Whether this is due to incorporation of abnormal metabolic products into the cell membrane, or to alterations in blood serum osmolality(5) are questions still under discussion. Changes in blood serum proteins in certain exudative diatheses occurring in selenium and antioxidant deficient diets support the view of others that changes in capillary permeability are respon-

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sible for transudations into tissue spaces(6). To assess the role such a mechanism might play in the development of encephalomalacia, we have studied blood serum protein profiles in chickens on an encephalomalacia producing diet.

Methods and material. One-day-old chicks† were divided into 3 groups and placed on specific diets as follows:

Group 1. An experimental diet (Table I) (7), high in linoleic acid and deficient in Vit. E, or any other antioxidant.

Group 2. The same experimental diet as in Group 1 with the difference that 0.025% ethoxyquin‡ was added as an antioxidant.

Group 3. A balanced commercial feed, with the usual fat content, and adequate amounts of Vit. E.§

It is to be noted that the unsaturated fatty acid content is identical in Groups 1 and 2, but different in Group 3.

The Vit. A content of the diet has been questioned. Moore(8) has found lower levels of Vit. A in livers of animals on Vit. E deficient diets. Stoewsand and Scott(9) showed that rancidifying fats high in linoleic acid quickly destroy Vit. A. However, Machlin and Gordon(10), feeding experimental diets identical with those used by us, found adequate levels of Vit. A in the liver of the chickens. They believed that their studies were not complicated by Vit. A deficiency. We cannot be certain that a Vit. A deficiency did not exist in our experimental chickens. In fact we shall suggest later in this paper that certain integumentary changes observed may be evidence of a Vit. A deficiency.

Chicks were sacrificed on alternate days, beginning on the fifth day, until symptoms of encephalomalacia developed in Group 1. Before the chicks were sacrificed blood samples were drawn by ventricular puncture.

† Chicks were derived from a cross between Arbor Acre 50 females and Vantress Dominant white males. Arbor Acre 50 females were derived from a cross between Arbor Acre White Rock females (pure line) and live 50 male (synthetic line).

‡ Generic name for 1,2-dehydro-6-ethoxy,2,2,4-trimethyl quinoline.

§ Startena, prepared by Ralston Purina Co., St. Louis, Mo., containing 1.9 mg/lb. alpha tocopherol.

TABLE I. Experimental Diet.

Ingredients	% by wt	
Isolated soybean protein	35.0	
Methionine hydroxy analogue (MHA)	.7	
Salts A*	6.0	
Vitamin Mix S35†	.5	
Sodium selenite (contains 30% Se)	—	1 ppm
Vit. D concentrate (7,500 I.C.U./g)	.03	
Vit. A concentrate (50,000 I.U./g)‡	.05	
Procaine penicillin (50%)	.004	
Choline chloride	.20	
NF 180 (a nitro furazone)	.05	
Alphacel (purified cellulose)	1.00	
Cerelose and/or fat§	to 100.	

* This will supply in mg/kilo of finished diet: vit B_{12}, 0.03; biotin, 0.30; menadione, 1.0; pyridoxine HCl, 8.0; folic acid, 4.0; riboflavin, 16.0; calcium pantothenate, 20.0; thiamine HCl, 24.0; nicotinic acid, 100.0. This mixture, Alphacel, and salts "A" can all be obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

† Contains in g per 60 g the following compounds: $CaCO_3$, 15; K_2HPO_4, 9; Na_2HPO_4, 7.3; $Ca_3(PO_4)_2$, 14; NaCl, 8.8; $MgSO_4 \cdot 7H_2O$, 5; ferric citrate, 0.4; $MnSO_4 \cdot 4H_2O$, 0.42; KI, 0.04; $ZnCO_3$, .02; $CuSO_4 \cdot 5H_2O$, 0.02.

‡ As Stabilized Vitamin A Preparation, (Vitamin A palmitate) manufactured by Hoffman Co.

§ 8% Ethyl linoleate (NBC) + 4% peroxidized coconut oil.

Blood samples were obtained under sodium pentobarbital anesthesia (100 mg/kilo i.p.). Chickens from Group 2 were preserved beyond the age of 5 weeks for another study and were bled by veni-puncture of the wing vein.

Blood samples were allowed to clot. After centrifugation the sera were collected and refrigerated. Eight microliter samples of blood serum were analyzed in the Beckman "Spinco" electrophoretic apparatus model R and the separations traced on the Beckman Analytrol model RB. Total serum protein concentrations were determined by the Biuret method as modified by Ferro and Ham(11). The results were evaluated statistically by the paired T test.

Results. Group 1. Encephalomalacia developed regularly, as anticipated, in chicks on the experimental diet. A variety of signs developed during the disorder, justifying the grouping of affected birds into 3 categories. Category I: Chicks gained weight moderately well. They showed integumentary changes of scaling and cracking of the feet epithelium; the feathers were coarse and rough. Skin

TABLE II. Serum Protein Alterations in Nutritional Encephalomalacia.

	No. studied	Total proteins (g/100 ml)	Albumin (g/100 ml)	Globulin (g/100 ml)	A/G ratio
Group 1					
Category I	6	3.0 ± .1*	1.1 ± .07	2.0 ± .05	.544 ± .033
" II	7	3.3 ± .1‡	1.1 ± .06	2.3 ± .11	.468 ± .030†
" III	6	3.7 ± .2‡	1.0 ± .10	2.7 ± .18	.389 ± .037‡
Group 2	7	2.8 ± .1	1.0 ± .05	1.8 ± .05	.542 ± .017
Group 3	12	2.5 ± .1	1.0 ± .06	1.6 ± .05	.596 ± .027

* As compared with Group 3, P value is less than .05.
† As compared with Group 3, P value is less than .02.
‡ As compared with Group 3, P value is less than .005.
Table includes standard error of mean.

TABLE III. Globulin Distribution (Absolute Values in Grams/100 ml of Serum).

	Gamma	Beta	Alpha 3	Alpha 2	Alpha 1
Group 1					
Category I	.28 ± .02	.80 ± .04*		.58 ± .05†	.32 ± .03
" II	.29 ± .01	.93 ± .04‡		.69 ± .07‡	.38 ± .04
" III	.30 ± .04	1.20 ± .06‡		.77 ± .09‡	.36 ± .07
Group 2	.28 ± .04	.60 ± .04	.17 ± .22	.53 ± .05*	.24 ± .04
Group 3	.24 ± .02	.59 ± .02		.45 ± .02	.30 ± .02

See Table II footnotes.

changes first appeared between the fourth and sixth day. Ataxia did not appear. Category II: Chicks gained weight poorly; integumentary changes were present and ataxia appeared between the sixth and 14th day. Category III: Chicks were in terminal stages of the disease. Weight gain was very poor; righting reflexes were lost. "Bicycling" appeared and motor activity was generally diminished or absent. These findings appeared between the 8th and 16th day. At no time during the course of the disease did the syndrome of exudative diathesis appear.

Serum protein changes are presented in Tables II and III. Using Group 3 chickens as controls for values obtained in chickens on experimental diets, the normal values for total proteins were 2.5 ± 0.1 g%, with an A/G ratio of 0.596 ± 0.027. In Group 1, total proteins were significantly increased in each category, ranging from 3.0 ± 0.1 g% in the mildest to 3.7 ± 0.2 g% in the most severely affected category. The A/G ratio was progressively decreased in this group, ranging from 0.544 ± 0.33 in mildly affected to 0.389 ± 0.037 in the most severely affected.

The serum profile is also altered in the chicks on the antioxidant deficient diet as compared with those on control (commercial diets). The beta globulin fraction showed a striking increase, while the alpha 2 globulin fraction showed a slight but significant increase. The albumin, alpha 1 and gamma globulin fractions were like those in the control. Thus, alterations in the A/G ratio were apparently a reflection of the increase in beta and alpha 2 globulin.

Group 2. These chicks were on antioxidant supplemented with high linoleic acid diet. They gained weight as well as chicks on the commercial control diet (Group 3), and developed no signs of encephalomalacia. Total serum protein and A/G ratios were not significantly altered from those of the controls. Total serum protein averaged 2.8 ± 0.1 g% and the A/G ratio was .542 ± 0.017. Serum protein profiles showed little change except for a slight increase of the alpha 2 fraction. This elevation was of questionable statistical significance ($P<0.05$).

Group 3. These chicks were fed the nutritionally balanced, commercial laboratory diet. They grew normally and developed no

symptoms. Total serum proteins averaged 2.5 ± 0.1 g%. Serum protein profiles were similar to those reported by Vanstone *et al* (12) for chicks during the first 5 weeks of growth.

Discussion. With progression of the symptoms of encephalomalacia, total serum protein content rose, whereas the level of serum albumin remained unchanged. Thus the falling A/G ratio was a reflection of the rise of serum globulins. Only beta and alpha 2 fractions of the globulin rose significantly. Alpha 1 and gamma globulin fractions were similar irrespective of the clinical state of the chickens.

The increase in the beta globulin fraction was more pronounced in those chickens with far advanced encephalomalacia (Group 1, Category III). When the diet was supplemented with antioxidant, the beta globulin fraction did not increase, despite the high linoleic acid content and even though these chickens remained on the diet for 5 weeks. Others(5,6) reported that chickens with exudative diathesis, (considered by some to be another manifestation of antioxidant deficiency)(13), showed a relative increase in the beta globulin fraction of the serum proteins. Creech(14,15) reported that one-day-old poults, hatched of hens on Vit. E deficient diet, had a severe decrease in A/C ratio which was the result of increase in the beta globulin fraction.

Chicks with encephalomalacia lost weight and hyperlipemia may have appeared as they deteriorated. The increase in total serum protein concentration could have reflected increased tissue destruction, leading to release of intracellular proteins. Gross pathological changes in organs other than the brain and epithelial structures were not observed. Liver degeneration(16) was not grossly apparent.

The rise in the alpha 2 globulin fraction, although not as pronounced as that of the beta fraction, was nevertheless statistically significant, particularly in those chicks with overt encephalomalacia (Group 1, Category III). A slight rise in the alpha 2 globulin fraction of uncertain statistical significance was seen in chicks after 5 days on the diet supplemented with ethoxyquin but high in unsaturated fatty acid content (Group 2). Thus the altered fat content of the diet of itself did not appear to determine the rise in the alpha 2 globulin fraction in chicks in Group 1. The absence of antioxidant appeared to be a critical factor. It is interesting to note that the alpha 2 fraction of sera chicks in Group 1, Category III contains a large percentage of serum lipoprotein when the electrophoretic pherogram is stained with lipoid specific oil red O dye according to the method that Durrum *et al*(17) applied to human sera.

The significance of these changes in serum is not clear. In our studies an exudative diathesis did not appear even though the A/G ratio fell to 0.313 ± 0.08. It has been said that the development of exudative diathesis in chickens depends upon deficiencies of selenium and antioxidant of the diet, and not upon the fat content of the diet. The pathophysiology of the disorder has been explained on the basis of increased capillary permeability(3). Goldstein and Scott(6) suggested alteration of plasma protein anabolism as a cause of serum changes. Bieri and Pollard(5) reported a decrease in A/G ratio with lowered serum albumin and normal total proteins during the period before the clinical syndrome became manifest. In those experiments, recovery from the disorder was accompanied by a more marked decrease in the albumin fraction and increase in alpha 2 and alpha 3 globulin fractions. Goldstein and Scott(6), finding a similarity in the electrophoretic patterns of serum and exudate, believed that increased capillary permeability in Vit. E deficiency states favored transudation of serum proteins.

Our findings suggest that the altered serum protein profile in chicks with nutritional encephalomalacia is due to antioxidant deficiency. The profile with the exception of the albumin level is similar to that observed in chicks with exudative diathesis. However, since none of our chicks showed evidences of exudative diathesis, it would appear that the altered serum proteins *per se* do not produce exudative diathesis. The high content of unsaturated fatty acid in the antioxidant deficient diet appears to be unrelated to the

changes observed in the blood serum.

These measurements reflect changes in concentration of the serum proteins only. Since blood volume values were not obtained in this study, conclusions regarding changes in the total serum protein mass cannot be derived.

Moore(14) has found lower levels of Vit. A in the liver of animals on the Vit. E deficient diet. The integumentary changes in our group of experimental chicks may have been a manifestation of Vit. A deficiency rather than Vit. E deficiency.

Summary and conclusions. 1. Chicks fed on an experimental diet, high in linoleic acid and deficient in antioxidants, developed a progressive disease manifested by integumentary changes and signs of central nervous system impairment. 2. Blood serum protein patterns were altered and were characterized by an increase in total protein, a decrease in the A/G ratio, and an increase in beta and alpha 2 globulin fractions. 3. Despite a decreasing A/G ratio, chicks on the experimental diet did not develop exudative diathesis. 4. It appears that the blood serum protein changes are a manifestation of Vit. E deficiency.

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The Mammary Tumor Agent in Completely Mammectomized Mice.* (28933)

ANNA DUX AND O. MÜHLBOCK (Introduced by W. U. Gardner)
A. V. Leeuwenhoekhuis, The Netherlands Cancer Institute, Amsterdam

Although the mammary tumor agent (milk factor, Bittner virus) has been studied for many years, our knowledge, especially of the biology of this agent, is scarce(1-3). Nothing is known about the tissues or organs in which this agent multiplies in the mouse. Its only known effect is the promotion of cancer in the mammary glands of mice. It would seem reasonable to assume that the mammary gland is the organ in which the agent develops and propagates. Lasfargues *et al*(4) conclude that the glandular stroma of the mammary gland is involved in the multiplication of this agent. It is known, however, that the tumor agent can propagate in male animals in which there only rudimentary mammary glands.

One of us (A.D.)(5) has developed a technique to extirpate all mammary glands from a mouse. Such mammectomized mice can be used to test the propagation of the

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