U-6420 did not lower blood sugars of alloxan diabetic or eviscerate rats, nor increase oxidation of glucose U-C^{14} by fasted intact rats. It was concluded from these studies that the mechanism of action of U-6420 was not like that of the sulfonylureas, biguanides, or insulin. The most probable mechanism was that of decreasing glucose output by the liver.

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Effect of Dietary Fats and Vitamin E on Oxidative Denaturation of Serum Low Density Lipoproteins.* (27320)

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We have previously shown that low density lipoproteins isolated from human or chick serum by ultracentrifugation can be denatured *in vitro* by exposure to the hydroperoxides of methyl linoleate(1). Furthermore, the hydroperoxide of methyl linoleate selectively or preferentially denatured β-lipoproteins and not other serum lipoproteins or protein constituents. It has recently been shown that low density lipoproteins contain a major share of the total serum tocopherol (2). In a deficiency of antioxidants or synergists therefore a diet rich in unsaturated fatty acids may cause serum low density lipoproteins to be susceptible to oxidative denaturation. In the present study, the stability or lability of low density lipoproteins of the Sf 0-20 class obtained from sera of chicks fed corn oil or hydrogenated coconut oil and with or without DL-α-tocopherol was compared in the presence of a catalytic amount of hemin.

Method. Day-old female chicks (New Hampshire Columbian Cross) were divided into 5 groups of 35 chicks each and kept on the experimental diets(3) for 2 weeks. These diets contained 2 or 10% stripped corn oil† or 10% hydrogenated coconut oil with and without 8 mg % of DL-α-tocopherol.‡ The birds were bled via heart puncture, serum samples from birds in each group pooled and the fraction composed of chylomicrons and low density lipoproteins of Sf 20-400 removed by an initial centrifugation. Low density lipoproteins of Sf 0-20 class were isolated(4),

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‡ DL-α-tocopherol was furnished through courtesy of Merck & Co., Inc., Rahway, N. J.

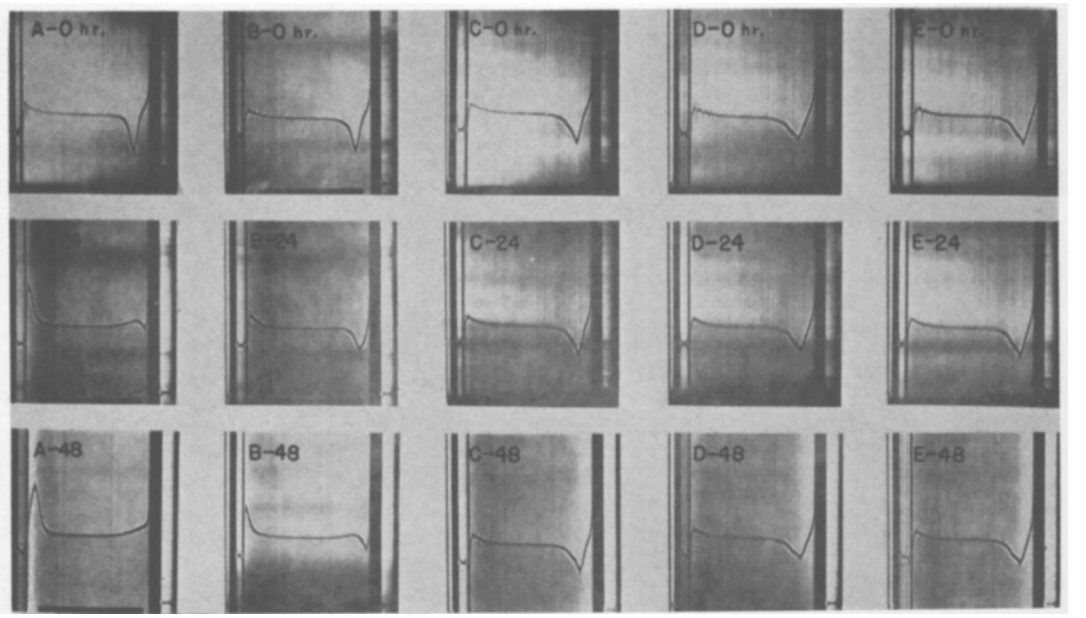

FIG. 1. Changes in ultracentrifugal flotation patterns of chick low density lipoproteins. The pictures labeled A-0, A-24, and A-48 represent the flotation patterns of serum low density lipoproteins of the Sf 0-20 class, which were obtained from chicks fed 10% stripped corn oil in absence of vit. E, after 0, 24 and 48 hr incubation, respectively. The series of pictures labeled B represent a change in the flotation patterns of lipoproteins which were obtained from chicks fed 10% of stripped corn oil in presence of vit. E; the series of pictures C, the flotation pattern of lipoproteins obtained from those fed 2% of stripped corn oil in absence of vit. E; the series of pictures D and E, the flotation pattern of the lipoproteins from those fed 10% of hydrogenated coconut oil in absence and presence of vit. E, respectively. In each case the picture was taken 22 min. after reaching maximum speed at a bar angle of 60°.

subjected to ultracentrifugal analysis(5) and diluted with sodium chloride solution of density 1.063 to give a lipoprotein concentration of 450 mg %. Exactly 0.8 ml of the lipoprotein fraction thus obtained and 30 lambda of 4×10^{-3} M hemin§ solution were transferred to a Warburg flask and the total volume adjusted to 1.2 ml with buffered saline solution of density 1.063; the final concentration of potassium phosphate buffer was 0.01 M. The flasks were shaken in Warburg apparatus under oxygen at 20°C for a period of 24 or 48 hours and the lipoprotein solutions subjected to ultracentrifugal analysis.

Fatty acid composition of the lipid moiety of lipoproteins was determined on pooled samples extracted with 2:1 chloroform-methanol (6). The methyl esters were prepared(7) and subjected to analysis on a Model 90C Aerograph with a 10-ft diethylene glycol succinate (DEGS) column at 218-222°C and with a helium pressure of 30 lb per square inch(8).

Results. The results indicated that the low density lipoproteins obtained from chicks fed 10% stripped corn oil were very labile *in vitro* towards oxidative denaturation in the presence of hemin (Fig. 1). A progressive decrease in both flotation rate and concentration of lipoproteins was noted, and after 48 hours shaking, almost all lipoproteins were denatured. Although addition of 8 mg % of tocopherol to a diet which contained 10% stripped corn oil partially prevented the oxidative denaturation of the isolated serum low density lipoproteins, a decrease in corn oil to 2% almost completely prevented the oxidative denaturation even in the absence of vit. E. The substitution of 10% hydrogenated coconut oil for 10% corn oil stabilized the lipoproteins towards oxidative denaturation

§ Crystalline hemin was obtained from California Corp. for Biochemical Research, Los Angeles. It was recrystallized twice(23), dissolved in 1/10 N sodium hydroxide, and immediately diluted to a sodium hydroxide concentration of .01 N.

TABLE I. Fatty Acid Composition in Dietary Fats and in Serum Low Density Lipoproteins.

Fatty acid component	Fatty acid composition in		Fatty acid composition in serum low density lipoproteins — Dietary supplements				
	Hydrogenated coconut oil	Stripped corn oil	10% Hydrogenated coconut oil +E*	10% Hydrogenated coconut oil -E	2% Stripped corn oil -E	10% Stripped corn oil +E	10% Stripped corn oil -E
	%						
Caprylic	6.3	—	—	—	—	—	—
Capric	5.8	—	—	—	—	—	—
Lauric	49.7	—	2.6	2.6	—	—	—
Myristic	18.5	—	4.7	2.3	—	—	—
Palmitic	8.7	11.5	22.8	22.0	22.0	23.3	28.4
Palmitoleic	—	—	9.3	9.9	5.3	2.3	2.2
Stearic	9.3	2.3	15.4	14.5	13.9	14.8	12.3
Oleic	1.7	28.8	31.5	34.1	35.6	20.5	27.0
Linoleic	—	57.4	9.7	10.9	18.5	33.4	25.3
C_{20} trienoic†	—	—	2.8	2.6	1.8	1.0	.8
C_{20} tetraenoic	—	—	1.2	1.1	2.9	4.7	4.0

* +E or -E indicates supplement or no supplement of 8 mg % vit. E to basal diet, respectively.

† Identification is tentative.

and only a slight decrease in flotation rate was observed under the present experimental condition.

The high stability of the low density lipoproteins obtained from chicks fed 10% hydrogenated coconut oil or 2% stripped corn oil appeared to be due to their fatty acid composition, particularly their linoleic acid content which was approximately one-third and one-half, respectively, of that in chicks fed 10% stripped corn oil and tocopherol (Table I). It is interesting to note that in spite of a lower linoleic acid content in the low density lipoproteins obtained from chicks fed 10% stripped corn oil in the absence of tocopherol, these lipoproteins proved to be less stable than those obtained from chicks fed the same amount of stripped corn oil in the presence of tocopherol. It is not clear how the linoleic acid content in the serum low density lipoproteins was lowered in the absence of tocopherol. Unlike the results obtained with stripped corn oil, the presence or absence of tocopherol in a diet containing 10% hydrogenated coconut oil did not produce marked differences in linoleic acid content of the low density lipoproteins. Hydrogenated coconut oil appeared to reduce the linoleic acid content originally present in serum low density lipoproteins of chicks to the extent that tocopherol no longer seemed necessary as a biological antioxidant.

Although dietary hydrogenated coconut oil decreased the percentage of linoleic and tetraenoic acid, the palmitoleic, oleic, and C_{20} trienoic acids in the serum low density lipoproteins were increased. These fatty acids, however, did not affect the stability of the serum low density lipoproteins. The lipid fraction of the lipoproteins obtained from chicks which had been fed corn oil contained approximately 4 times more tetraenoic acid than those fed coconut oil. Therefore, the concentration of linoleic and C_{20} tetraenoic acids in the serum low density lipoproteins seemed to be a major factor which affected stability or lability of the lipoproteins.

Discussion. It has been recognized that diets deficient in vit. E, or which contain large amounts of unsaturated fatty acids(9, 10,11), specifically linoleic acid(12,13), cause nutritional encephalomalacia in chicks. We have previously reported that intravenous injection of as little as 10 mg of the hydroperoxide of methyl linoleate into chicks kept on a diet which contained 10% corn oil stripped of vit. E induced cerebellar disorders and cerebellar lesion identical to the lesion observed in nutritional encephalomalacia(14, 15). Furthermore, serum low density lipoproteins are known to be the least stable serum lipoproteins(1) and are a major carrier of vit. E(2). Therefore, a diet deficient in vit. E and abundant in linoleic acid can in-

crease the percentage of linoleic and C_{20} tetraenoic acids in serum low density lipoproteins or in tissue lipoproteins to the extent that a limited supply of dietary vit. E may not fully prevent the peroxidation of these lipoproteins. An increase in peroxidation of lipids in tissue homogenates has been noted in absence of vit. E(16,17,18). The lability of the homogenates towards peroxidation may also have been influenced by an exogenous source of linoleic acid which determines the character of the lipid composition of the serum lipoproteins and in turn the tissue homogenates.

It is well known that lipids are rapidly peroxidized in the presence of hemin or various hemoproteins *in vitro*(19,20,21). Since thrombosis or hemorrhage is often associated with various diseases, hematin compounds such as hemin or hemoglobin, released from erythrocyte under certain physiological disturbances may accelerate the formation of lipohydroperoxides in the unsaturated fatty acid moiety of serum lipoproteins or in tissue lipoproteins *in vivo*, and thus facilitate or initiate oxidative denaturation or degradation of the lipoproteins. Furthermore, when normal cell metabolism in certain tissue is impaired, hemoproteins such as cytochromes may become available to accelerate the oxidative degradation of various lipoproteins.

A physico-chemical study on the interaction between serum low density lipoproteins and hemin molecules indicated that complex formation took place immediately after their mixing(22). Various dietary factors such as presence or absence of vit. E, and the amount of unsaturated fatty acids in a diet appeared to affect the formation of lipohydroperoxide in the lipoprotein-hemin complex and its subsequent oxidative degradation. Further studies are in progress in our laboratory to clarify the mechanism involved in the oxidative degradation of lipoproteins catalyzed by various hematin compounds.

Summary. The stability or lability of serum low density lipoproteins appeared to be greatly affected by dietary fat and by the presence or absence of vit. E. The low density lipoproteins isolated from the serum of chicks which had been fed corn oil stripped of vit. E under high vacuum were less stable *in vitro* toward oxidative denaturation catalyzed by hemin than the lipoproteins obtained from the serum of chicks fed hydrogenated coconut oil. Although a dietary source of tocopherol partially prevented the oxidative denaturation of serum low density lipoproteins in chick serum, a decrease in the content of unsaturated dietary fat prevented more effectively the oxidative denaturation of serum low density lipoproteins. The percentage of linoleic and C_{20} tetraenoic acids in the lipid moiety of the low density lipoproteins as determined by gas chromatography of the methyl esters was shown to be dependent on the percentage of linoleic acid in the dietary fat and was shown to be the most important factor which influenced the stability of the low density lipoproteins. Dietary fats rich in linoleic acid, therefore, should be stabilized with a sufficient amount of vit. E in order to prevent possible *in vivo* peroxidation of serum and tissue lipoproteins.

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Effect of Triparanol Pretreatment of Rats on *in vitro* Adrenal Steroid Production.* (27321)

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Recently, Melby, *et al.* have shown that pretreatment of rats with Triparanol‡ decreases adrenal cortical steroid production by adrenal slices *in vitro* to less than 50% of control values(1). They also demonstrated that the adrenal glands of Triparanol-treated rats can utilize acetate-1-C^{14} or mevalonate-2-C^{14} for synthesis of corticosteroids. The specific activities of the corticoids produced by the adrenals of Triparanol-treated rats were higher than those of the controls. Since Triparanol inhibits the synthesis of cholesterol from acetate and mevalonate by partially blocking the conversion of desmosterol to cholesterol(2), Melby *et al.*, suggested that the labeled steroids were being produced by biosynthetic pathways which did not involve cholesterol.

We have made a similar study in which adrenal slices from rats pretreated with Triparanol were incubated with progesterone-4-C^{14}. Our purpose was to investigate the possibility that Triparanol might act as an inhibitor at one or more of the steps subsequent to the formation of progesterone.

Materials and methods. Young adult female Sprague-Dawley rats, averaging 160 g in body weight, were divided into 10 groups of 4 animals each. Five of the groups served as controls. The remaining animals received subcutaneous injections of 12.5 mg of Triparanol (saline suspension) per 100 g of body weight 5 times a week. Two of the treated animals died during this interval. The animals were housed in air-conditioned quarters and fed a regular diet with tap water *ad libitum*. After 6 weeks the animals were weighed and sacrificed by decapitation. The heart, left kidney, thymus and adrenals were removed from each rat. The organs were trimmed free of extraneous tissue and weighed. The adrenals were halved and stored in iced saline until termination of the autopsy. The adrenals of the control animals were divided into 5 pools, those from the treated animals into 4 pools.

The separate pools of adrenal tissue were pre-incubated for ½ hour in 5.0 ml Krebs-Ringer-bicarbonate-glucose at 37°C with 95% O_2-5% CO_2 bubbled continuously through the medium. The preincubation fluid

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‡ Triparanol (MER/29); 1-ρ(β-diethylaminoethoxy)phenyl- 1 -(ρ-toyl)2-(ρ-chlorophenyl)ethanol, was graciously supplied by William S. Merrell Co., Cincinnati, Ohio.