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Effect of Beta-Sitosterol on Regression of Hyperlipemia and Increased Plasma Coagulability in the Chicken.* (25769)

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As an etiological factor in atherogenesis, lipids have received a great deal of study. However, as an etiological agent in the formation of thrombi, they have only recently become of interest to investigators. Within this decade, Fullerton *et al.*(1) correlated high blood lipid levels with increased blood coagulability in the human. This past year, reports were published on production of experimental infarcts in animals fed a lipemia-inducing diet(2,3,4). This report deals with production of an experimental hyperlipemia and increased plasma coagulability in the chicken and its subsequent reversal by feeding beta-sitosterol.

Methods. Six-week-old cockerels were housed in a broiler battery. A broiler ration and water were allowed *ad libitum* during a one-week acclimatization period. Plasma lipid levels and plasma coagulation times were then determined on randomly selected birds. The cockerels were then randomly divided into 5 diet groups: A, broiler ration (7% fat, 24% protein, 3% crude fiber); B, broiler ration + 5% cottonseed oil, (basal ration); C, basal

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ration + 1% cholesterol; D, basal ration + 1% cholesterol + 4% sitosterol; E, basal ration + 4% sitosterol; C-D, one-half group C placed on diet D at 4-week test period. Each bird was bled at 2, 4, and 6-week intervals after being placed on the diets. At 4 weeks, Group C was divided into 2 equal groups. One group was placed on diet C-D to determine whether regression of hyperlipemia and hypercoagulability would occur; whereas the other group remained on diet C. Blood was drawn by cardiac puncture and was citrated in the syringe. An aliquot of the plasma was extracted with 1:1 alcohol-acetone for lipid analysis; plasma coagulation, or "Stypven," time was determined according to Page and Russell(5) on the remaining plasma. When the experiment was terminated, liver and aorta were removed for determination of total cholesterol and total lipid. Total cholesterol was determined by the method of Sperry and Webb(6); total lipids were determined gravimetrically after treating the alcohol-acetone extract as outlined by Folch(7); lipid phosphorus was estimated on the residue from the total lipid analysis by the method of Fiske and Subba-Row(8).

Results. Data for blood lipids are presented in Table I. Irrespective of dietary treatment and time, a marked increase in total lipids and cholesterol levels was found in Group C during the experimental period. Group D, which received 4% sitosterol in addition to the Group C diet, did not exhibit a hyperlipemia. At 6 weeks, Group C-D, which was transferred from diet C to diet D at the end of the 4-week test period, had normal lipid and cholesterol levels. This represented a regression of hyperlipemia of the birds derived from Group C. In Group C, an increased lipid phosphorus was found at 4 and 6 weeks, whereas Group C-D had lipid phosphorus levels equal to those of the control group at 6 weeks.

TABLE I. Effect of Dietary Additions on Plasma Lipid Concentration* of Chickens.

Diet group	Time	Total lipids	Total chol.	Lipid-P
Control	0	279	82	4.8
A	4 wk	215	89	3.4
B		239	90	3.2
C		985	385	4.6
D		307	111	3.3
E		325	101	2.8
A	6 wk	252	83	3.3
B		300	88	3.9
C		983	534	5.8
D		377	102	4.2
E		340	85	4.7
C-D		338	113	4.2

* Data expressed as mg/100 cc plasma.

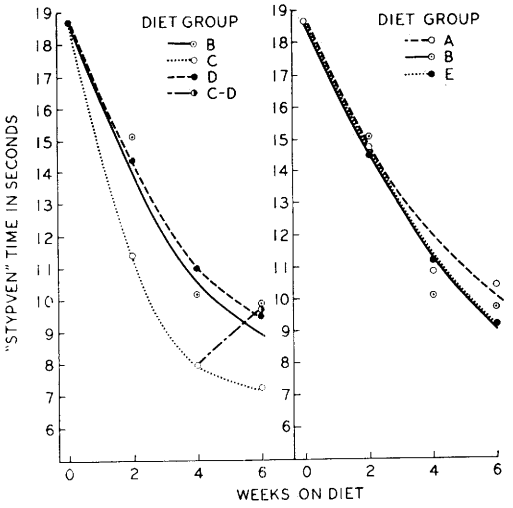

FIG. 1. Effect of diet on "Stypven" in chickens.

Plasma coagulation data, or "Stypven" times, are presented in Fig. 1. Throughout the course of experiment, a decrease in "Stypven" time was found in all groups. However, as may be ascertained from the curves, rate of decrease was the same in all control groups. Group C, which exhibited a marked increase in blood lipids, had a significantly greater rate of decrease of "Stypven" time during the experimental period. Group D, which was receiving diet C with an addition of 4% sitosterol, had "Stypven" times commensurate with those of Group B on a basal ration. Group C-D, which was placed on diet D after 4 weeks on diet C, had "Stypven" times equal to the control groups at 6 weeks. The effects of dietary additions on aorta and liver total lipids and cholesterol are presented in Table II. A marked increase of total lipids and cholesterol in these tissues was observed only in Group C. When 4% sitosterol was added to the diet and fed during the entire experimental period, an increase in total lipids and cholesterol of these tissues was prevented in Group D. Group C-D, which was transferred

TABLE II. Effect of Dietary Additions on Aorta and Liver Lipid Concentrations.

Diet group	Aorta* Total lipids	Aorta* Total chol.	Liver† Total lipids	Liver† Total chol.
A	117	3.5	28.5	3.5
B	108	4.2	32.0	3.2
C	165	7.4	77.9	35.0
D	126	4.1	30.2	3.5
E	133	3.8	28.9	3.1
C-D	113	4.9	29.8	3.7

* Data expressed as mg/g dry tissue.
† " " " " wet " .

to diet D at the end of the 4-week test period, was found to have normal lipid and cholesterol levels in liver and aorta.

Discussion. In the chicken, development of a hyperlipemia, and a concomitant hypercholesteremia, appears to be dependent upon presence of cholesterol in the diet. Both Peterson(9,10) and Pollack(11) have reported independently that a hyperlipemia was not observed when a cholesterol-free diet, or a diet containing cholesterol and sitosterol, was fed to chickens or rabbits. Regression of cholesterol-induced hyperlipemia and atheroma was observed when sitosterol was added to the diet containing cholesterol(12). Addition of sitosterol to the diet presumably inhibits cholesterol absorption. Thus, normal lipid levels are obtained and, depending upon the experimental situation, atheromas are either not formed or existing ones regress. Fullerton *et al.*(1) observed a decrease in clotting time of human whole blood, and of plasma in the presence of Russell's viper venom, after ingestion of a fatty meal. Tilden and Shipley (13) have reported similar observations in dogs. Comparable results were obtained by Shoulders *et al.*(14) and Swank(15) upon intravenous administration of lipid emulsions to dogs; Swank(15) produced similar results in rabbits. Among the agents suspected of shortening plasma clotting time are chylomicrons, certain fats, and certain phospholipids. McDonald and Fullerton(16) observed that decreased clotting time was obtained in humans with restricted physical activity; a fat meal decreased this time still further. This lack of physical activity may be a contributing factor in the decreased "Stypven" time observed in the control chickens of this report.

It is generally accepted that atherosclerosis and/or thrombosis are pathological states leading to tissue infarcts. Interrelationships of those pathological states are unknown. Whether one predisposes to the other, and to what extent each acts independently of the other to cause an infarct, remains to be investigated. From evidence accumulated to date, chronically elevated blood lipids are factors predisposing to atherosclerosis and thrombosis. Indeed, O'Neal *et al.*(2), Thomas and Hartroft(3), and Wilgram(4) have demonstrated that experimental infarcts may be produced in rats with a lipemia and atheroma-inducing diet, *i.e.*, a high fat diet with cholesterol. It is unknown whether hypercholesteremia is a concomitant, or causative agent, of hyperlipemia. It is interesting to note, however, that experimental production, or regression, of atheromas and changes in plasma coagulation times may be controlled in young cockerels with beta-sitosterol, an agent which specifically inhibits cholesterol absorption.

Summary. 1. A cholesterol-induced hyperlipemia was fully counteracted (reduced to normal levels) by feeding beta-sitosterol. 2. The decreased plasma clotting times associated with a hyperlipemia returned to control values when a normal lipid level was attained. 3. An inverse relationship was demonstrated between plasma clotting time in presence of Russell's viper venom and degree of lipemia; *i.e.*, a hyperlipemia resulted in decreased plasma clotting time, and vice versa. 4. Addition of 4% beta-sitosterol to the diet prevented or reversed an increased lipid and cholesterol concentration in liver and aorta.

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Parotoid Secretions of *Bufo blombergi* and *B. peltocephalus.* (25770)

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Bufo blombergi, a giant toad of Colombia, was recently discovered by Rolf Blomberg(1, 2). Each toad weighs one kg or more. *B. peltocephalus* (or *peltacephalus*(3)), a toad well known in Cuba, is comparable in size to *B. marinus.* In contrast with other species, the longitudinal axis of its parotoid glands is perpendicular to the body axis(4). Both *B. blombergi* and *B. peltocephalus* were in the collections of the Dept. of Amphibians and Reptiles of New York Zoological Park. Since no chemical and pharmacological information is available about their parotoid secretions, it was felt desirable to make a preliminary examination. By permission of Dr. James A. Oliver, Director of the New York Zoological Park, one of us (K.K.C.) expressed the parotoid secretions from 2 animals of each species into 2 glass vessels, according to procedure previously described(5). The light yellow secretion of the Colombian toad was milky, slimy, and quickly developed a consistency of chewing gum. It had no special odor. Secretion of the Cuban toad was light gray, creamy, and had a slight scent. Both secretions were air-dried and then desiccator-dried. The material from *B. blombergi,* total 2.156 g, remained light yellow. It was very difficult to pulverize in a mortar because of stickiness. Chunks were unavoidable. No other toad secretion has shown this property. The venom of *B. peltocephalus,* total weight 0.237 g, turned black on drying, and could easily be ground into a fine powder. Sneezing, a bitter taste, and numbness of tongue were noticed during grinding. Various extracts were prepared for detection of catecholamines, indolethylamine derivatives, cholesterol, and cardiotonic substances.

Results. 1. *Catecholamines.* Ten-mg samples were repeatedly extracted with 0.1 N HCl, filtered, and diluted to 10 ml. When ammoniacal alcohol was layered over extracts of both toad secretions, a rose-red color, typical of catecholamines, developed at the junction(6). Intravenous injection of these solutions in pithed cats was followed by a rapid rise of carotid blood pressure with prompt recovery, the pressor action of *B. peltocephalus* being greater than that of *B. blombergi.* Quantitative comparisons were attempted by paper chromatography(7,8). The acid extracts were spotted on Whatman No. 1 paper and developed with water-saturated phenol by ascending chromatography in an atmosphere equilibrated with concentrated HCl and phenol. Air-dried tapes were sprayed with potassium ferricyanide in phosphate buffer, pH 7.8, to make the spots visible. Estimations of epinephrine content were made by comparing spot intensities with those produced by known quantities of epinephrine. Epinephrine concentrations in dried secretions of *B. peltocephalus* and *B. blombergi* were 10% and 2% respectively. Best estimates of *nor*-epinephrine in *B. peltocephalus* were 0.5 to 1%. The existence of this base in the secretion of *B. blombergi* could not be detected by the