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Effect of triparanol on atherosclerosis and on sterol composition and concentration in serum and aorta of the chicken*

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SUMMARY

The administration of triparanol resulted in an increase in serum total sterols and desmosterol in both cockerels and egg-laying pullets. In cockerels treated for 20 weeks with 25.0 mg of triparanol/kg body weight, the total serum sterol level was 106 mg/100 ml compared to 80 mg/100 ml for the controls. Desmosterol represented 76% of these sterols in the triparanol-treated birds while none was detectable in the serum of the controls. In the aortas of these cockerels, desmosterol was not detectable in the controls but represented 67% of total sterols in those receiving triparanol. In egg-laying pullets, a similar but more pronounced effect was observed. Administration of 40.0 mg of triparanol/kg body weight for 10 weeks resulted in an increase of total serum sterols from 65 mg/100 ml to 522 mg/100 ml, of which 90% was desmosterol. Pullets treated for 2 weeks with 50–100 mg of triparanol/kg body weight showed about a fourfold increase in serum sterols, up to 83% of which was desmosterol. In all cases, the administration of triparanol caused a cessation of egg production. It appears that triparanol enhances the degree and incidence of atherosclerosis of the aorta of the chicken.

Since the studies by Blohm et al. (1, 2) indicating that administration of triparanol (1-[*p*-(β-diethylaminoethoxy) - phenyl] - 1 - (*p* - tolyl) - 2 - (*p* - chlorophenyl) ethanol) produced a lowering of blood and tissue cholesterol levels in rats and monkeys, a number of investigators have reported that this drug lowers serum cholesterol in man (3, 4). The replacement of cholesterol by desmosterol in the blood (and tissues) of patients treated with triparanol was first reported by Steinberg, Avigan, and Feigelson (5) and more extensively presented later (6). This effect has been shown by Avigan et al. (4) to result from a block in a late stage of cholesterol synthesis, namely, the reduction of desmosterol to cholesterol.

We have reported that the administration of triparanol for 10 weeks to cockerels fed plain mash or an atherogenic diet did not cause significant changes in the serum cholesterol and phospholipid levels (7). Unexpectedly, there was an increased incidence of microscopic aortic atherosclerosis in cockerels fed plain mash and receiving triparanol as compared to controls on plain mash alone. It seemed desirable, therefore, to undertake an extended study of triparanol-treated chickens on plain mash diets to evaluate further the effect of triparanol on atherosclerosis and its relationship, if any, to serum desmosterol levels.

This report summarizes data obtained from cockerels and egg-laying pullets maintained on various dose levels of parenteral triparanol. Of particular interest was the relatively large increase in serum and aortic sterols in egg-laying pullets treated with triparanol.

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TABLE 1. TOTAL STEROLS AND PERCENTAGE DESMOSTEROL IN AORTAS OF COCKERELS AFTER 10 WEEKS ON TRIPARANOL

Regimen	No. of Birds	Total Sterols	Desmosterol in Sterols
		mg/g	%
Controls on plain mash*	8	1.07 ± 0.09‡	N.D.§
12.5 mg of triparanol/kg	8	0.93 ± 0.02	12.0 ± 2.6
25 mg of triparanol/kg	8	0.82 ± 0.06	29.7 ± 2.1
Controls on atherogenic diet†	8	2.3 ± 0.50	N.D.
12.5 mg of triparanol/kg	7	2.2 ± 0.37	N.D.
25 mg of triparanol/kg	7	2.6 ± 0.40	N.D.

* Red Rose Starter Grower Mash, John W. Esherman & Sons, Stanford, N.C.

† Plain mash containing cholesterol 2%, cottonseed oil 5% by weight.

‡ Standard error of the mean.

§ Desmosterol was not detectable.

METHODS

Ten-week-old Hy-Line cockerels or 31-week-old egg-laying Hy-Line pullets were divided into three to six groups of 8–10 birds each and injected subcutaneously five times per week with triparanol (generously provided by Wm. S. Merrell Co., Cincinnati, Ohio) dissolved in olive oil at dosages ranging from 12.5 mg/kg to 100 mg/kg body weight. The controls were injected with 1.0 cc of olive oil. Blood samples were drawn at biweekly intervals during the experiment. Phospholipid and total lipid determinations were made in the serum by the methods of Fiske and Subbarow (8) and Bragdon (9), respectively. Desmosterol and total sterols in serum and tissues were determined by the method of Avigan et al. (4). After sacrifice of the chickens, the aortas were opened longitudinally and examined for the presence of atherosclerotic lesions.

RESULTS

The Effect of Triparanol on Cockerels. Results of the sterol analysis of aortas from these birds are shown in Table 1. Cockerels on plain mash, and treated with 12.5 or 25.0 mg of triparanol/kg showed a moderate but consistent decrease in total sterols in the aorta as compared to the controls ($p<0.05$). The desmosterol content after 10 weeks of treatment represented 12.0% of the total aortic sterols in the group receiving 12.5 mg/kg body weight and 29.7% at the 25.0 mg/kg level. On the other hand, triparanol treatment of cockerels on an atherogenic diet had no effect on the accumulation of sterols in the aorta.

Repetition of the plain-mash experiment for a period of 20 weeks indicated (Table 2) a slight increase in total serum sterols and a marked increase in the proportion of desmosterol in both serum and aorta sterols.

The Effect of Triparanol on Egg-Laying Pullets on Plain Mash Diets. Treatment of 31-week-old laying pullets with 40 mg/kg of triparanol daily for 10 weeks caused an elevation of serum sterols to an average of 522 mg/100 ml (Table 3), 90% of which was desmosterol. Serum phospholipids and total lipids were also markedly elevated, indicating that lipoprotein concentrations had been greatly increased. At the end of 10 weeks of treatment, the total sterol concentration in the aortas of triparanol-treated pullets was four times that in the aortas of pullets on plain mash. The desmosterol in the aorta, expressed as a percentage of the total sterols, was similar to that of the serum.

In another group of 31-week-old laying pullets, which were treated with 50, 75, and 100 mg/kg of triparanol for 2 weeks, egg production ceased within two weeks (Table 4). At the 100 mg dose level, the pullets ceased laying by the end of the first week. The birds were again started on a course of triparanol

TABLE 2. TOTAL STEROLS AND PERCENTAGE DESMOSTEROL IN SERUM AND AORTA OF COCKERELS AFTER 20 WEEKS ON TRIPARANOL

Regimen	No. of Cockerels	Serum		Aorta	
		Total Sterol	Desmosterol in Sterols	Total Sterol	Desmosterol in Sterols
		mg/100 ml	%	*mg/g*	%
Controls on plain mash	11	80 ± 13.0*	N.D.†	1.1 ± 0.1	N.D.†
12.5 mg of triparanol/kg	8	120 ± 8.7	60 ± 1.0	1.8 ± 0.8	37.9 ± 2.0
25 mg of triparanol/kg	9	106 ± 5.0	76 ± 4.0	1.1‡	67.5‡

* Standard error of the mean.

† Desmosterol was not detectable.

‡ Only two aortas were analyzed.

TABLE 3. EFFECT OF TRIPARANOL ON EGG-LAYING PULLETS TREATED FOR 10 WEEKS

Regimen	No. of Birds	Serum				Aorta	
		Total Sterol	Desmosterol in Sterols	Phospholipids	Total Lipids	Total Sterol	Desmosterol in Sterols
		mg/100 ml	%	*mg/100 ml*	*mg/100 ml*	*mg/g*	%
Controls	5	65 ± 10.0*	0	722 ± 56.6	1968 ± 158.4	2.0 ± 0.3	N.D.†
40 mg of triparanol/kg	7	522 ± 80.8	90 ± 2.3	2307 ± 232.0	7910 ± 715.0	8.0 ± 2.4	76 ± 5.7

* Standard error of the mean.
† Desmosterol was not detectable.

treatment when they were 43 weeks old, and again there was a significant decrease in egg production 2 weeks later (Table 4). The pullets were sacrificed at that time, and sterols in serum and aorta were analyzed.

At the end of the 14-week period, the amount of total sterols and the percentage of desmosterol in serum sterols were increased in the triparanol-treated pullets (Table 5). Administration of triparanol resulted in the appearance of gross aortic lesions, and with increasing dose of the drug the number of birds affected increased. No gross atherosclerotic lesions were seen in any of the pullets on plain mash.

DISCUSSION

Our initial data (7) suggested that triparanol treatment increased the incidence and extent of aortic atherosclerosis of cockerels fed either plain mash or an atherogenic diet for 10 weeks. Triparanol treatment of birds on an atherogenic regimen caused no accumulation of aortic desmosterol (Table 1), presumably because the exogenous cholesterol depressed sterol synthesis and the sterol deposited in the plaques was almost solely derived from the diet. When, in the present studies, the drug was administered to birds on plain mash for 20 weeks, the amount of total sterol in the serum as well as the percentage of desmosterol was increased significantly (Table 2). The total sterol in the aortas was found to be slightly higher in the triparanol-treated cockerels than in the controls on plain mash. Furthermore, there was a significant amount of desmosterol in the aortic tissue of the triparanol-treated cockerels, but little, if any, in the aortas of controls on plain mash.

Administration of 40 mg/kg of triparanol to egg-laying pullets on a plain mash diet caused a marked increase of serum sterols. The extremely high proportion of desmosterol in the serum sterols, averaging 90%, has not been observed before in other species. The dramatic rise in total serum sterols caused by triparanol may be related to the cessation of ovulation without a parallel reduction in sterol biosynthesis.

Increasing the triparanol dose from 50 to 100 mg did not affect significantly the total sterol concentration and percentage of desmosterol in serum sterols (Table 5). The proportion of desmosterol in the sterols of the grossly normal parts of the aortas as well as in the atherosclerotic lesions appeared to be similar to that in the serum, suggesting that desmosterol has a tendency similar to that of cholesterol to deposit in arteries of cockerels or egg-laying pullets. Analogous results were reported for the rabbit by Avigan and Steinberg (10).

The results of this study indicate that there is an accumulation of desmosterol in the plasma of triparanol-treated chickens, resulting in the formation of aortic lesions containing considerable amounts of desmosterol.

TABLE 4. NUMBER OF EGGS LAID WEEKLY BY PULLETS TREATED WITH TRIPARANOL

Age in Weeks	29	30	31*	32	33†	34	35	36	37	38	39	40	41	42	43‡	44	45
Daily Dose of Triparanol/kg Body Weight	Total Number of Eggs per Week																
Controls (10)§	84	87	73	66	76	71	71	79	77	79	79	67	67	70	77	69	79
50 mg (8)	66	70	66	20	0	0	7	11	50	61	75	73	59	54	54	43	7
75 mg (9)	75	70	56	17	0	0	3	13	21	46	51	49	65	68	68	32	11
100 mg (8)	77	63	54	0	0	0	5	9	11	29	30	41	50	64	63	41	4

* Beginning of treatment with the drug.
† Drug withdrawn.
‡ Second treatment period.
§ Number in parentheses indicates number of pullets in each group.

TABLE 5. SERUM AND AORTIC STEROLS OF EGG-LAYING PULLETS TREATED WITH TRIPARANOL FOR TWO TWO-WEEK PERIODS

Regimen	No. of Birds	No. with Gross Lesions	Serum		"Normal" Aorta Desmosterol in Sterols	Aortic Lesions Desmosterol in Sterols
			Total Sterols	Desmosterol in Sterols		
			mg/100 ml	%	%	%
Controls	10	0	62 ± 11.0	3 ± 1.0*	...†	...
50 mg of triparanol/kg	7	3	226 ± 94.0	78 ± 5.5	...	...
75 mg of triparanol/kg	9	6	276 ± 81.5	66 ± 5.5	...	...
100 mg of triparanol/kg	8	8	231 ± 68.2	83 ± 3.8	63 ± 3.0	58 ± 6.7

* Difference from zero is within the error of determination.
† Not determined.

Recent observations by Herndon and Siperstein (11) also show that desmosterol deposits at least as readily as does cholesterol. The cessation of egg-laying in pullets on high doses of triparanol has been reported by Wong et al. (12) and Burgess, Burgess, and Wilson (13). The latter investigators have also shown that feeding hens with 0.5% triparanol over a two-week period resulted in a replacement of 85% of the egg cholesterol by desmosterol. Furthermore, it was noted that pullets treated with triparanol produced only small immature ova. Holloszy and Eisenstein (14) reported that triparanol feeding produced a significant reduction in the amount of corticosterone secreted by the adrenals of rats. However, this reduction may have been secondary to the marked decrease in adrenal cholesterol concentration that results from triparanol therapy. It can only be speculated that triparanol may interfere with the secretion of both adrenocortical hormone and estrogen. Further studies are necessary to determine if this drug did reduce estrogen production; such an effect might account for the cessation of egg-laying and the small ova found in our studies.

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