Appearance of Cholesterol Precursors as a Result of Treatment with 20,25-Diazacholesterol. (29430)

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The observation that 22,25-diazacholestanol dihydrochloride (SC-11952) induced the accumulation of desmosterol in the sera of patients(1,2) and in the sera and livers of experimental animals(3,4) has prompted studies with another, more potent diazasterol, 20,25-diazacholesterol dihydrochloride (SC-12937(5)), with the intent of more specifically identifying the cholesterol precursors present in the serum and liver.

Methods. Eight male rats were treated orally with an aqueous solution of SC-12937 (1 mg/kg) for a period of 10 days. A control group was treated with the vehicle alone. All animals received as drinking water 6-propylthiouracil as a 0.02% solution to induce a mild hypercholesterolemia(3). After the treatment period all animals were sacrificed by bleeding from the aorta and serum and liver samples were taken for analysis.

The serum samples (1 ml) were extracted with 20 ml of chloroform-methanol (2:1), and 10 ml aliquots of this extract were taken to dryness and saponified with 10% KOH in 50% aqueous ethanol at 100° for 3 hours. The non-saponifiable lipids were extracted from the aqueous phase with 10 ml n-hexane. Liver samples (0.5 g) were homogenized in 20 ml chloroform-methanol, saponified and extracted with n-hexane in the same way. Serum and liver cholesterol, desmosterol and total sterol concentrations were determined spectrophotometrically by the method of Avigan *et al*(6).

Gas chromatographic analysis of the non-saponifiable lipid extracts was carried out on hexane aliquots taken to dryness under N_2 and redissolved in a small volume of chloroform-methanol (1:1). This analysis was accomplished using a Barber-Coleman model 10 instrument with an Sr^{90} detector. The adsorbent was prepared by adding 30 g Gas Chrom Z (100-140 mesh) to 150 ml chloroform containing 1.2 g G E 287-149-300. This mixture was stirred thoroughly, heated to remove the solvent, and finally dried under an infra red lamp overnight. A 4 ft × 8 mm O.D. glass column was packed with the coated support and was cured at 220° for 48 hours in a flow of argon at 10 lb pressure. Operating temperatures for the column, cell and flash heater were 225°, 265-275°, and 285-300°C, respectively. Argon pressure was 20 lb/sq in, and flow was 68-100 ml/min.

Standard samples of cholesterol, desmosterol, lanosterol and zymosterol were run both independently and in known mixtures to determine the qualitative and quantitative characteristics of the system. The peaks obtained were symmetrical and the quantitation by planimetry and by the disc integrator of the instrument was within experimental error (± 2%). When the retention time of cholesterol was set as 1.0, the relative retention times of the standard sterols were: desmosterol, 1.17, lanosterol, 1.40 and zymosterol, 1.71.

For evaluation of the effect of SC-12937 in man* two serum samples from each of 6 patients were analyzed. The first sample was taken before treatment was begun. The second sample was taken after the patients had been given SC-12937 at a dose of 25 mg per day for periods of 2 to 9 months. Both spectrophotometric and gas chromatographic analyses of the non-saponifiable lipids of each sample were carried out.

Results. The effects of SC-12937 on the liver and serum sterols in male rats are shown in Table I. It is evident that a major action of this compound was to induce a shift in the proportions of cholesterol and desmosterol making up the total sterol content of both the liver and the serum. Thus,

* Human serum samples were provided through the courtesy of Dr. J. M. Martt, Univ. of Missouri, Columbia, Mo.

TABLE I. The Effect of SC-12937 on Serum and Liver Sterols in Male Rats.

Tissue	Method	Component	Control	Treated
Serum	Spectrophotometric	Total sterols	1.41 mg/ml	1.25 mg/ml
		Cholesterol	1.36 "	.98 " *
		Desmosterol	.05 "	.27 " *
	Gas chromatography	Cholesterol	100%	78%*
		Desmosterol	0%	22%*
Liver	Spectrophotometric	Total sterols	2.73 mg/g	2.51 mg/g
		Cholesterol	2.44 "	1.50 " *
		Desmosterol	.29 "	1.01 " *
	Gas chromatography	Cholesterol	100%	56%*
		Desmosterol	0%	34%*

* Values are significantly different from control concentrations, $P < .01(10)$.

TABLE II. Effect of SC-12937 on Serum Sterols in Man.

Method	Component	Serum sterols	
		Pretreatment	Treatment
Spectrophotometric	Total sterols	296 mg/100 ml	244 mg/100 ml*
	Cholesterol	296 "	127 " *
	Desmosterol	0 "	117 " *
Gas chromatography	Cholesterol	100%	68 %*
	Desmosterol	0%	23.0%*
	Fraction III	0%	6.6%*
	Fraction IV	0%	2.4%*

* Values are significantly different from pre-treatment concentrations, $P < .05(10)$.

in the control sera, cholesterol accounted for about 94% of the total sterols by spectrophotometric analysis, whereas in the sera from the treated rats only 78% of the total sterols was analyzed as cholesterol. Similarly, 89% of the total liver sterols in the control group was detected by this method as cholesterol while in the treated animals 60% was found as this sterol.

In the control animals no desmosterol was detected by gas chromatography. The reductions in cholesterol contents of serum and liver of treated rats were statistically significant as were the increases in desmosterol concentrations, and the ratio of cholesterol to desmosterol as detected by this procedure was little different from that found by the chemical method.

In Table II are given results of the analyses of sera from patients treated with SC-12937. No desmosterol or other sterol was detected spectrophotometrically in the pretreatment serum samples. Treatment with the diazasterol induced a reduction in total sterols of about 18%. By spectrophotometric analysis cholesterol appeared to contribute 52% to the total sterols while desmosterol was present to the extent of 48%. The reductions of serum total sterols and of cholesterol were statistically significant as was the increase in desmosterol concentration.

It is pertinent that the chemical test for desmosterol is not specific for this substance (6). Any naturally occurring constituent of the non-saponifiable lipid extract of tissues that has significant absorption at 425 mμ in the Lieberman-Burchard test will yield an *apparent* amount of "desmosterol" in the extract. This is substantiated in the gas chromatographic analyses of human sera summarized in Table II. The pretreatment samples showed only a single sterol component that was identified as cholesterol. Treatment with SC-12937 reduced the proportion of cholesterol in total sterols to 68% and resulted in the appearance of 3 additional sterols. The most abundant of these was identified as desmosterol which amounted to 23% of the total sterols detected. The other 2 sterols showed relative retention times (Fraction III 1.57; Fraction IV 1.89) inconsistent with the observed sterol standard materials.

Conclusions. It is evident that use of SC-12937 as a hypocholesterolemic agent induced the appearance of desmosterol in sera of rats and man. In addition, in man traces of 2 other sterols were detected. In this regard it is noteworthy that Klein *et al*(7) have found that in the rat subjected to short interval, low dose treatment with SC-12937 the appearance of an unidentified sterol was detected *without* desmosterol accumulation. Further, Thompson *et al*(8) detected in the carcasses of rats treated with a high dose (10 mg/kg for 8 weeks) of SC-12937 a sterol they tentatively identified as 7,24-cholestadiene-3β-ol. These investigators found only cholesterol and desmosterol present in the livers, however. It seems evident that the discrimination between cholesterol and the other sterols that might appear in the serum or tissues as a result of SC-12937 treatment may be more accurately carried out by gas chromatographic analysis or high resolution column chromatography(9) of the nonsaponifiable lipid than by spectrophotometry.

The hypocholesterolemic effect of SC-12937 may be tentatively attributed in large part to inhibition of the synthesis of cholesterol at multiple loci in the series of poorly understood transformations whereby lanosterol is converted to cholesterol. The particular cholesterol precursor(s) that may be detected during SC-12937 treatment seemingly will be dependent upon the intensity and duration of the treatment with the drug.

Summary. Analysis of the plasma of rats and patients treated with 20,25-diazacholesterol dihydrochloride demonstrated the presence of desmosterol in the non-saponifiable lipids. Small amounts of 2 unidentified sterols were also detected in the human plasma samples.

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Treatment of Experimental Salmonellosis in Chicks with Synnematin B* (29431)

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Following the observation that synnematin B† exerted an *in vitro* bacteriostatic effect on *Salmonella*(1), Olson and Jennings found that in mice or chicks the visceral organs of animals inoculated with *S. typhimurium*, *S. typhi* or *S. pullorum* could be freed of these microorganisms even if treatment with the antibiotic was delayed 1-2 days or more(2). Others confirmed the life-saving effect of synnematin B in mice, when drug administration was begun within an hour after exposure to *S. typhimurium*(3,4), although Hobby *et al* observed that the organisms localized in the spleens could not be completely eradicated

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† Syn.: adcillin, cephalosporin N, penicillin N.