observation of Hayano *et al.*(10) that ascorbic acid may inhibit 11β-hydroxylation in beef adrenals leads us to think that the stimulatory effect of 3MC on adrenal ascorbic acid synthesis may be associated with the inhibition of corticosterone synthesis in the adrenal cortex. Whether 3MC suppresses corticosterone synthesis by inhibition of 11β-hydroxylation is now under study.

Summary. A single feeding of either DMBA and 3MC caused significant lowering of adrenal corticosterone concentration in the adrenal glands of the rats so treated. The fact that DMBA produced massive necrosis of the adrenal cortex and 3MC did not induce any morphological evidence of damage of cortical cells, suggests that the mechanism by which these 2 carcinogenic hydrocarbons suppress adrenal corticosterone synthesis is not the same. It was also observed that adrenal ascorbic acid was greatly reduced in DMBA-treated rats; its synthesis was significantly enhanced in 3MC-treated rats.

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Accumulation of 24-Dehydrocholesterol in Rats Treated with 22,25-Diazacholestanol. (28237)

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It has been reported(1) that 20α-(2-dimethylaminoethyl)amino-5α-pregnan-3β-ol dihydrochloride (22,25-diazacholestanol, or SC-11952), in a rat liver homogenate interfered with the biosynthesis of cholesterol primarily by inhibiting β-hydroxy-β-methylglutaryl coenzyme A reductase. Furthermore, *in vivo*, the compound reduced levels of cholesterol in the serum of normal and hypercholesterolemic rats(2).

Contrary to the conclusions as to its sites of action based on studies with rat liver homogenates, Sachs and Wolfman(3) have recently discovered high levels of 24-dehydrocholesterol (desmosterol) in the serum of hypercholesterolemic human patients after treatment with 22,25-diazacholestanol. We have therefore considered it important to establish whether or not desmosterol is accumulated in rats treated with 22,25-diazacholestanol.

Methods. Twenty male hooded rats with an average body weight of 150 g were used. The animals were kept on a Purina Chow Laboratory grade diet containing 0.07% cholesterol. Ten rats received daily 100 μmoles/kg of 22,25-diazacholestanol dihydrochloride (mol wt 464.60)* orally by stomach tube for a period of 7 days. The other 10 animals used as controls underwent the same treatment except that saline was used. On the 7th day of treatment after 4 hours fasting and 3 hours after the last oral injection all animals were sacrificed, blood samples taken by heart puncture and livers and adrenals removed. Cholesterol and desmosterol levels were determined by the following procedures.

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TABLE I. Effect of 22,25-Diazacholestanol Dihydrochloride* (100 μmoles/kg/day for 7 days) on Levels of Cholesterol and Desmosterol in Male Rats.

	Serum (mg/100 ml)		Liver (mg/100 g wet wt)		Adrenals (mg)	
	Control	Treated	Control	Treated	Control	Treated
Total sterols	75.0 ± 4.9†	56.8 ± 8.4 (−24%)	225.7 ± 8.4	212.1 ± 3.2 (− 6%)	.56 ± .07	.19 ± .02 (−66%)
Cholesterol	73.9 ± 5.2	16.4 ± 1.8 (−78%)	217.7 ± 8.5	49.8 ± 3.8 (−78%)	.52 ± .06	.05 ± .01 (−90%)
Desmosterol	1.1 ± .3‡	40.4 ± 2.9	8.0 ± 1.3‡	162.3 ± 4.3	.04 ± .01‡	.14 ± .02

* Mol wt 464.60. † Standard error.

‡ Due to decreased accuracy of method for trace amounts of desmosterol, value may not represent true concentration.

Serum samples (0.5 ml) were saponified with 0.4 M ethanolic KOH (5 ml) for 1 hour at 37°C. Water (5 ml) and n-hexane (10 ml) were added, the mixture was shaken and centrifuged. Aliquots (7 ml) of the hexane layer were evaporated to dryness and cholesterol and desmosterol determined by the differential colorimetric technique of Avigan *et al.*(4), as modified by Blankenhorn and Kuzma(5) by using the Liebermann-Burchard reagent according to Abel *et al.*(6). Standards of cholesterol and desmosterol and mixtures thereof were used to verify the method.

Liver samples (0.5 g) and adrenals were saponified with 1.25 M ethanolic KOH (5 ml) for 1 hour at 70°C and assayed as described for blood serum.

Results. The concentrations of cholesterol and desmosterol in tissues of control and treated rats are presented in Table I. In treated animals true cholesterol levels in the serum, liver and adrenals were strikingly reduced; in spite of significant accumulation of desmosterol, true total sterol levels remained low in both serum (76% of control) and adrenals (34% of control). In the liver, on the other hand, the true total sterol level was only slightly depressed, the loss of cholesterol being almost replaced by desmosterol. In treated animals the high desmosterol content in the serum (71%), liver (76.5%) and adrenals (74%) was remarkably similar.

Since the method is not sufficiently accurate to determine traces of desmosterol in cholesterol, the levels of desmosterol in control rats may not be significant(4).

Discussion. In vitro(1), 22,25-diazacholestanol depressed (a) acetate-1-C^{14} incorporation into cholesterol to a greater extent than that of mevalonate-2-C^{14}, (b) acetate-1-C^{14} incorporation into mevalonate but not into β-hydroxy-β-methylglutarate and, (c) mevalonate-2-C^{14} incorporation into lanosterol. From this, it was concluded(1) that the apparent primary site of action of 22,25-diazacholestanol is at the level of β-hydroxy-β-methylglutaryl coenzyme A reductase. Earlier it was suggested that the same enzyme is inhibited by exogenous cholesterol(7,8). In view of this similarity of action, 22,25-diazacholestanol was termed as "cholesteromimetic." Later it was reported that *in vivo* 22,25-diazacholestanol reduced the levels of cholesterol in the serum of normal and hypercholesterolemic rats(2).

Assuming that the suggested "cholesteromimetic" type of interference with endogenous formation of cholesterol *in vitro* also occurs *in vivo,* treatment with 22,25-diazacholestanol should produce sterol contents which differ from controls only in their reduced concentrations. Contrary to this expectation, high levels of desmosterol have been found in the serum of hypercholesterolemic patients after treatment with 22,25-diazacholestanol(3).

Since rats are usually used in studies of potential antihypercholesterolemic agents which act by interfering with the endogenous synthesis of cholesterol, it was important to establish whether this apparent discrepancy is due to species difference which may impose different mechanisms of action.

According to our results, oral administration of 22,25-diazacholestanol to normal male rats produces in the serum, liver and adrenals high concentrations of desmosterol, a phenomenon similar in principle to that found in humans.

It thus appears that *in vivo,* in normal rats

and in hypercholesterolemic humans, 22,25-diazacholestanol inhibits the biosynthesis of cholesterol by a triparanol-like(9) rather than a cholesterol-like mechanism as suggested on the basis of studies *in vitro*.

Summary. Oral administration of 22,25-diazacholestanol dihydrochloride to rats caused striking lowering of cholesterol and significant accumulation of desmosterol in the serum, liver and adrenals. The findings are similar to those in humans and suggest a triparanol-like mechanism of action.

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Isolation and Chemical Characterization of a β-CRF from Pig Posterior Pituitary Glands.* (28238)

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We wish to report here the isolation from commercial powders of pig posterior pituitary gland of a basic peptide with ACTH-releasing activity. Amino acid composition showed that this peptide was probably related to lysine-vasopressin; it was thus designated β-CRF in accord with the terminology proposed earlier(1,2). Only the terminal sequences could be determined with the minute quantities of the peptide available.

Methods. The assays for vasopressin, CRF, ACTH and MSH have been described(3,4, 5). Pituitary preparations, chromatographic methods and estimation of peptides have also been reported(4,6,7). The countercurrent distribution and recovery of materials thereafter were performed essentially as in Schally *et al.*(8); n-butanol was freshly redistilled over zinc powder. For paper chromatography of peptides the Partridge system was used (9).

Results and discussion. Fifty grams of a material similar to protopituitrin(4) from *hog* posterior pituitary glands were subjected to the precipitation procedure as in(4); 12.3 g of precipitate B(4) were obtained; about 4.1 g of precipitate B were applied on a preparative column of carboxymethylcellulose (CMC) (0.45 meq/g; 2.9 × 55 cm, equilibrated with 0.02 M pH 4.6 ammonium acetate buffer). Elution was effected by a gradient to 0.1 M, pH 7 buffer, through 2000 ml mixing flask. The fractionation pattern was identical to that shown in Fig. 3 in Schally and Guillemin(4). A large peak of basic peptides had as its main components lys-vasopressin and α-MSH; CRF activity was contained on the ascending edge of this peak. Two such columns yielded after lyophilization 1.4 g of basic peptide material. One gram of this material was distributed for 103 transfers (Fig. 1) in a system of n-butanol/0.03 M p-toluene sulphonic acid containing 0.001 M EDTA and 6.10^{-5} M cystine. CRF

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