rhotic patients. Fat deprivation for a long period is known to lower post-heparin lipoprotein lipase response(16) and the steatorrhoea demonstrated in some, but not all, of our patients with cirrhosis of the liver might contribute to the findings.

Plasma free fatty acids fail to rise after administration of heparin in fasting patients with cirrhosis(18). This might be at least partly explained by the low lipoprotein lipase activity.

Summary. An improved method is described for estimating post-heparin plasma lipoprotein lipase activity permitting initial velocity measurement with zero order kinetics. Plasma values after heparin in 20 normal subjects are considerably higher than previously recorded. Values in 31 cirrhotic patients are significantly lower than normal.

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Effect of Carcinogenic Polynuclear Hydrocarbons on Corticosterone and Ascorbic Acid Content of Adrenal Glands in Rats.* (28236)

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The induction of massive necrosis and hemorrhage in the adrenal cortex by 7,12-dimethylbenz(a)anthracene (DMBA) reported earlier by Huggins and Morii(1) has since been confirmed(2,3). The adrenolytic effect of DMBA was found to involve selectively the inner zones of the adrenal cortex. Interestingly, this profound effect of DMBA is not a property common to all carcinogenic hydrocarbons. A single large dose or repeated doses of 3-methylcholanthrene (3MC) failed to induce adrenocortical necrosis. It was demonstrated, however, that so far as induction of mammary cancer in rats was concerned, 3MC was a much less potent carcinogen than DMBA.

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In the present investigation, it was observed that concentrations of corticosterone in the adrenal gland and in plasma decreased rapidly after a single dose of either DMBA or 3MC. It was also demonstrated that whereas ascorbic acid synthesis was greatly depleted in the adrenal glands of DMBA-treated rats, it was, to the contrary, stimulated in the adrenal glands of rats receiving 3MC. These observations suggest that the mechanisms by which these 2 polycyclic hydrocarbons suppress corticosterone secretion in the adrenal cortex may not be the same, since 3MC does not induce adrenocortical necrosis.

Materials and methods. The experimental animals were female Sprague-Dawley rats 50-55 days old and weighing 150-185 g. The car-

cinogens were dissolved in olive oil in a concentration of 30 or 60 mg/ml for 3MC, and 20 mg/ml for DMBA. A single feeding of either of these compounds through a stomach tube was performed in all experiments.

There were 5 groups of 50 rats each of experimental animals in the studies of plasma and adrenal corticosterone contents: (a) controls, (b) rats receiving 1 ml of olive oil, (c) rats receiving a single feeding of 20 mg/ml of DMBA, (d) rats receiving a single feeding of 30 mg/ml of 3MC and (e) rats receiving a single feeding of 60 mg/ml of 3MC. Five rats were decapitated each day for 6 days and then on the 8th, 15th, 20th, and 25th days after the single feeding of the carcinogen. Both adrenals in each animal were carefully trimmed, weighed, and then homogenized and prepared for corticosterone estimation according to the method of Guillemin *et al.*(4). For serum or plasma corticosterone, the procedure for extraction and preparation was carried out according to the method of Zenker and Bernstein(5). The final development of fluorescence, however, was modified.

The modification involved the development of fluorescence on the dry residue of the extract, either of plasma or of the adrenal tissues, with 0.1 ml ethylalcohol and 0.5 ml of 90% H_2SO_4 for 2 hours at room temperature. The mixture was then diluted with 2 ml of 65% H_2SO_4, and the fluorescence was measured on a Farrand fluorometer equipped with a Corning No. 5113-3389 glass filter as a primary filter, and a 540-mμ interference filter as a secondary filter.

There were 3 groups of experimental animals for studies of adrenal and plasma ascorbic acid contents: (a) controls, (b) rats receiving a single dose of 20 mg/ml of DMBA, and (c) rats receiving a single feeding of 30 mg/ml of 3MC. The ascorbic acid content of the adrenal gland and the plasma was determined for 20 adult female rats as controls. No determinations for controls were done on the consecutive days paralleling the determinations for carcinogen-fed rats. In Groups b and c, each containing 45 rats, the ascorbic acid content in adrenal glands and plasma was determined daily for the first 5 consecutive days, and then on the 7th, 10th, 14th,

TABLE I. Corticosterone Content of Adrenal Gland in Rats.*

Groups	Day 1		Day 2		Day 3		Day 4		Day 5	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Control	18.9-35.5	27.0 ± 4.6	20 -34.6	25.9 ± 5.8	18.0-34.8	26.8 ± 6.4	17.6-36.0	27.2 ± 6.8	19.0-34.2	26.0 ± 6.0
Olive oil (1 ml)	20.5-26.0	23.1 ± 2.5	21 -27.4	24.0 ± 2.9	14.4-37.8	22.2 ± 7.4	19.0-28.6	24.0 ± 4.0	18.2-35.0	22.4 ± 7.4
DMBA (20 mg)	30.4-34.1	33.5 ± 3.6	18.6-30.8	27.0 ± 4.4	12.3-26.7	17 ± 7.8	11.0-18.4	14.6 ± 4.8	8.5-16.5	12.0 ± 5.0
3MC (30 mg)	19.4-41.2	34.0 ± 8.7	18.0-34.2	22.0 ± 6.8	11.2-29.8	19.4 ± 7.4	11.0-20.7	13.8 ± 6.5	14.5-21.0	19.6 ± 8.1
3MC (60 mg)	24.6-55.0	43.2 ±11.6	19.8-32.6	21.5 ± 5.7	11.3-28.5	18.0 ± 4.6	9.5-18.9	14.4 ± 4.2	10.5-19.0	15.0 ± 4.6

Groups	Day 6		Day 8		Day 15		Day 20		Day 25	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Control	18.8-29.8	23.8 ± 4.6	19.2-36.4	26.7 ± 6.7	19.7-29.4	24 ± 4.4	19.4-37.0	30.2 ± 5.9	20.5-36.0	27.5 ± 6.4
Olive oil (1 ml)	14.0-23.5	18.6 ± 3.8	17.9-25.7	21.7 ± 3.1	13.2-25.0	18.2 ± 4.1	18.9-36.0	28.3 ± 6.1	15.9-26.8	21.1 ± 4.2
DMBA (20 mg)	8.5-21.5	12.5 ± 5.0	14.8-25.8	21.9 ± 3.9	18.2-45.5	28.3 ± 11.5	48 -56.9	51.5 ± 3.3	18.4-36.6	25.0 ± 7.8
3MC (30 mg)	15.5-23.0	21.0 ± 7.9	18.8-36.6	27.3 ± 7.0	17.0-38.0	28.0 ± 7.0	19.5-43.8	31.4 ± 8.5	15.1-43.1	29.8 ± 9.5
3MC (60 mg)	9.6-18.9	14.4 ± 4.2	20.1-27.4	23.2 ± 2.8	31.7-41.2	36.6 ± 5.4	24.9-39.6	33.6 ± 5.3	29.2-33.6	30.7 ± 1.7

± standard deviation.

* All values of corticosterone are expressed in μg/g of adrenal gland.

and 21st days after the feeding of the carcinogenic hydrocarbons. Ascorbic acid in the adrenal glands and in plasma was determined by the method of Schaffert and Kingsley, a slight modification of the method of Roe and Kuether(6). The histology of the adrenal glands was studied in rats of both the control group and the carcinogen-treated groups.

Results. Corticosterone concentration in the adrenals and plasma of normal adult rats. The mean contents of corticosterone expressed as $\mu g/g$ of adrenal tissue in the 100 adrenal glands of 50 control adult female rats determined on days parallel to the experimental groups ranged from 23.8 $\pm$ 4.6 to 30.2 $\pm$ 5.9 (Table I). Within the experimental period of 25 days, the serum corticosterone expressed as $\mu g/100$ ml serum in these same animals ranged from 20.6 $\pm$ 3.5 to 34.8 $\pm$ 4.0. In the group in which a single dose of 1 ml of olive oil was fed, the mean amounts of adrenal corticosterone (in the same number of animals) ranged from 18.6 $\pm$ 3.8 to 26.3 $\pm$ 6.2. In this group, the serum corticosterone was 17.6 $\pm$ 6.8 to 31.8 $\pm$ 7.0. Although the adrenal corticosterone content in rats receiving 1 ml of olive oil was slightly lower than in the control rats, the difference was not significant. It is to be concluded that feeding of the olive oil vehicle has not affected the corticosterone concentration of the adrenal gland.

Corticosterone in adrenals and serum of rats treated with polycyclic hydrocarbons. The mean contents of corticosterone in the adrenals and of rats receiving either DMBA or 3MC are shown in Table I. When the contents of corticosterone in the adrenal glands in carcinogen-treated rats are calculated as percentages of the amounts of adrenal corticosterone in the control rats on the corresponding days, the relative corticosterone content in the adrenal glands first rose after the feeding of the carcinogens, but it soon fell to a low level beginning on the 3rd day, and remained at approximately the same level until the 8th day, when a significant rise occurred (Fig. 1). The decline of corticosterone content on the 3rd day in the DMBA-treated rats was correlated with the appearance of necrosis and hemorrhage in the

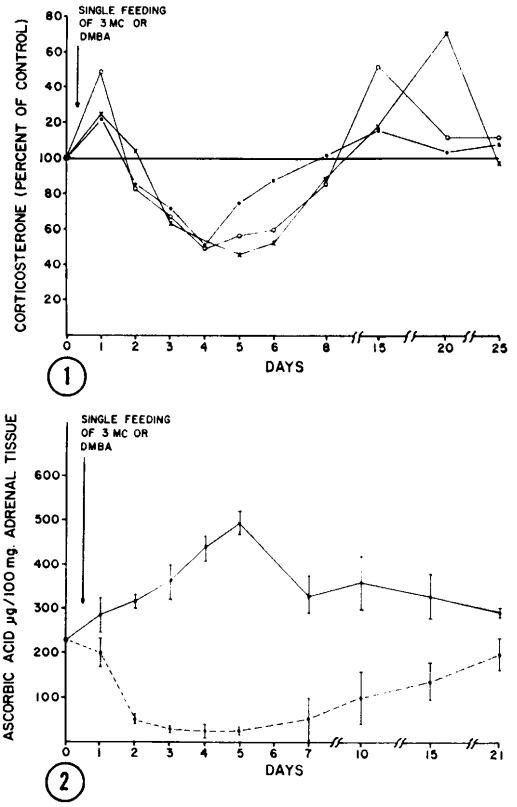

FIG. 1. Effect of DMBA and 3MC on corticosterone content of adrenal gland (calculated as percentage of control). Five rats in each group. Adrenal corticosterone content in rats receiving 20 mg of DMBA (×—×), 60 mg of 3MC (○—○), 30 mg of 3MC (●—●).

FIG. 2. Adrenal ascorbic acid content in rats receiving 20 mg of DMBA (●- - -●) and 30 mg of 3MC (●——●). Mean and standard deviations are plotted.

inner zones of the adrenal cortex. Histologically, evidence of regeneration appeared on the 6th day. It is interesting to note that degree of fall of corticosterone content in the adrenal glands in rats treated with DMBA and in those treated with 3MC was almost identical, despite the fact that DMBA caused necrosis and hemorrhage of the cortical cells. It should also be noted that a sudden rise in corticosterone content of the adrenal gland between the 15th and 20th days after the initial carcinogen feeding was correlated with morphological evidence of completion of regeneration of the cortical cells. It is interesting that the corticosterone content of the adrenal glands in 3MC treated rats began to

rise on the 5th day whereas it remained at a low level in DMBA treated rats until the 8th day.

Within the experimental period of 25 days, the plasma corticosterone in the DMBA treated rats ranged from 11.6 ± 5.8 to 50.0 ± 11.5; and that in the 3MC (30 mg/ml) treated groups from 19.0 ± 6.8 to 48.6 ± 7.9. The rise and fall of the plasma corticosterone in the carcinogen fed rats followed exactly the same pattern as the content of the adrenal corticosterone in these same rats.

Ascorbic acid concentration in adrenals and plasma in normal and carcinogen-treated rats. In our laboratory, ascorbic acid concentration in the adrenals in 20 rats ranged from 203 to 350 μg/100 mg of tissue, with a mean of 251 ± 12.8; and that in plasma from 6.5 to 11.0 μg/ml, with a mean of 8.6 ± 5.4. In the 3MC-treated rats, there was a continuous rise in adrenal ascorbic acid from the 1st to the 5th day after the single feeding of the carcinogen. The level then returned to normal, and remained normal throughout the rest of the experimental period. In the DMBA-treated rats, a rapid decline in ascorbic acid content of the adrenals was observed beginning on the second day after the carcinogen feeding. The level remained low until the end of the experimental period (Fig. 2). The plasma ascorbic acid of either DMBA- or 3MC-treated rats was not changed, indicating that synthesis of ascorbic acid in other sites was not affected.

Morphological studies of adrenals in rats receiving carcinogenic hydrocarbons. The histological appearance of the adrenal cortex in rats receiving 20 mg of DMBA has been described in detail elsewhere(1,2,3). In the present experiments, adrenal necrosis and hemorrhage were observed in the severest degree on the 3rd day following a single feeding of the carcinogenic hydrocarbon. Calcification and regeneration began on the 6th day; and on the 15th day, complete regeneration was observed in most of the animals. In a few rats, the adrenal cortex even appeared hyperplastic. No evidence of necrosis of the inner zones of the adrenal cortex was ever observed in rats treated with 3MC in the present experiments.

Discussion. The present study demonstrates that both DMBA and 3MC are capable of suppressing adrenal corticosterone secretion. The fact that plasma corticosterone level was similarly decreased in these same rats suggests that synthesis of corticosterone was reduced. At the dose levels of the carcinogens used in the present study, 3MC and DMBA are able to suppress adrenal corticosterone synthesis with equal effectiveness. It is interesting that whereas DMBA produced necrosis of the adrenal cortex in 100% of the animals receiving the drug in this study, 3MC did not produce any morphological evidence of damage of the cortical cells. It would appear that the depletion of corticosterone content in the adrenals of DMBA-treated rats might be secondary to damage to the cortical cells, since dead cortical cells cannot synthesize corticosterone. The question then arises, What is the mechanism of the inhibition of corticosterone synthesis in 3MC-treated rats?

If the inhibition of adrenal corticosterone synthesis in DMBA-treated rats is indeed secondary to the necrosis and hemorrhage of the cortical cells, it is conceivable that administration of a "non-necrotizing" dose of DMBA would not affect corticosterone synthesis in the adrenal cortex. Preliminary results from experiments that we have recently conducted show that a single feeding of 10 mg/ml DMBA to rats failed to induce any depletion in the corticosterone content of the adrenal glands, within the first 5 days. This observation leads us to postulate that the mechanism by which 3MC inhibits adrenal corticosterone synthesis is different from that by which DMBA inhibits it.

Stimulation of ascorbic acid synthesis and excretion in rats treated with 3MC has been observed by several investigators(7,8,9). The mechanism by which 3MC stimulates ascorbic acid synthesis remains a mystery. In the present study, it was demonstrated that adrenal ascrobic acid synthesis was stimulated by 3MC. The ascorbic acid content of the adrenal glands in DMBA-treated rats, however, was greatly reduced. Although the relationship between ascorbic acid and adrenal steroid synthesis is not yet understood, the

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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