capable of preventing the dietary necrosis (2) were effective also in reducing mortality of mice fed the necrogenic diet and infected with M.H.V.$_3$ virus. In chicks a Torula yeast diet produces multiple exudates. Like necrotic degeneration in mice these lesions can be prevented by both alpha tocopherol and selenium(3). Our experiments have provided more evidence that very small amounts of selenium can often replace Vit. E as an essential nutrient. We have also shown that mortality of mice infected with experimental viral hepatitis and receiving a casein diet supplying 15% of protein is similar to that of animals receiving a yeast diet of similar concentration and supplemented with alpha tocopherol. Although casein and yeast had a similar effect on mortality, casein was considerably more effective in stimulating growth than yeast supplemented with alpha tocopherol. This result confirms previous observations suggesting that the growth-promoting and anti-infective effects of protein are independent of one another(5).

Summary. A necrogenic Torula yeast diet increased the susceptibility of mice to experimental viral hepatitis. Supplementation of this diet with alpha tocopherol or selenium reduced the mortality of this infection. The susceptibility of mice fed a casein diet resembled that of animals receiving a yeast diet of similar protein content and supplemented with alpha tocopherol.

1. Himsworth, H. P., *The Liver and its Diseases,* 2nd Edit., Cambridge, Mass., 1950, p120.

2. DeWitt, W. B., Schwarz, K., *Experientia,* 1958, v14, 28.

3. Dam, H., Nielson, G. K., Prange, I., Søndergaard, E., *ibid.,* 1957, v13, 493.

4. Ruebner, B., Bramhall, J. L., *Arch. Path.,* 1960, v69, 190.

5. Ruebner, B., Miyai, K., *J. Lab. and Clin. Med.,* 1961, in press.

6. Meader, R. D., Williams, W., *Am. J. Anat.,* 1957, v100, 167.

7. Dick, G. W. A., Niven, J. S. F., Gledhill, A. W., *Brit. J. Exp. Path.,* 1956, v37, 90.

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Precursors of Etiocholanolone and Androsterone in Adrenal Carcinoma. (26650)

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Most patients with adrenal carcinoma excrete increased amounts of etiocholanolone (E) and variably increased amounts of androsterone (A) resulting in an E/A ratio well above the normal range(1,2). This has been interpreted to mean that some of the E originates from C_{21}-steroids whose metabolism leads to 17-ketosteroids (17-KS) of the 5β-series predominantly. Since the relative amounts of 5α- and 5β-17-KS may be related not only to the precursors but to the metabolic transformations of any particular precursor, tritium-labeled dehydroepiandrosterone (DHA) has been used to study the origin of a urinary A and E in patients with adrenal carcinoma. The secretion rate of DHA was also estimated in these patients.

Materials and methods. Five mg of DHA acetate were added to one millicurie of 7-tritium-DHA acetate (New England Nuclear Corp.), the material chromatogrammed in phenyl Cellosolve/heptane (I), eluted, and hydrolyzed with 0.067 N NaOH. It was then successively chromatogrammed in ligroin/propylene glycol (II), and isooctane/methanol:water, 10/8:2 (III). The DHA then had a specific activity (S.A.) of 51 $\times$ 10^6 cpm/mg.

The samples were counted in the Packard Tri-Carb liquid scintillation spectrometer with an efficiency of approximately 15%. Sufficient counts were accumulated to give a standard error of no more than 5%.

Urine was collected for 48 hours after intravenous injection of 2.1 $\times$ 10^6 cpm of DHA. The urines were treated as previously

TABLE I. Case Material.

Patient	Age	Sex	Diagnosis	Clinical syndrome	Site of metastases	Urinary excretion, mg/24 hr 17-KS	17-OHCS	THS
Wh	27	♀	Chorioca	Good health	—	9	6.2	.2
Cha	23	♀	"	"	—	11	5.5	.2
Sc	7	♂	Adrenal ca	Virilism	Lung	44	4.3	.2
Che	49	♀	"	Cushing's & virilism	Lung, liver	480	170	56
Ma	37	♀	"	"	Lung, abdomen	58	15	4.2
St	55	♀	"	"	Lung	46	14	3.5

described(3) and DHA separated from A and E by digitonin precipitation. The ketosteroids were then chromatogrammed in systems II and III, acetylated, rechromatogrammed in I, and measured by a micro-Zimmerman technic(4). The methods used in this laboratory for 17-KS, 17-hydroxycorticoids (17-OHCS), and tetrahydro-compound S (THS) have been listed(3).

The secretory rate of DHA was calculated by the formula:

$$\text{Secretory rate} = \frac{\text{cpm DHA injected}}{\text{S.A. of urinary DHA}}.$$

This is a maximal value since less than 100% of the isotope is excreted in 48 hours(5). Since the S.A. of A and E should equal the S.A. of DHA if they were completely derived from DHA, a decrease in S.A. could result only through the contribution of unlabelled precursor to urinary A and E. The percentage of A derived from DHA must then be $\frac{\text{S.A.}_{\text{A}}}{\text{S.A.}_{\text{DHA}}} \times 100$, and similarly for E. The errors in the methods are such that the S.A. of the urinary ketosteroids had a standard error of ± 8%.

Six patients were studied, 2 ovariectomized women and 4 patients with metastatic adrenal carcinoma. The 2 ovariectomized women had been treated for choriocarcinoma previously, but were in good health and apparently free of disease at the time of study. Pertinent features of these cases are presented in Table I.

Results. (Table II). In both normal subjects, S.A._{A} and S.A._{E} did not differ significantly from S.A._{DHA}. Thus the fraction of A and E derived from precursors other than DHA was negligible. Estimated secretion rates of DHA were 7 mg and 11 mg daily.

The patients with adrenal cancer excreted large amounts of E and A. In 3 of them, the E/A ratio was above normal (2.6, 4.1, 5.2). The S.A._{A} did not differ significantly from the S.A._{DHA} in 3 of the 4 patients. Only in the patient with the highest excretion of 17-KS (Che) could it be shown that as much as 19% of the androsterone was not derived from DHA. In contrast, in 3 of the 4 patients with adrenal cancer, the S.A._{E} was lower than S.A._{DHA} so that only 35%, 34%, and 76% of the E was derived from DHA.

The calculated daily secretion rates of DHA varied from 28 mg to 390 mg in the patients with carcinoma. There was a rough correspondence between these secretion rates and urinary excretion of DHA.

TABLE II. Steroid Excretion, Specific Activities and Derived Data.

Patient	Urinary excretion, mg/24 hr* A	E	DHA	E/A	Specific activity, cpm/μg A	E	DHA	DHA secretion rate, mg/24 hr	% derived from DHA A	E
Wh	1.7	2.3	.6	1.5	93	95	98	11	95	97
Cha	1.9	1.8	.9	1.0	161	139	150	7	107	93
Sc	11	14	18	1.3	35	34	38	28	92	90
Che	27	112	161	4.1	2.2	.92	2.7	39	81	34
Ma	4.2	22	21	5.2	20	9.1	26	41	77	35
St	3.5	9.1	15	2.6	34	25	33	32	103	76

* A = Androsterone. E = Etiocholanolone. DHA = Dehydroepiandrosterone.

Discussion. The method for measuring the extent to which a steroid metabolite is derived from a precursor was described by Vande Wiele and Lieberman(5) who showed that, in the normal adult, DHA is the precursor of the urinary A and E. The results in the 2 ovariectomized women are in accord with this. In 3 of the 4 cases of adrenal carcinoma, however, urinary E must have originated from both DHA and other precursors, these other precursors accounting for as much as ⅔ of the E in 2 of the patients. It is significant that the 3 patients with $S.A._{E} < S.A._{DHA}$ were those with excess corticoid production as shown by the excretion of 17-OHCS and THS.

The 17-KS produced by the metabolism of 17α-hydroxyprogesterone(6,7) and compound S(8) are predominantly of the 5β-series, and it has been suggested(2) that compound S may be an important precursor of urinary E in adrenal cancer. In a series of 10 patients with adrenal carcinoma, both THS and pregnanetriol were generally found in large amounts when the cancer was producing corticoids (Lipsett and Wilson, unpublished data). This implies that 17α-hydroxyprogesterone and compound S were not being utilized normally in biosynthesis of cortisol. Thus it seems reasonable to suppose that the large amounts of E in adrenal cancer are due not only to over-production of DHA but to the metabolism of 17α-hydroxyprogesterone and compound S as well, in many of the patients.

This viewpoint is supported by the study in Sc. This boy had no evidence of Cushing's syndrome and excretion of THS and 17-OHCS was normal. The E/A ratio was 1.3 although both steroids were excreted in large amounts. The S.A. of A and E did not differ significantly from $S.A._{DHA}$, and thus only small quantities of A and E could have been derived from steroids other than DHA.

With respect to A, the specific activity was significantly lower than that of D in 2 of the 4 cases. The unlabelled A may have arisen from 17α-hydroxyprogesterone and compound S as discussed, and a further contribution of androstenedione to urinary A and E seems possible(9). Degree of dilution of the labelled A was not of the same magnitude as that of E. This would not be expected in view of the above considerations.

The secretion rate of DHA in the 2 normal subjects and in 4 others(5) suggests that DHA is quantitatively, at least, an important secretory product of the adrenal cortex. The accuracy of the secretion rates in adrenal cancer may be less than in the normal, since it seems possible that equilibration of the injected isotope with the large pool may not be sufficiently rapid with respect to its excretion. If such an effect occurred, however, the true secretion rates would be even higher than those estimated.

Summary. The precursor of urinary androsterone and etiocholanolone was shown to be dehydroepiandrosterone in 2 ovariectomized women. In 3 women with Cushing's syndrome due to adrenal carcinoma, there were other major precursors of etiocholanolone and, to a lesser extent, of androsterone. It was suggested that these other precursors were 17α-hydroxyprogesterone and compound S. In one patient with adrenal cancer who excreted only 11-deoxy-17-ketosteroids, etiocholanolone was derived almost entirely from dehydroepiandrosterone. The secretion rate of DHA was 7 and 11 mg daily in 2 ovariectomized women. In 4 patients with adrenal carcinoma, it ranged from 28 mg to 390 mg daily.

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1. Gallagher, T. F., *Cancer Res.*, 1957, v17, 520.

2. Vande Wiele, R., Christy, N. P., Lieberman, S., Jailer, J. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 520.

3. Lipsett, M. B., Damast, B., *ibid.*, 1958, v99, 285.

4. Wilson, H., *Arch. Biochem. Biophys.*, 1954, v52, 217.

5. Vande Wiele, R., Lieberman, S., in *Biological Activities of Steroids in Relation to Cancer*, G. Pincus and E. P. Vollmer, Eds., 1960, Academic Press, N. Y. and London, p93.

6. Jailer, J. W., Gold J. J., Vande Wiele, R., Lieberman, S., *J. Clin. Invest.*, 1955, v34, 1639.

7. Axelrod, L. R., Goldzieher, J. W., *J. Clin. En-*

docrinol. Metab., 1960, v20, 238.
8. Birke, G., *Acta Endocrinol.*, 1954, v15, 17.
9. Hirschmann, H., De Courcy, C., Levy, R. P., Miller, K. L., *J. Biol. Chem.*, 1960, v235, P.C. 48.

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Effect of Certain Liquid Organopolysiloxanes on Cholesterol Atherosclerosis of the Rabbit.* (26651)

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It has been postulated that the stability of lipids in emulsion form depends on maintenance of a sufficient surface charge, so that deposition of lipid from plasma at certain sites on the linings of the blood vessels might well result from a lack of surface active agents in plasma(1). Fatty acids are anionic surface active agents and their surface activity is increased by presence of double bonds in the fatty acid radicle(2). Thus, the least saturated fatty acid is the most effective as a charged surface active agent. The pronounced lipemia and cholesterolemia producing effect of a surface active detergent like Triton WR-1339 has been thoroughly explored(3), whereas hydrophobic surface active agents like the silicone fluids have received little attention. Dimethylpolysiloxanes, like DC 200 and DC antifoam,† had only a suggestive effect on aortic atherosclerosis of rabbits(4), but a phenylmethylpolysiloxane‡ altered cholesterol deposition profoundly(5). The present study compares the effect of these 2 silicone fluids in a large number of animals.

Methods and results. In experiments lasting 2 months New Zealand albino rabbits in groups of 6 were fed a stock diet to which 2% cholesterol and .5,% 1%, 2% and 5% of the silicone fluids in weight of diet were added. Weekly serum samples were analyzed for cholesterol by the method of Pearson(6), but the results of the terminal samples only are presented since there were no specific trends in reaching the final value. Tissue cholesterol was determined by the method of Kingsley(7).

The results (Table I) show that the 2 silicone fluids used exert a different biological effect. Addition of phenylmethylpolysiloxane in all concentrations prevents development of severe hypercholesterolemia. The arbitrarily chosen lowest concentration of .5% has as much effect as the 10 times larger amount. The dimethylpolysiloxane did not prevent hypercholesterolemia, except in one experiment where a 1% concentration was used. Even in this instance the effect was less pronounced than that obtained with the other silicone fluid. Cholesterol content of the liver remains esentially unchanged if dimethylpolysiloxane is added to the stock diet containing 2% cholesterol, but increases markedly if the phenylmethylpolysiloxane fluid is fed at the 2% and 5% level. The opposite changes occur in the aorta where the dimethylpolysiloxane produces an increased cholesterol content, whereas addition of phenylmethylpolysiloxane in higher concentrations does not change the cholesterol content of the aorta. The histological lesion of the aorta in a 2% feeding experiment is shown in representative photomicrographs (Fig. 1). Addition of 2% silicone fluids to the stock diet without cholesterol does not alter cholesterol content of the blood serum or of the tissues examined and does not produce histological changes.

Discussion. The cause of the described biological effects of silicone fluids is unknown, therefore the discussion is specula-

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