TABLE II. Effect of DMAE on Renal Hemodynamics.

Dog No.	Urine flow, ml/min. C	I	R	G. F. R., ml/min. C	I	R	R. P. F., ml/min. C	I	R	Dose, mg/kg
1	2.41	2.88	3.08	58.5	62.2	56.0	195	209	185	5
2	4.00	3.47	2.72	71.0	59.0	67.1	238	198	190	5
3	2.71	2.76	3.04	48.8	48.3	47.2	138	121	113	5
3	3.64	2.33	2.45	58.6	44.3	49.5	125	115	134	5
2	3.03	3.15	2.95	68.0	66.2	69.1	215	225	214	7.5
2	4.31	3.95	4.12	64.0	67.3	65.2	231	240	229	"
3	2.95	3.08	3.30	54.2	53.4	58.6	140	131	135	"
4	4.00	3.90	3.98	59.0	54.0	61.2	195	208	212	"
5	3.60	3.80	3.51	53.0	56.0	54.2	185	190	178	"

at this concentration. On the basis of these results in dogs it would appear that DMAE in doses that are well tolerated does not have any specific effect on the kidney in acute experiments. No changes in sodium, potassium or chloride excretion were noted in these experiments.

Summary. Renal effects of DMAE were determined in rats and dogs. In high doses an antidiuretic effect was noted in rats. Dogs that had been administered DMAE in maximal tolerated doses exhibited no changes in urine flow nor renal clearances. There were no indications that this compound had any toxic effect on the kidney.

Statistical analysis kindly performed by E. H. Jenny, Emory University.

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Metabolism of Cortisol by Loose Connective Tissue *in vitro.* (23921)

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Extensive transformation of progesterone (1) and cortisol(2) (4-pregnene-11-beta, 17-alpha, 21-triol, 3, 20-dione, or hydrocortisone) to a series of metabolites by eviscerated rats has been demonstrated. These metabolites represent a pool of steroids which have been transformed by a variety of tissues. The extent to which each tissue adds to the pool of steroid products is being investigated. Connective tissue obtained from mice was incubated *in vitro* in the presence of cortisol-4-C^{14}. Histological examination of this tissue has shown that 90% of the cells are fibroblasts(3).

Various products were isolated from these incubations and the following 5 compounds were identified: 4-pregnene-11-beta, 17-alpha, 20-alpha, 21-tetrol, 3-one (20-epi substance "E" of Reichstein); 4-pregnene 17-alpha, 21-diol, 3, 11, 20-trione (cortisone); pregnane 11-beta, 17-alpha, 21-triol, 3, 20-dione (dihydrocortisol); 4-pregnene 11-beta, 21-diol, 3, 20-dione (corticosterone) and 4-androstene 11-beta-ol, 3, 17-dione.

Materials and methods. Fourteen mice (AK) were killed by decapitation and the connective tissue collected as described previously(4). The areolar tissue was placed in 8 incubation flasks immediately after collection. Each flask contained 0.3-0.6 g of tissue in 10 ml of 0.1 M phosphate buffer, pH 7.4 and cortisol 4-C^{14} (25,000 cpm; Specific Activity 1.467 mc/mole). Incubation was carried out in shaking water bath at 37°C for a total of

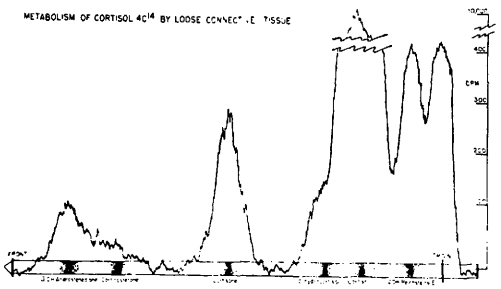

FIG. 1. Radiochromatographic tracing, chloroform/formamide system.

4 hours. Two control flasks were incubated without tissue and 2 other zero hrs. controls with tissue were not incubated. The contents of each flask were extracted 3 times with 60 ml of hot acetone. The acetone was subsequently filtered and evaporated under a stream of nitrogen. The aqueous phase remaining after evaporation of acetone, was then extracted 4 times with 30 ml portions of chloroform. The chloroform fraction was evaporated and directly chromatographed in the chloroform/formamide system of Zaffaroni(5). Radioactive analysis of the chromatograms, identification and determination of specific activities of the different metabolites was carried out as described in detail previously(2,6,7).

Results. Seven zones of radioactivity were detected from the original chromatograms obtained from chloroform/formamide system in each of the incubated connective tissue experiments. The Rfs of the radioactive peaks and shoulders were: .00, .08, .19, .27, .51, .76 and .88 (Fig. 1). Rechromatography of the origin and high polar region in chloroform formamide for 12 hrs. was composed of 2 peaks. After acetylation(6) and rechromatography in the benzene/formamide system for 3 hr the zones of Rf's .19 and .29 were resolved into 3 radioactive compounds. Two radioactive peaks could be separated from the low polar region (Rf's .76 and .88) after rechromatography in the benzene/formamide system for 5 hr. From incubated and non-incubated control flasks only cortisol was isolated. Identification of the isolated steroids was based on the following data:

Cortisol. To an aliquot obtained from the peak of Rf .19 of the original chromatogram, 100 μg of non-radioactive carrier cortisol were added and the mixture acetylated and chromatographed in benzene/formamide for 6 hrs. The carrier and radioactive peak migrated at the same rate. At this time the specific activity was determined (32.1 cpm/μg). The mixture was oxidized(6) and rechromatographed in benzene/formamide for 4 hr. The oxidized mixture (cortisone acetate) had a specific activity of 32.3 cpm/μg.

4-pregnene 11-beta, 17-alpha, 20-alpha, 21-tetrol, 3-one (20-epi Substance "E" of Reichstein). Standard 20-epi substance "E" and the radioactive peak (Rf 0.08, Fig. 1), occurred at the same rate in chloroform/formamide for 8 hr (S.A. 12.8 cpm/μg). Acetylation of the mixture and chromatography in the benzene/formamide system for 9 hr demonstrated that both acetylated compounds migrated at the same rate (S.A. 12.7 cpm/μg). Oxidation of the acetylated derivatives gave as final product 4-pregnene 17-alpha, 20-alpha, 21-triol, 3, 11-dione. 20, 21-diacetate (S.A. 12.9 cpm/μg). When carrier Substance "E" of Reichstein (20-beta-ol form), was chromatographed with an aliquot obtained from the original high polar region, the specific activity of the mixture dropped from 14.1 cpm/μg to 0.1 cpm/μg after acetylation and no radioactivity could be detected in this carrier after oxidation.

Pregnane 11-beta, 17-alpha, 21-triol, 3, 20-dione (Dihydro-cortisol). The radioactive zone obtained from the region of Rf 0.27 (Fig. 1), was mixed with non-radioactive carrier dihydro-cortisol, the mixture was rechromatographed in the "free," acetylated and oxidized states. In every case carrier and radioactivity migrated at the same rate. Specific activities were not determined on this compound.

4-pregnene 17-alpha, 21-diol, 3, 11, 20-trione (Cortisone). The radioactivity in the cortisone region (Rf 0.51) was eluted and divided into 2 equal portions. To each portion was added 100 μg of nonradioactive cortisone (S.A. 36.6 cpm/μg). One portion was oxidized and then chromatographed in benzene/formamide. The Rf (0.5) and the specific activity (36.3 cpm/μg) indicated that the mixture was oxidized to 4-androstene 3, 11,

17-trione (Adrenosterone). The other portion was acetylated and chromatographed in benzene/formamide for 8 hr, the specific activity of the derivative (cortisone acetate) was 36.6 cpm/μg.

4-pregnene 11-beta, 21-diol, 3, 20-dione (Corticosterone). The radioactive unknown (Rf 0.76, Fig. 1) was combined with 300 μg of non-radioactive corticosterone and chromatographed in the chloroform formamide system for 3 hr. The specific activity of the mixture was 6.3 cpm/μg. The eluted mixture was divided into 3 aliquots. The first aliquot was acetylated and chromatographed in benzene/formamide for 3 hours: the derivative corticosterone acetate had a specific activity of 6.6 cpm/μg. This same aliquot was subsequently oxidized and chromatographed in the hexane-benzene/formamide system for 4 hr. The derivative 11-dehydro-corticosterone acetate had a specific activity of 6.5 cpm/μg. The second aliquot was mildly oxidized with 1 mg of CrO_3 in 15 drops of glacial acetic acid for 3 minutes at 80°C and chromatographed in benzene/formamide for 10 hr. This type of oxidation will give a 45% yield of 11-dehydro-corticosterone. The specific activity of this derivative was 6.6 cpm/μg. The third aliquot was strongly oxidized with 4 mg of CrO_3 in 15 drops of glacial acetic acid for 3 hr at 80°C and chromatographed in chloroform/formamide for 35 hr. This type of oxidation will give a 30-40% yield of the etioacid of 11-dehydro-corticosterone. The specific activity of this derivative was 6.8 cpm/μg.

4-androstene 11-beta-ol, 3, 17-dione. To the eluate obtained from the zone of lower polarity (Rf 0.88), 150 μg of the carrier non-radioactive compound was added and rechromatographed in benzene/formamide for 3 hr. The specific activity of the mixture was 32.3 cpm/μg. Oxidation of the eluted mixture and rechromatography in the same system gave as final product adrenosterone, with a specific activity of 32.6 cpm/μg.

Unknown compounds. At the present time a highly polar compound isolated from the original chromatogram is under study. One of the compounds obtained after acetylation of the region with Rf's 0.19 and 0.27, behaved chromatographically as a diacetate, which resembled 4-pregnene 17-alpha, 20-alpha, 21-triol, 3, 11-dione; however, the carrier was not available at this time in our laboratory for the actual identification of this steroid.

Discussion. Numerous studies have been reported concerning metabolism of cortisol and other steroids by the liver(8). The major metabolic changes of cortisol appear to be loss of the side chain, oxidation reduction at 3, 4, 11 and 20 positions and formation of water soluble conjugates(8,9). Kidney homogenates(10) have also been reported to metabolize cortisol in a similar fashion. Leukemic cells(11) have the capacity to alter the chemical structure of this hormone in a cyclic manner. It has been reported that osteosarcomas can remove the 17-alpha hydroxyl group of cortisol to form corticosterone(12). That the liver is not the main or only organ capable of transforming cortisol and other steroid hormones to various metabolites has been further demonstrated by the experimental use of eviscerated rats(1,2).

In our experiments it is demonstrated that cortisol can be changed chemically by connective tissue. The molecular changes include oxidation of the hydroxyl group in position C-11, reduction of the 4-5 double bond, reduction of the C-20 ketone (C-20-alpha-ol form) and oxidative cleavage of the side chain. A particularly interesting reaction is the removal of the 17-alpha hydroxyl group to form corticosterone. It has been proven in our laboratory that human tissue culture fibroblasts metabolize cortisol in a similar fashion except that the isomerism of reducing the C-20 ketone by these cells is in the 20-beta form(13). Formation of corticosterone has also been confirmed in these experiments(13). The possibility that removal of the C-17 hydroxyl group could be an artefact(14) was eliminated by showing that the extracts of control flasks contained only cortisol.

The predominant cell found in connective tissue is the fibroblast(3). Histological, histochemical and radioautographic studies(4, 15) have shown that fibroblasts treated with cortisol round up and in this state resist destruction in areas of inflammation and it has been demonstrated that radioactive cortisol

tends to localize mainly either at the surface of fibroblasts or within them. It has been suggested that this hormone-induced change in fibroblasts enhances their resistance to cellular damage and thus interrupts a chain reaction of cellular destruction which potentiates inflammation(16,17). The relation of this increased resistance and the ability of this cell to transform the chemical structure of cortisol may be related to its mechanism of anti-inflammatory action. This is further suggested by the fact that none of the conversion products resulting from fibroblastic action on cortisol have anti-inflammatory activity(4). It therefore appears that it is cortisol itself which possesses anti-inflammatory activity. Since cortisol is at least 7 or 8 times more potent in its antiphlogistic effect than cortisone(3) and it has been demonstrated that cortisone and cortisol are inter-convertible(8), it is very possible that cortisol only is anti-inflammatory and that cortisone must be converted to cortisol for this hormone to be antiphlogistic. This possibility explains the difference in potency of these two compounds.

Summary. Action of the loose connective tissue (90% of cells are fibroblasts) on cortisol was investigated. Data are presented on the isolation and identification of 5 of the 7 metabolites observed when cortisol-4-C^{14} was incubated with mouse (AK) loose connective tissue. The identified metabolites are: 20-epi substance E of Reichstein; cortisone; dihydro-cortisol; corticosterone; and 4-androstene, 11-beta-ol, 3, 17-dione. The relationship of this metabolic picture to the fibroblastic role on the anti-inflammatory action of cortisol is discussed.

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