tween adrenal tumors and adrenal hyperplasia seems to be possible.

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Progesterone a Precursor of Testicular Androgens in Sheep.* (23200)

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Recent studies have provided evidence for a possible relation between fecal androgen and progesterone metabolism in the bovine (1). Furthermore, several androgenic compounds have been identified in the feces of a pregnant cow following the administration of progesterone(2). While feces from normal bulls and steers are inactive(3,4) it is interesting to note that progesterone administration to a bull produced statistically significant increases in the androgen content of the feces (1). *In vitro* formation of testosterone and Δ^4-androstene-3, 17-dione from progesterone has been demonstrated for rat testis(5) and human testicular tissue(6).

In view of this finding, the results of an experiment in which progesterone was given to a ram and a castrate male sheep are presented as indicating possible conversion of progesterone to androgens by the ovine testis.

Materials and methods. Collections of feces were made daily from 2 male sheep, one of which had been castrated as a lamb. The ram was a 2-year-old New Zealand Romney x Lincoln and weighed approximately 140 lb during the period of experimentation. The second animal was a New Zealand Romney wether, 14 months of age, weighing 80 lb. After preliminary collection period of one week each animal received daily during the next week a subcutaneous injection of 0.5 g and 0.25 g respectively, of progesterone in arachis oil. Total feces were dried daily for 48 hours in an oven at 45°C and ground to a fine powder in a Wiley mill. White Leghorn cockerels from the College Farm were fed the dried feces as 10% of the control ration for 21 days, starting when chicks were 4 days old. The increase in comb weight was used to assess androgen content of the fecal samples when compared with the comb response to methyl-testosterone incorporated in the feed. In a preliminary trial a sample of dried feces from a ewe in the last month of pregnancy was tested against 3 levels of methyltestosterone and one level of Δ^4-androstene-3,17-dione in the chick feed. The latter fecal sample had been stored in a dry condition for 2 years.

Results. The chick comb response revealed some androgenic activity in the feces of a pregnant ewe (Table I), whereas the response to the feeding of feces from both the untreated ram and wether was slight (Table II). When progesterone was administered, androgen excretion in the feces of the ram was very markedly increased. This finding was in con-

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TABLE I. Effect of Oral Administration of Androgens to 6 Groups of Male Chicks.

No. of chicks	Treatment	Body wt (g)	Comb wt (mg)	Comb ratio ± S.E.	Testes wt ± S.E. (mg)
15	Control	110.9	52.5	47.3 ± 5.0	21.4 ± 1.8
20	Dried feces* (pregnant ewe)	124.5	202.7	159.2 ± 17.2	27.9 ± 2.3
20	Methyltestosterone, 10 mg†	119.5	391.3	320.8 ± 28.4	24.4 ± 1.6
20	" ,20 " †	125.5	546.4	418.8 ± 32.7	19.5 ± 2.3
20	" ,40 " †	117.9	792.6	661.5 ± 38.1	20.9 ± 1.5
11	Δ^4-Androstenedione, 10 mg†	115.2	892.3	788.1 ± 71.4	14.9 ± 1.5

* Incorporated as 10% of control ration. † Wt of compound/kg feed.

TABLE II. Effect of Progesterone on Excretion of Fecal Androgens in 6 Groups of Male Sheep.

No. of chicks	Treatment	Body wt (g)	Comb wt (mg)	Comb ratio ± S.E.	Testes wt ± S.E. (mg)
20	Control	124.2	122.6	92.2 ± 12.1	27.3 ± 1.8
20	Ram dried feces*[1]	146.0	226.9	149.6 ± 15.7	36.2 ± 2.6
22	*Idem* *[2]	135.9	959.0	695.7 ± 50.5	19.8 ± .9
20	Methyltestosterone, 10 mg†	141.1	496.9	345.6 ± 21.7	31.8 ± 2.4
20	Castrate male dried feces*[1]	154.6	207.3	127.9 ± 15.6	32.2 ± 2.1
13	*Idem* *[2]	163.2	204.4	113.8 ± 15.9	31.4 ± 3.4

* Incorporated as 10% of control ration. † Wt of compound/kg feed.
[1] Collected before progesterone injections. [2] Collected during progesterone injections.

trast to the results from injecting progesterone in the castrate male sheep (Table II).

When comb growth was greatest there was a depression of testes weight(4), but hypertrophy of the combs was not always associated with a decrease in testes weight. The activity of Δ^4-androstene-3,17-dione was much greater than methyltestosterone in causing an increase in comb weight.

Discussion. A difference in the ability to transform progesterone to fecal androgen seems apparent in the present data from an experiment with an entire and castrate male sheep. The amount of progesterone administered daily to the ram was twice that given to the castrate animal but so, also, was the approximate body weight. The data show a greater power of conversion of progesterone to androgen in the male with testes.

Since oral administration of progesterone to chicks does not stimulate comb growth (1,4), no significant activity would result from excretion of progesterone as such by the sheep. Conversion of progesterone to androgens by microorganisms of the gastrointestinal tract(1,2) might explain the slight androgenic activity in the feces of castrate sheep, but would hardly account for the great increase in fecal androgen of the ram. It would appear that the tissues of the ram played a major part in transformation of progesterone to androgenic compounds in the feces of this animal. While adrenal tissue may be implicated(7), it seems likely that activity of the testes was of more significance. Further support is given to this conclusion by the recent demonstration of conversion of progesterone to androgenic compounds *in vitro* by rat and human testis(5,6). The present data therefore provide evidence that progesterone may be a precursor of testicular androgens *in vivo*.

Summary. Daily administration of progesterone by subcutaneous injection to a ram and a castrate male sheep for one week resulted in a great increase in the fecal androgenic activity of the ram. This observation suggests that progesterone may serve as a precursor of testicular androgens in the sheep. Some androgen excretion in the feces of a pregnant female sheep was noted but little fecal androgen from untreated males.

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Intracellular Distribution of Proteolytic Enzymes in Rat Liver Tissue.* (23201)

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The presence of intracellular proteolytic activity in mitochondria isolated from rat liver homogenates has been demonstrated(1, 2). deDuve *et al.*(2) utilized a modified hemoglobin substrate that may have been attacked by many enzymes. Maver and Greco (1) used benzoyl-L-argininamide, but their mitochondrial preparations were contaminated with large cytoplasmic granules, probably nuclear material.

Two intracellular proteinases have been extensively purified and studied(3,4,5). These enzymes have been designated cathepsins B and C. Previous work has shown that a synthetic substrate suitable for the assay of cathepsin B is benzoyl-L-argininamide, while one for cathepsin C is glycyl-L-argininamide, while one for cathepsin C is glycyl-L-tyrosinamide. This paper offers additional information about localization of the intracellular proteolytic enzymes of rat liver obtained by using synthetic substrates and cellular fractionation procedures.

Materials and methods. Liver tissue from 170 g Wistar strain rats was used in all experiments. Four grams of liver were homogenized in 36 ml of sucrose solutions by the method of Potter and Elvehjem(6). The 0.25 M sucrose plus 7.5% polyvinylpyrrolidone (PVP) solution of Novikoff† and 0.88 M sucrose were used as fractionation media. Fractionation of the homogenates was obtained by differential centrifugation following the schemes presented by Schneider and Hogeboom(7) with minor modifications. Following fractionation, 5 components were available for testing: 1) homogenate, 2) nuclei and debris, 3) mitochondria, 4) microsomes, and 5) supernatant fluid. 0.88 M sucrose was added to bring the final volume of each fraction to 40 ml. Immediately thereafter, the enzyme activity was determined at 37°C in a reaction mixture with the following composition: 1) 0.4 ml of citrate buffer 0.04 M, 2) 0.1 ml of cysteine hydrochloride 0.04 M, 3) 0.2 ml of the above components. 4) 0.2 ml of substrate 0.05 M, and 5) sucrose to a final volume of 1 ml and a pH of 5. Samples were removed from the incubation mixtures at one and 2 hours for analysis in duplicate Conway vessels. The analysis was performed with the use of a glass electrode system and the addition of alkali by a microcapillary burette calibrated in lambdas(8). The substrates utilized were benzoyl-L-argininamide hydrochloride monohydrate (BAA), and glycyl-L-tyrosinamide acetate (GTAA). The rates of hydrolysis were linear in all instances. Results are listed as cathepsin units (C.U.). A cathepsin unit is defined as that amount of enzyme or enzyme-containing material that provides 1% hydrolysis per minute. Analysis for protein nitrogen was done by the micro Kjeldahl technic(8) or the biuret method(9).

Results. The data given in Table I indicate that the enzymes which hydrolyse BAA

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