tain satisfactory differentiation between normal liver and tumor with this method. This is evident from the ratios of tumor tissue/normal liver tissue.

The administration of Lugol's solution to some of the animals did not change the concentration of the iodinated albumin or the ratios between tumor, "invasive" zone and normal liver tissue (Table I).

Comment. Duran-Reynals(1) showed that heterologous proteins and dyes injected intravenously localize selectively in transplantable and spontaneous tumors of mice. He interpreted his findings as, "indicating that newly formed capillaries of tumors are, in general, more permeable than the capillaries of any normal tissue." In our animals, increased concentrations of homologous albumin were demonstrated in tumor tissue as compared to the surrounding normal liver. The ratio of specific activity of tumor/normal liver was even greater after the animals had been perfused. The reason for this may, indeed, be increased permeability of newly formed capillaries with escape of homologous albumin into tumor tissue. Once the albumin has escaped into tumor tissue, it cannot be removed by perfusion. Another possibility is actual metabolic utilization of homologous albumin by the more rapidly growing tumor tissue.

The labelled protein used in our study was homologous and presumably not denatured. It is assumed that it is handled by the rabbit like native albumin. Whether the intact protein molecule or a metabolite of this protein is deposited in tumor tissue cannot be stated at this time.

Conclusion. The data presented indicate that experimental tumors of the liver (Brown-Pearce) in rabbits accumulate more radioiodinated rabbit albumin than normal liver tissue of the same animal. This increased concentration appears to be unrelated to vascularity since it was demonstrated in perfused animals.

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Fecal Androgen, A Progesterone Metabolite in the Bovine.* (21964)

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Early investigations clearly indicated that pregnanediol is a product of metabolism of progesterone in man(1-4). Further *in vivo* studies have indicated that progesterone may be reduced to, and excreted as, allopregnanediol-3(α), 20(α)(5) and pregnanol-3(α)-one-20(6). However these metabolites in the urine account for only 5-20% of administered progesterone(7). Recent studies indicate that pregnanediol is not present in urine of pregnant goats(8) and cattle(9). The importance of a biliary pathway for elimination of progesterone metabolites has been indicated: (a), recovery of pregnanediol from bile of humans after progesterone was given parenterally (10); (b), identification of allopregnanediol-3(β), 20(β) in cattle bile(11); and (c), isolation of pregnanol-3(α)one-20 and pregnanediol-3(α), 20 (β) from bile of pregnant cows (12). It has been shown in studies of proges-

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terone-21-C^{14} metabolism in rats that most of the injected progesterone or its metabolites are carried to the intestine by bile and eliminated in the feces(13). In a study of steroid metabolism in the bovine, we previously determined the neutral ketonic non-alcoholic character of androgens in cow feces(14). Biliary androgenic activity has also been detected in this species(15). However, feces from intact or spayed heifer, following treatment with testosterone, contained comb-growth depressing substances(16). Studies in pregnant cattle have demonstrated a preparturient increase in fecal excretion of androgens(17).

These reports led us to suspect that fecal androgen might be related to progesterone metabolism. This paper describes experiments which indicate that fecal androgen in the bovine is dependent on exogenous and/or endogenous progesterone metabolism.

Methods. Progesterone was administered to a 6-months-old Holstein calf, a yearling Jersey heifer, a Jersey cow in 7th month of pregnancy, 3 non-pregnant Jersey cows, and an 18-months-old Holstein bull. Feces were collected before, during, and after progesterone administration, dried in oven at 45°C, and pulverized in a ball-mill. The dried feces from each collection period was then well mixed and 1250 g portions taken for assay. Total weight of feces excreted from day to day did not vary appreciably in a given animal. The androgen content of each fecal sample was determined by measuring comb response of White Plymouth Rock cockerels to the fecal sample incorporated as 10% of control ration. Fecal samples were substituted for an equivalent amount of alfalfa meal present in the control ration(17). Comb response was expressed as 100 times the ratio of comb weight in mg to body weight in g. For comparison, response of chick comb to androstane-3, 17-dione and to Δ4-androstene-3,17-dione at the level of 10 mg/kg of feed is presented. Recently, androgens present in bovine feces have been identified as androstane-3, 17-dione, Δ 4-androstene-3, 17-dione, and Δ 1, 4-androstadiene-3, 17-dione(18). One g of progesterone in olive oil was given subcutaneously, daily, for 3 successive days to each animal with exception of the pregnant cow which received one g progesterone daily for 5 successive days. Control samples of feces were collected for a 2-day period preceding injections. Fecal samples were then collected during and after progesterone administration on the following days:

	Days
Holstein calf	0–1, 2–4, 5–7, 8–10
Yearling heifer	0–2, 3–5, 6–8
Jersey cow # 544	1, 2, 3, 4, 5, 6
" " # 310	1, 2, 4, 5, 6, 7, 8
" " #10160	1, 2, 3, 4, 5, 6
Holstein bull	1, 2, 3, 4, 5, 6, 7
Pregnant cow	1, 5

Results. Feces of untreated 6-months-old calf exhibited a slight but insignificant androgenic response when fed to baby chicks; whereas marked, significant androgenic responses were obtained from feces of the yearling heifer, 2 of the non-pregnant Jerseys and the pregnant Jersey cow. These data indicate that androgen elaboration in the cow is dependent upon sexual maturation or pregnancy. Feces from the untreated pregnant cow contained the greatest amount of androgenic activity. The peak of fecal androgen excretion thus synchronizes with the period of maximum progesterone production by the animal. It is interesting to note that an insignificant amount of androgen is excreted in feces of the untreated bull as previously reported(19).

Administration of progesterone to the 6-months-old calf, the yearling heifer, the 3 non-pregnant Jersey cows, and the Holstein bull produced statistically significant increases in androgenic content of feces. Upon progesterone withdrawal, the androgen content of feces gradually diminished to or below the pre-treatment levels.

Progesterone administration to the pregnant cow produced a slight but not statistically significant augmentation of androgenic content of feces. A diminution in fecal androgenic activity on prolonged administration of progesterone was indicated in the fecal sample collected after 5 daily doses of progesterone. This slight initial increase as well as the ensuing decrease in androgenic activity on continued progesterone injection might be due to a depression of endogenous progesterone

TABLE I. Excretion of Fecal Androgen in Normal and Progesterone-Treated Bovine.

Animal	Bovine feces: Progesterone treatment	Collections (days)	Assay chicks: No. of cockerels	Avg body wt (g)	Avg comb wt (mg)	Comb ratio ± S.E.	% diff. after inj.
Holstein calf (6 mo old)	Before inj.	2	17	271.2	200.8	74.1 ± 9.7	—
	After " *	0– 1	19	313.4	327.7	102.8 ± 10.1	+ 39
		2– 4	18	299.4	306.9	99.6 ± 12.0	+ 35
		5– 7	15	292.9	223.0	75.2 ± 7.8	+ 1
		8–10	14	284.6	156.4	54.6 ± 4.7	– 26
	Control chicks		17	279.6	158.0	55.9 ± 6.7	
Jersey heifer (yearling)	Before inj.	2	20	311.9	355.5	114.0 ± 8.1	—
	After " *	0– 2	20	328.2	539.8	162.8 ± 11.2	+ 43
		3– 5	20	308.0	444.2	144.2 ± 11.0	+ 27
		6– 8	18	324.4	384.8	120.0 ± 12.7	+ 5
Jersey cow (pregnant)	Before "	2	15	268.9	401.6	150.6 ± 17.9	—
	After " †	0– 1	16	240.3	413.0	168.2 ± 15.8	+ 12
		4– 5	18	263.7	284.2	107.9 ± 9.9	– 28
	Control chicks		14	283.9	104.9	36.6 ± 3.5	
	Androgen-fed chicks‡		19	257.7	169.7	65.5 ± 5.6	
Jersey cow #310	Before inj.	2	21	276.1	166.6	59 ± 6.3	—
	After " *	1	24	284.5	130.6	46.3 ± 3.4	– 22
		2	22	290.6	160.4	55.4 ± 4.0	– 7
		4	24	296.6	264.5	89.3 ± 8.4	+ 51
		5	24	284.0	158.9	55.4 ± 4.6	– 7
		6	24	309.1	153.7	50 ± 5.4	– 15
		7	25	308.6	141.7	46 ± 3.1	– 22
		8	22	295.8	130.6	44 ± 3.6	– 25
	Control chicks		23	298.7	158.2	52 ± 4.4	
	Androgen-fed chicks§		23	293.7	442.8	150.8 ± 12.2	
Holstein bull	Before inj.	2	22	327.0	200.9	61.8 ± 5.1	—
	After " *	1	21	320.9	182.7	58 ± 5.6	– 6
		2	22	337.0	246.9	74 ± 5.6	+ 20
		3	23	358.0	271	75.6 ± 6.0	+ 23
		4	22	349.1	534.4	154 ± 11.4	+149
		5	21	324.2	267.9	82.6 ± 9.9	+ 34
		6	24	353.7	199.8	56.6 ± 4.2	– 8
		7	25	334.2	155.8	47 ± 4.1	– 24
	Control chicks		20	308.6	162.3	52.8 ± 5.2	
	Androgen-fed chicks§		23	345.6	524.4	149.1 ± 13.2	
Jersey cow #10160	Before inj.	2	25	248.7	192.0	76.6 ± 5.4	—
	After " *	1	19	267.6	235.5	87 ± 5.0	+ 13
		2	24	261.8	189.2	71.9 ± 4.4	– 6.5
		3	24	256.4	173.5	67.0 ± 3.8	– 13
		4	23	274.5	292.4	100 ± 10.1	+ 30
		5	24	269.9	297.9	109 ± 7.9	+ 42
		6	23	279.4	309.8	110 ± 8.5	+ 43
	Control chicks		24	276.2	116.1	41.3 ± 2.0	
	Androgen-fed chicks§		23	271	403	143 ± 14.5	
Jersey cow #544	Before inj.	2	21	256.5	250	96.9 ± 5.9	—
	After " *	1	25	264.5	205	75.8 ± 6.7	– 22
		2	21	289.5	254.4	88.9 ± 7.0	– 8
		3	21	269.5	288.4	106.4 ± 7.9	+ 9
		4	22	263.0	280	105.5 ± 8.4	+ 9
		5	22	278	358	127.1 ± 10.8	+ 31
		6	20	273.5	318.8	112.0 ± 13.4	+ 15
	Control chicks		24	252	148.1	58.4 ± 4.7	
	Androgen-fed chicks§		21	274	410	151.3 ± 16.9	

* 1 g progesterone admin. daily for 3 successive days. † 1 g progesterone admin. daily for 5 successive days. ‡ Chicks fed androstane-3,17-dione, 10 mg/kg feed. § Chicks fed Δ^4-androstene-3,17-dione, 10 mg/kg feed. ‖ Third day fecal sample not collected.

TABLE II. Effect of Oral Administration of Progesterone on Male Chick's Comb.

Conc. progesterone in feed (mg/kg)	No. of cockerels	Avg body wt (g)	Avg comb wt (mg)	Comb ratio ± S.E.
0	23	298.7	158.2	52 ±4.4
80	22	312.3	140.6	44.8±4.9
160	25	306.8	114.1	37 ±2.4

secretion which would normally be at a maximum in late pregnancy (Table I).

Discussion. These data demonstrate a statistically significant increase in excretion of fecal androgen in the male and non-pregnant female bovine following injection of progesterone. It is suggested that the androgen found in feces of sexually active bovine female is a metabolite of progesterone and is significantly increased upon injection of exogenous progesterone.

Since androgen is bioassayed by feeding feces to chicks, it has been suggested that progesterone might be excreted as such in the bovine and then converted to an androgen in the chicks' gut, liver or other organ. However, oral administration of progesterone to chicks at a level of 40 mg/kg of feed did not stimulate comb growth(16). This report was verified by the authors at progesterone levels of 80 and 120 mg/kg of feed (Table II).

In general, two possible modes of biogenetic conversion of progesterone to androgens in the bovine may be outlined: (1) Conversion of progesterone by microorganisms of the bovine gastrointestinal tract(2), the 17-hydroxylation of progesterone (adrenal gland?) followed by oxidative degradation of the C_{17} side chain. Further research is now underway to elucidate these or other possibilities.

Summary. Sexually mature and pregnant cows excrete in their feces appreciable amounts of androgen, whereas males and immature females do not. To determine whether progesterone might be the precursor of the androgen, 7 male and female bovines were given subcutaneously 1 g progesterone daily for 3 or more days. Following these injections a statistically significant increase in fecal androgen was observed in 6 animals while a non-significant increase was observed in the pregnant cow. These data are believed to indicate that at least a part of the fecal androgen normally found in the sexually active bovine may be derived from progesterone.

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