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Radioactive Distribution of Tritium Labeled Acetophenone Derivative of 16*a*, 17*a*-Dihydroxyprogesterone. (30043)

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A new class of progesterone derivatives has been reported by Fried and his associates(1). One compound of this class, the acetophenone derivative of 16*a*, 17*a*-dihydroxyprogesterone (D),* was shown to be biologically active in the Clauberg assay. This derivative is twice as potent as progesterone parenterally and twice as active as norethisterone orally (2). D maintains pregnancy in ovariectomized rats(3) and does not virilize the internal or external sex structure of the newborn rat(4). The labeling of this steroid with tritium at the 6,7 positions enabled us to investigate the distribution of D in the Sprague-Dawley rat after intravenous, intramuscular, and oral administration.

Materials and methods. Tritiated D was prepared by reduction of $\Delta^{4,6}$-3-keto-16,17-dihydroxyacetophenonide with tritium and 5% palladium-charcoal mixture in toluene under mild conditions. The resultant steroid had a specific activity of approximately 1.5 millicuries per mg.

A 20% ethanol solution containing .25 mg of D was injected intravenously into one female rat. Another female rat was given 25 mg of D in a mixture of 60% corn oil—40% benzyl benzoate orally. Each animal received a total of 1.3×10^7 dpm. Twenty-four hours after administration of the compound, the animals were killed. Organs, blood, feces, and urine were analyzed for total radioactivity.

In a second study D was administered intramuscularly. Five 250 g female rats were used. Each animal received 1 mg of D dissolved in 70% corn oil—30% benzyl benzoate. The amount of radioactivity given to each animal was 6.7×10^8 dpm. Blood samples were taken from 2 of the animals at various time intervals up to 24 hours, at which time one of the animals was killed. The 4 remaining animals were killed at weekly intervals. During this period all urine and feces were collected daily. Blood samples were taken from 2 of the animals once a day for one week. The same animal was never bled on 2 successive days. After the first week, blood was sampled from the remaining animals twice weekly. At time of sacrifice, each animal was dissected and various tissues were frozen for subsequent determination of radioactivity.

The method used to determine radioactivity consisted of a modification of the closed oxygen flask combustion technic developed by Kelly *et al*(5). Approximately 50 mg portions of wet tissue were wrapped in Schoniger sample holder papers and enclosed in platinum sample holders. These holders were imbedded in Corning 6710 ground glass joints

* Droxone® Squibb Dihydroxyprogesterone Acetophenide.

of 24/40 standard taper. Samples of whole blood or urine up to 0.5 ml volumes were dried directly on Schoniger papers. Intestinal contents and feces were dried in a hot air oven and ground with a mortar and pestle. Quantities up to 20 mg of the dry material were used in the combustions. The combustion flasks were 1 liter flat-bottom boiling flasks each containing a single 24/40 female ground glass joint. After flushing the flask with oxygen for approximately 20-30 seconds, the paper was ignited and the sample thrust rapidly into the flask and combustion allowed to take place. The flask was partially immersed in a dry ice-alcohol sludge and water which formed during the combustion condensed as a frost on the bottom of the flask. Eleven ml of a 30% ethanol—70% toluene mixture containing 5 g 2,5-diphenyloxazole and 300 mg 1,4-bis-2-(5-phenyloxazolyl)-benzene per liter of toluene was added and the contents were allowed to reach room temperature. The tritium in the sample was oxidized to water during the combustion and was completely soluble in the above mixture. Ten ml of the scintillation solution was removed and placed into counting vials. These were then read in a Packard automatic liquid scintillation counter. After counting, an internal standard of tritiated toluene was added to determine the counting efficiency. All results are expressed as disintegrations per minute (dpm).

TABLE I. Radioactivity Isolated from Tritiated D Treated Rats 24 Hours After Administration.

Source	Oral		Intravenous	
	% dose	dpm/mg or ml	% dose	dpm/mg or ml
Urine	3	30,000	3	29,700
Blood	.1	1,400	.2	2,200
Feces	36	7,500	37	5,000
Stomach	.3	28	.2	22
Stomach contents	11*	4,600*	.9	5,600
Duodenum	.1	22	.07	24
Duodenal contents	.05	1,100	.05	1,200
Jejunum	.4	23	.5	20
Jejunal contents	.7	900	None detected	
Ileum	2.2	137	2	132
Ileal contents	5.5	2,300	4.3	7,000
Colon	1	62	.3	17
Colon and cecal contents	30	7,500	27	5,000
Liver	1	17	1.5	32
Kidney	.06	5	.07	7
Spleen	.15	3	.3	6
Ovary	<.01	3	<.01	10
Uterine fat	.2	19	.3	51
Mesenteric fat	.3	18	1	66
Recovery	93%		79%	

* Animal had eaten a portion of its feces. Analysis showed approximately 200 μg D, containing 1.0×10^5 dpm, in feces eaten by rat (300 dpm/mg due to fecal D).

TABLE II. Blood Levels of Radioactivity from Tritium D Treated Rats.

Time	Oral dpm/ml	Intravenous dpm/ml
1½ min	—	52,500
3 "	—	21,000
5 "	—	23,500
8 "	—	18,000
10½ "	—	15,200
4 hr	2,080	—
6 "	1,440	—
8 "	2,400	—
24 "	1,400	2,200

Results and discussion. Analysis of the tissues, organs, and body fluids of the 2 animals sacrificed 24 hours after the intravenous or oral dose of D, showed that the greatest degree of radioactivity appeared in the gastrointestinal tract. Results of this study can be found in Table I.

Radioactivity in the organs and tissues of the rat that had received the intravenous dose of D was higher than in those of the animal that had received the oral dose, except in the intestinal tract and its contents. It is of interest that the fatty tissues from both animals had a higher degree of radioactivity than did the organs from these animals, indicating a concentration of the steroid in the fat. The amount of radioactivity in blood from these animals determined at various time intervals is shown in Table II. A rapid initial increase of radioactivity followed by a decrease was found in the blood of the intravenously treated animal. Radioactivity in the blood of the animal after peroral treatment was low and unchanged from 4 to 24 hours.

Results of studies with animals given 1 mg of D in oil intramuscularly are in Table III. The major pathway of concentration and elimination was the intestinal tract during the entire test period. Radioactivity in the fat from these animals was high during 28 days post treatment. Fat may be acting as a reservoir with a gradual release of the steroid. The largest amount of radioactivity

TABLE III. Radioactivity Obtained from Rats Receiving 1 mg D Intramuscularly.
(Results in disintegrations/mg)

	Rat number				
	1	2	3	4	5
	24 hr	7 days	14 days	21 days	28 days
Ovary	107	65	84	39	36
Uterus	49	27	17	10	10
Uterine fat	177	209	316	157	113
Omentum fat	282	403	264	150	108
Bladder	45	22	11	33	25
Kidney	151	77	61	36	38
Kidney fat	212	250	350	203	168
Thyroid	66	—	65	40	51
Adrenal	211	93	108	43	44
Pituitary	126	241	109	97	73
Thymus	87	15	36	36	27
Liver	456	109	83	38	33
Lung	68	46	57	36	21
Brain	14	26	14	23	21
Spleen	62	27	28	18	19
Thigh bone	2	1	2	<1	1
Skull	1	1	1	<1	1
Skin	438	514	340	86	114
Duodenum	580	219	166	24	31
Jejenum	931	100	126	84	99
Jejunal contents (dry)	13,000	3,900	1,980	880	1,050
Ileum	460	134	99	53	60
Ileal contents (dry)	7,250	1,420	1,380	415	363
Stomach	68	79	87	30	15
Stomach contents (dry)	101	961	651	—	18
Cecum	1,133	131	113	60	50
Cecal contents (dry)	32,876	6,110	3,113	1,186	1,400
Colon	581	141	76	68	42
Colon contents (dry)	3,009	47,240	4,835	1,507	1,284
Pectoral muscle	92	109	38	17	14
Injection site	795	974	1,430	800	816
Urine (dpm/ml)	2.9×10^6	4.3×10^4	1.2×10^4	2.2×10^3	2.9×10^3
Blood plasma (dpm/ml)	1.0×10^5	2.3×10^4	6.2×10^3	9.0×10^3	2.7×10^3

in the blood appeared within 15 minutes after intramuscular injection and then decreased gradually but was still detectable after 28 days. These results are shown in Table IV.

The radioactivity recovered from each animal is presented in Table V. The results show that after 28 days radioactivity was still detectable. The animal killed 24 hours post treatment had the least amount of radioactivity in the tissues examined. Presumably the major portion of activity was in muscles, at the site of injection and in fat depots. The radioactivity in the blood, organs, and intestinal contents decreased with time correlating with an increased excretion in the urine and, particularly, in the feces.

Summary. The acetophenone derivative of 16*a*, 17*a*-dihydroxyprogesterone (D) was tritiated in the 6,7 position and was administered to rats by various routes. The major portion of the radioactivity was concentrated in the gastrointestinal tract 24 hours after the steroid was given orally, intravenously or intramuscularly. Radioactivity was found in the feces even at 28 days after a single intramuscular injection of 1 mg of D in oil. A

TABLE IV. Radioactivity in Rat Blood After Tritium Labeled D Administered Intramuscularly in Oil.

Time	Animal 1 dpm/ml	Animal 2 dpm/ml
1 min	101,000	108,000
5 "	211,000	138,000
15 "	214,000	172,000
30 "	193,000	149,000
60 "	109,000	101,000
24 hr	65,000	47,000
7 days	36,000	27,000
13 "	16,000	14,000
23 "	—	8,500
27 "	—	5,700

TABLE V. Radioactivity Recovered from Rats Given 1 mg Tritiated D (Expressed in dpm).

	24 hr	7 days	14 days	21 days	28 days
Organs	1.4×10^7	8.4×10^6	6.4×10^6	4.4×10^6	3.5×10^6
Intestinal contents	2.1×10^7	3.9×10^7	6.7×10^6	3.2×10^6	1.7×10^6
Blood	8.3×10^5	7.6×10^5	2.4×10^5	2.5×10^5	2.2×10^5
Urine	2.0×10^7	2.4×10^7	3.7×10^7	3.4×10^7	3.7×10^7
Feces	3.4×10^7	2.9×10^8	3.9×10^8	4.8×10^8	5.0×10^8
Total radioactivity	8.9×10^7	3.6×10^8	4.4×10^8	5.3×10^8	5.5×10^8
% of dose recovered	13	54	66	79	82

high quantity of tritiated material was found in fatty tissue, suggesting that fat may serve as depot for the slow release of D. Radioactivity appeared in the blood one minute after the intramuscular injection of D in oil. The maximum concentration appeared within 15 minutes and was circulating 28 days after the material was given. Approximately 82% of the total radioactivity given was recovered on day 28.

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Development and Characteristics of the Rabbit Kidney Cell Strain, LLC-RK$_1$* (30044)

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Primary rabbit kidney cell cultures have been found useful for cultivation of some viruses, and, in particular, those in the herpes group. One strain of embryonic rabbit kidney cells ERK-1 developed by Westwood *et al*(1) was described as being sensitive to poliovirus following a "transformation" which occurred. The attempt to establish a strain of rabbit kidney cells in our laboratory was initiated in 1959 for the dual purpose of providing the laboratory with such a cell strain and to attempt duplication of the finding of Westwood *et al.*

Material and methods. Tissue cultures. All rabbit kidney tissue cultures were prepared by trypsin dispersion of the kidney cells from 3-week-old New Zealand white rabbits by a procedure essentially the same as that described by Younger(2). Medium 199(3) supplemented with horse serum was used as a nutrient. Culture populations were quantitated by the nuclei enumeration procedure of Sanford *et al*(4).

Viruses. All viruses employed in this study were from stocks carried in these laboratories as tissue culture adapted strains. Virus titrations were performed by inoculation of 0.5 ml quantities of log dilutions of virus into each of 10 cultures/dilution. Titers were determined by the calculation of 50% end-

* Strain LLC-RK$_1$ is now under study by the Cell Collection Committee of the Tissue Culture Association as a candidate strain for replacement in the American Type Culture Collection. Until such placement is made, the culture is available only from our laboratory. The supply is restricted to those investigators who agree, upon receipt, not to further distribute the culture.