acid, and phenylacetic acid on oxygen consumption by slices or homogenates of rat brain were measured. 2. None of these substrates significantly inhibited oxygen uptake by rat brain homogenates. 3. *L*-leucine, *L*-valine, and *L*-phenylalanine were also found not to significantly depress oxygen uptake by brain slices. 4. On the other hand, alpha-ketoisocaproic acid, alpha-ketoisovaleric acid, phenylpyruvic acid, and phenylacetic acid (at concentrations of 0.01 M) did depress oxygen utilization by brain slices to the extent of 10-27% of control values. 5. It is suggested that the central nervous system dysfunction observed in patients with certain hereditary diseases characterized by defects in amino acid metabolism may be related to the accumulation of alpha-ketoacids which occurs in these diseases.

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Biological Activities of the 2-Acetofuran Derivative of 16α, 17α-Dihydroxyprogesterone. (28457)

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A new class of progestational steroids derived from 16α, 17α-dihydroxyprogesterone was reported by Fried *et al.*(1). One of these compounds, the acetophenone derivative of 16α, 17α-dihydroxyprogesterone (D)* was shown to be more active as a progestational agent in the rabbit than progesterone (P) parenterally administered or 17α-ethinyl-19-nortestosterone (N)† orally administered(2). This progestogen maintained pregnancy in the ovariectomized rat(3), did not masculinize the rat fetus(4) and was devoid of undesirable hormonal activities(2).

Another member of this series of steroids is the 2-acetofuran derivative of 16α, 17α-dihydroxyprogesterone (AF). The synthesis of this compound was described by Fried *et al.*(5). Results of biological studies with this compound are presented here.

Materials and methods. New Zealand white rabbits weighing 800-1200 g were employed in the progestational evaluation assays. The animals were primed with a total of 15 μg estradiol benzoate, administered subcutaneously in 3 divided doses on alternate days for a 6-day period. The progestational steroids were administered in a sesame oil solution or suspension, subcutaneously or orally once daily for the next 5 days. Animals were sacrificed 24 hours after the final dose. The uteri were removed and weighed, and sections were taken for histological evaluation(6).

Duration of activity was determined in ovariectomized immature rabbits. These animals were primed with estrogen as described above. A single 5 or 10 mg dose of progestogen was administered intramuscularly on the day following the last priming dose. A main-

* Droxone or Deladroxone (Dihydroxyprogesterone Acetophenide—Squibb®).

† Norethisterone.

tenance dose of .5 μg estradiol benzoate was administered every other day throughout the 35-day study. Rabbits were killed at 5-day intervals beginning 5 days after the start of progestogen administration and their uteri were dissected, prepared for microscopic examination and histologically evaluated for glandular development according to the grading of McPhail(6).

Pregnancy maintenance was studied in 200-250 g virgin Sprague-Dawley Caesarean-derived strain rats obtained from the Charles River Breeding Laboratories. These animals were distributed 4 to a cage together with 2 fertile male rats. Their vaginal smears were examined every morning for the presence of sperm. The day sperm were found was recorded as day one of pregnancy and these animals were placed in individual cages. On the eighth day pregnancy was confirmed by the presence of implantation sites and the rats were bilaterally ovariectomized.

The progestogens and estrone were dissolved or suspended in sesame oil and administered subcutaneously once daily. Steroid treatment was started on the day of ovariectomy and continued through the 19th day of gestation. On day 21 the animals were autopsied and inspected for possible ovarian tissue. The uteri were examined for live and dead fetuses, and for abnormalities.

Virilizing activity was investigated in the unborn rat. Adult females of the same weights and strain as used in the pregnancy maintenance study were employed. Two males were placed into each cage of 4 females and vaginal smears were taken daily. The day sperm were found was considered the first day of pregnancy. All pregnant animals were placed in individual cages. Beginning with day 14 and continuing through day 19 of gestation, the steroids dissolved or suspended in sesame oil were administered subcutaneously once daily. The animals were submitted to Caesarean section on day 22 of pregnancy. The sex of the pups was determined by measurements of the ano-genital distance. Pups with meaurements of 2.2 mm or less were considered females; those with measurements of 2.3-2.8 mm were classified as intersexuals; and those with measurements greater than 2.8 mm were graded as males. All pups were examined internally with a dissecting microscope for the presence of abnormal sex and accessory sex organs.

TABLE I. Progestational Activity of 2-Acetofurans and Acetophenone Derivative of 16α, 17α-Dihydroxyprogesterone.*

Total dose, mg	Subcut admin			Oral admin		
	P	D	AF	N	D	AF
8.0						3
4.0				4	4	2–3
2.0		4		3	4	1–2
1.0	4	4		3	3	1
.5	3	3		2	2	1
.25	1–2	2	4	1	2	0–1
.125	1	1	4		1	
.06	0	0	4			
.03			3			
.015			3			
.008			3			
.004			1			

P, progesterone; N, norethisterone (17α-Ethinyl-19-nortestosterone); D, acetophenone derivative of 16α, 17α-dihydroxyprogesterone; AF, 2-acetofuran derivative of 16α, 17α-dihydroxyprogesterone.

* Numerical values of the McPhail grades are listed under the respective compounds. Each value represents mean response of at least 6 animals.

AF was assayed for uterotrophic activity in mice by a modification of the method of Rubin *et al.*(7); for estrogenic activity in ovariectomized rats by the method of Allen and Doisy(8); for androgenic activity in castrated rats by the assay described by Hershberger *et al.*(9); for glucocorticoid activity in adrenalectomized rats by a modification of the liver glycogen deposition test of Pabst *et al.*(10), the glycogen being determined by a procedure described by Singer *et al.*(11); and for its effect on urinary sodium and potassium, a modification of the method of Kagawa *et al.*(12) was employed.

Results. The 2-acetofuran derivative of 16α, 17α-dihydroxyprogesterone stimulated endometrial gland development in the rabbit in total subcutaneous doses as low a 4 μg (Table I). By this route it was 32-64 times as potent as P or D. Orally it was much less potent. AF was 1/8-1/4 as active as N and 1/8 as active as D. This compound was 1/125-1/500 as active orally as it was parenterally.

A single 10 mg intramuscular injection of P produced a response in the rabbit uterus

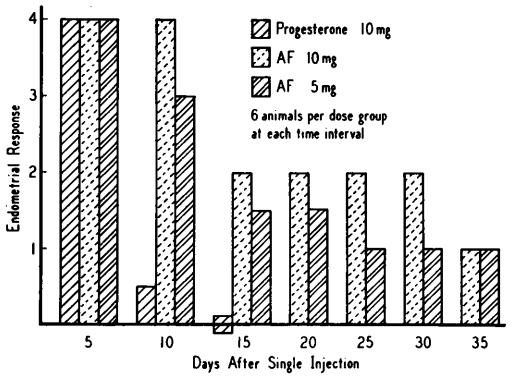

FIG. 1. Duration of progestational response following administration of progesterone and the 2-acetofuran derivative of 16α, 17α-dihydroxyprogesterone.

which was dissipated within 10 days (Fig. 1). AF in single doses of 5 or 10 mg, however, produced an endometrial response which was demonstrable for at least 35 days post injection.

All intact pregnant rats delivered litters, the average number of live pups corresponding closely to the average number of implantation sites (Table II). None of the animals ovariectomized on the eighth day of pregnancy delivered pups or had any signs of implantation. P in daily doses of 10 and 5 mg maintained pregnancy in 80% and 50% of the rats, respectively. AF was somewhat more effective in this respect, since it maintained gestation in 50% of the rats in daily doses of 1.25 mg. Neither compound fully sustained the average control number of live pups at the doses employed. Administration of estrone alone did not promote pregnancy maintenance in the ovariectomized animal but it did augment the effect of the progestogens. Approximately ¼ as much progesterone or AF was required to produce a corresponding degree of pregnancy maintenance when 1 μg of estrone was also administered. In these studies AF was 2-4 times as potent as P in sustaining pregnancy.

Daily doses of 10 mg AF administered to pregnant rats did not alter the sex ratio of the offspring. The percentage of males was 48% and that of females was 52%. This contrasts with the result of daily injection of 2.5 mg of N where 63% of the pups were

TABLE II. Maintenance of Pregnancy in Ovariectomized Rats by Subcutaneously Administered Progestogens Alone and with Estrone.

Compound	Daily dose mg	Daily dose Estrone (μg)	No. of rats	% rats with live pups	Avg No. implantation sites	Avg No. of live pups Per rat	Avg No. of live pups Per rat having live pups
Intact control (sham operated)	0	0	33	100	12.6	12.4	12.4
Castrate control	0	0	10	0	0	0	0
Estrone	0	1	10	0	0	0	0
Progesterone	10.0	0	10	80	12.6	7.0	8.8
	5.0	0	10	50	13.6	2.6	5.1
	2.5	0	10	0	11.7	0	0
	1.25	0	10	0	5.5*(5)	0	0
AF	5.0	0	10	80	11.1	5.5	6.8
	2.5	0	10	70	11.5	1.7	2.4
	1.25	0	10	50	11.1	.9	1.8
	.6	0	10	0	10.9	0	0
Progesterone	10.0	1	10	100	12.9	12.2	12.2
	5.0	1	10	100	13.3	10.4	10.4
	2.5	1	10	90	11.0	8.3	9.2
	1.25	1	10	40	9.2	2.6	6.5
	.6	1	10	0	0	0	0
AF	2.5	1	10	100	12.9	10.0	10.0
	1.25	1	10	90	10.1	8.7	9.6
	.6	1	10	10	1.2 (9)	.6	6.0

* ( ) Indicates number of animals per group with no visible implantation sites, since number of implantation sites given is an average for total number of rats per group.

male, 10% were female and 27% were of an intersex type(4). Internal sex structures were also masculinized by this steroid but not by AF.

Subcutaneous administration of AF in total doses of .1 and 1 mg produced uterotrophic effects in the immature mouse similar in degree to that induced by like doses of P or D. Administration of 5 mg of AF did not alter the castrate state of the vaginal smear of ovariectomized rats, indicating the lack of estrogenicity. Androgenicity as ascertained by the increase in the weights of the ventral prostate and seminal vesicles of the castrate immature rat was not elicited with daily doses of 5 mg AF. This progestogen in doses of 2 mg did not increase glycogen deposition in the liver of adrenalectomized rats indicating a lack of glucocorticoid-like activity. At a dose of 5 mg this steroid administered subcutaneously to the adrenalectomized rat did not alter urinary sodium excretion but slightly increased potassium excretion.

Discussion. Parenteral assays performed in the rabbit indicate that AF is an extremely potent progestational steroid. However, this compound is only weakly active when administered orally. In this respect it resembles P which is almost inactive by the oral route but not D which is approximately as active orally as it is subcutaneously in the rabbit. Unlike P, a single intramuscular injection of AF produced progestational activity of prolonged duration (35 days). It maintained this uterine effect for a longer period of time than did the closely related progestogen, D(2). Similarly to P or D, this progestogen is able to maintain pregnancy in the ovariectomized rat. It is more potent for such pregnancy protection than is the natural steroid. This interesting progestogen is qualitatively similar to D with respect to its lack of undesirable hormonal activities(2) and the absence of fetal masculinization(4).

Summary. The 2-acetofuran derivative of 16α, 17α-dihydroxyprogesterone (AF) is 32-64 times as active a progestogen as progesterone (P) or the acetophenone derivative of 16α, 17α-dihydroxyprogesterone (D) when administered subcutaneously in the rabbit. AF is only 1/8-1/4 as active as norethisterone (N) and 1/8 as active as D when orally administered in this same assay. This progestogen has a long duration of activity in the rabbit when administered intramuscularly. A single injection of 5 or 10 mg produced an endometrial response which was still present 35 days later. Pregnancy is maintained in the ovariectomized rat by smaller doses of AF than that required for P and the concomitant administration of estrone enhanced this activity. This progestational steroid does not masculinize the rat fetus and is devoid of androgenic, estrogenic or glucocorticoid activities.

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