of explanted papilloma tissues. Among them, the majority of round or polygonal cells exhibited intense fluorescence reaction in nucleus. A certain number of triangular or columnar cells also reacted with specific fluorescence in nucleus but the intensity of the staining was weaker and less conspicuous. The implication of these findings was discussed in connection with previous immunofluorescent studies on the tumorous tissues of the Shope system.

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Progesterone, 16α-Hydroxy-Progesterone, and Maintenance of Pregnancy In Mice.* (32113)

THOMAS R. FORBES
Department of Anatomy, Yale University, New Haven, Conn.

The hormone 16α-hydroxy-progesterone has been demonstrated in extracts of human blood and corpora lutea(1), but little information on its endocrine activity seems to have been published. This study was undertaken to subject 16α-hydroxy-progesterone to the Hooker-Forbes bio-assay for progestin activity(2) and to investigate the effect on fetal survival of administration of pellets of this compound, alone or in combination with progesterone, in intact and ovariectomized mice.

Materials and methods. A sample of 16α-hydroxy-progesterone was assayed for progestin activity(2). Solutions for assay were injected into 40 uterine horns of ovariectomized adult CHI mice.

All other experiments were done with adult female Brown Belt mice. They were mated with males of this stock and were examined daily for vaginal plugs. The day on which a plug was found was recorded as day 1 of pregnancy. Mice found to be not pregnant, and mice that became ill, were excluded from the experiment.

Hormones were mixed by dissolving the appropriate amounts in ether, stirring, and recrystallizing. Cylindrical pellets of pure steroid(s) were prepared by a technique which subjected each pellet to the same amount of compression(3). The pellets, which weighed about 9 mg each, were implanted with a large hypodermic needle beneath the dorsal thoracic or cervical skin during light ether anesthesia on day 12 of pregnancy.

Bilateral ovariectomies were performed by a lumbar dorsal approach under ether anesthesia on day 14. Care was taken not to disturb the pellets.

Body weights were recorded daily beginning on day 12.

Two groups of mice were allowed to go to term and deliver their young (Groups A, B, Table I). All other pregnant mice were anesthetized with ether on day 19. The abdomens and uteri were opened. The numbers of live fetuses, dead fetuses, and empty implantation sites (*i.e.*, those from which abortions or

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TABLE I. Effects of ovariectomy and of steroid administration on fetal survival. Prog., progesterone; 16α, 16α-OH-progesterone.

Group	Preg. Adults	Steroid(s) Administered	Total wt of pellets (mg)	% Progesterone	Ovariectomized?	Total implantations	% fetal survival
A	10	—	—	—	No	54	98*
B	"	16α	33.2 ± 2.6	0	"	63	83*
C	6	Prog.	18.4 ± 2.9	100	Yes	40	90
D	10	16α	72.4 ± 2.5	0	"	59	0
E	"	"	37.4 ± 2.6	"	"	66	0
F	"	"	71.6 ± 1.4	"	No	64	94
G	"	"	37.2 ± 4.1	"	"	57	82
H	"	"	4.6 ± .3	"	"	50	96
I	"	" + Prog.	18.6 ± 1.4	99	Yes	69	71
J	"	" " "	25.3 ± .2	80	"	60	52
K	11	" " "	94.7 ± 2.7	20	"	66	33

* Allowed to go to term; delivered day 20 or 21.

resorptions had occurred) were separately recorded. The original number of fetuses in an animal was considered to be equal to the number of fetuses remaining on day 19 plus the empty sites. An attempt was made to recover the pellets for reweighing, but they frequently disintegrated when removed, and reliable values for weights at the end of the experiment could not be obtained.

Results. In the standard bio-assay for progestin activity in CHI mice, the minimal effective dose of 16α-hydroxy-progesterone was 0.006 μg.

The fetal survival rates in Table I were based on the total number of fetuses and implantations in relation to the number of surviving fetuses. There were rather wide variations in numbers of implantations and survivals from animal to animal within each group. Maternal weight losses of 1 g or more, when they occurred, were noted almost invariably on days 15 or 16, one or two days after ovariectomy. Animals showing such losses were found at autopsy to have had abortions or resorptions or both.

Intact untreated controls had 98% fetal survival at normal delivery on days 20-21 (Group A). Intact mice implanted with 4 pellets of 16α-hydroxy-progesterone and allowed to go to term delivered on days 20-21 with 83% fetal survival (Group B). In other intact pregnant mice, fetal survival on day 19 was 82 to 96% after implantation in the mothers of 8, 4, or 1/2 pellets of 16α-hydroxy-progesterone (F, G, H).

On the other hand, no fetuses survived to day 19 after animals received 8 or 4 pellets of 16α-hydroxy-progesterone on day 12 and were ovariectomized on day 14 (D, E).

Still other pregnant mice were implanted with pellets containing approximately 18 mg of progesterone and from 0.2 to about 76 mg of 16α-hydroxy-progesterone (I, J, K). Even 1% of the latter conspicuously reduced fetal survival, and the death rate increased with the proportion of 16α-hydroxy-progesterone.

Discussion. Although 16α-hydroxy-progesterone is closely related chemically to progesterone, the Minimal Effective Dose of the former in the Hooker-Forbes bio-assay was 0.006 μg, or 30 times the M.E.D. of progesterone(2).

In the experiments with pregnant mice all hormones were administered in pellet form, partly because of the relatively poor solubility of 16α-hydroxy-progesterone in sesame oil, thus making impractical the injection of solutions containing large doses, and partly because absorption from a pellet is believed to be continuous, thus presumably permitting a steady release of the hormone into the tissue fluids and blood. Courrier and Kehl(4) long ago suggested that replacement therapy be initiated before the ovaries are removed so as to avoid interruption in the supply of hormone; it was for this reason that the pellets

were implanted two days before ovariectomy.

The duration of pregnancy in intact mice treated with 16α-hydroxy-progesterone and allowed to go to term (20-21 days) was the same as in intact, untreated Brown Belt mice (5). In addition, administration of this compound to intact mice did not appear significantly to reduce fetal survival (Table I).

It is well known that mice will abort promptly following ovariectomy at any stage of pregnancy unless adequate replacement therapy is provided. When 2 pellets of pure progesterone (about 18 mg) were implanted subcutaneously in pregnant Brown Belt mice on day 12 and the ovaries were removed on day 14, an average of about 0.27 mg was absorbed per day and the fetal survival rate on day 19 was 91% (5). In the present experiments each mouse which received progesterone, whether alone or in combination, was likewise given approximately 18 mg of that hormone, an amount theoretically sufficient for normal fetal survival in ovariectomized mothers. However, if the progesterone had been mixed with 16α-hydroxy-progesterone, even a very little of the latter was associated with an elevated fetal death rate, and the latter rose further as the proportion of 16α-hydroxy-progesterone was increased.

When administered alone to ovariectomized mice 16α-hydroxy-progesterone, like 17α-hydroxy-progesterone(5) and the α and β isomers of 20-hydroxy-Δ^4-pregnen-3-one(6), completely failed to maintain pregnancy.

Thus under the experimental conditions described 16α-hydroxy-progesterone a) did not interfere significantly with pregnancy in intact mice, b) by itself failed to support pregnancy in ovariectomized mice, and c) partially and proportionately interfered with fetal survival when administered to ovariectomized mice in combination with an amount of progesterone theoretically adequate to permit normal fetal survival to day 19. Since exogenous 16α-hydroxy-progesterone was supplied in all 3 groups of mice, its external source and the method of its administration would not in themselves appear to be determining factors.

The known variables significant in relation to the results would seem to be a) endogenous (ovarian) progestin and estrogen, b) exogenous progesterone, c) exogenous 16α-hydroxy-progesterone. When a) and c) were available, fetal survival was good. When b) and c) were provided in the absence of a), survival was poor. Thus the difference seemed to depend on a) versus b). Possibly a) gave better results because of the presence of estrogen or because the ovaries supplied more progestin than did the pellets.

Evidence is lacking as to the action of endogenous 16α-hydroxy-progesterone or, indeed, as to whether mice produce this hormone. If it develops that this compound occurs endogenously and inhibits the action of progesterone, 16α-hydroxy-progesterone would have to be recognized as a factor in the regulation of progestational action.

Summary. In the Hooker-Forbes bio-assay in CHI mice, the minimal effective dose of 16α-hydroxy-progesterone was 0.006 μg. Subcutaneous pellets of this compound did not affect the normal duration of pregnancy or survival of fetuses in intact mice. In mice implanted with 2 progesterone pellets (about 18 mg) of pure progesterone on day 12 of pregnancy and ovariectomized on day 14, fetal survival was good. If pellets of 16α-hydroxy-progesterone were substituted, total abortion promptly occurred. If the pellets were a mixture of 16α-hydroxy-progesterone and progesterone, some fetuses survived, the survival rate diminishing as the proportion of 16α-hydroxy-protesterone to a fixed amount (about 18 mg) of progesterone increased.

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