Progestational Activity of Halogenated Corticosteroids and Related Compounds in Rabbit and Monkey.* (22174)

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Fried(1) has reviewed the biological effects of the 9 alpha halo derivatives of hydrocortisone, cortisone, 11-beta-hydroxyprogesterone and other related steroids. The high mineralocorticoid and glucocorticoid activity shown by several of these compounds in experiments with rats and dogs were described. Results of the present investigations demonstrate progestational activity of several of the 9 alpha halo steroids in both the rabbit and monkey.

Materials and methods. Clauberg rabbit test. Female New Zealand white rabbits weighing 600-800 g at the beginning of treatment were used in all experiments. Ten μg of estradiol 17-beta were given daily subcutaneously in 0.25 ml sesame oil solution for 6 days. Beginning on the 7th day, the test compounds were given subcutaneously daily for 5 days. The animals were killed with chloroform 20 to 24 hours after the last injection. Body, uterus and adrenal weights were recorded. Uteri and adrenals were fixed in Bouin's solution. Sections were stained with hematoxylin and eosin. Progestational proliferation of the endometrium was measured according to the standard scale of McPhail (5). *Inhibition of estrogen withdrawal bleeding in the monkey.* Bilaterally ovariectomized female monkeys (*Macacus rhesus*) weighing 3.5-5.5 kg were given daily subcutaneous injections of 10 μg estradiol 17-beta in 0.25 ml sesame oil solution for 20 days. Subcutaneous injections of the test compound were begun on the 21st day. Daily vaginal smears were made with normal saline. Treatment was discontinued either with the onset of menstrual bleeding or when the experiment was terminated in order to obtain samples of the endometrium. With the exception of progesterone and desoxycorticosterone acetate (in solution), all compounds were administered as suspensions in sesame oil.

Results. It is well to keep in mind the quantitative variability of the bioassay method when the potency of compounds is compared by means of the Clauberg rabbit assay. We have chosen a total dose of 0.5 mg progesterone as the reference level because this amount will produce full progestational proliferation of the endometrium in more than 75% of the rabbits receiving this dose whereas 0.25 mg progesterone gives a maximum progestational response in 28% of animals on this lower dose. These percentages were derived from a total of 61 rabbits on the 0.5 mg dose and 22 rabbits on the 0.25 mg dose.

Data from several experiments are presented in Table I. It will be noted that at a total dose of 5 mg, the activities of 9 alpha-fluoro-hydrocortisone acetate, 9 alpha-chloro-hydrocortisone acetate and desoxycorticosterone acetate are comparable whereas hydrocortisone acetate is inactive even at a level of 25 mg. At a total dose of 15 mg, both the 9 alpha-fluoro- and 9-alpha-chloro derivatives of hydrocortisone produce a response comparable to that of 0.5 mg of progesterone (full proliferation). This would indicate an activity for these halo compounds of about 3% that of progesterone.

Delta-1-9-alpha-fluorohydrocortisone acetate was also assayed by the Clauberg method. This compound gave minimal progestational responses both at total doses of 10 mg and 20 mg.

Table I includes results on progestational proliferation produced in response to treatment with 9-alpha-fluoro-11-beta-hydroxyprogesterone. A 1.0 mg total dose of this halogenated compound possesses low grade activity and at the 3.0 mg level there is a moderate response. At 5.0 mg a maximal response was educed. An estimated potency of 5 to 10% that of progesterone could be made for this

* The 9 alpha-halo steroids used in this study were supplied through the courtesy of Dr. J. Fried of the Squibb Institute for Medical Research. The delta-1 steroids were supplied through the courtesy of Dr. Marvin Kuizenga of the Upjohn Co. and Dr. Preston Perlman of the Schering Corp.

TABLE I. Progestational Activity of Halogenated Steroids in the Clauberg Assay.

Total dose (mg)	Progestational proliferation					
	9 alpha-chloro F acetate	9 alpha-fluoro F acetate	Cpd. F acetate	DOC acetate	9 alpha-fluoro 11-beta-hydroxy-progesterone	Progesterone
25			0, 0, 0			
15		4, 3, 4				
	4, 4, 4*	4, 4, 4				
10		3, 2, 2				
	3, 4, 4, 1, 2	3, 3, 2	0, 0, 0	4, 4, 3	4, 4	
5	2, 2, 1	2, 1, 1				
	2, 1, 1	1, 1, 1	0, 0, 0	2, 1, 1	4, 4	
3.0					3, 2	
2.5	0, 0, 0	0, 0, 0				
	0, 0, 0		0, 0, 0	2, 2, 1		
1.0	0, 0, 0	0, 0, 0			2, 2, 1	
.5						4, 4, 4
	0, 0, 0					3, 4
.25						4, 4, 3, 3, 3

* Each number represents degree of progestational proliferation for one animal.

halo steroid. Neither delta 1-hydrocortisone nor delta 1-cortisone showed progestational activity when tested at a total dose of 20 mg using 3 animals for assay of each compound. Cortisone acetate was inactive as a progestogen at a total dose of 50 mg (3 rabbits).

Gross examination of animals receiving the 10 and 15 mg doses of fluoro- and chlorohydrocortisone and the 25 mg dose of hydrocortisone revealed ascites and liver enlargement. Adrenal weights were consistently lower in these animals as compared with those on progesterone treatment.

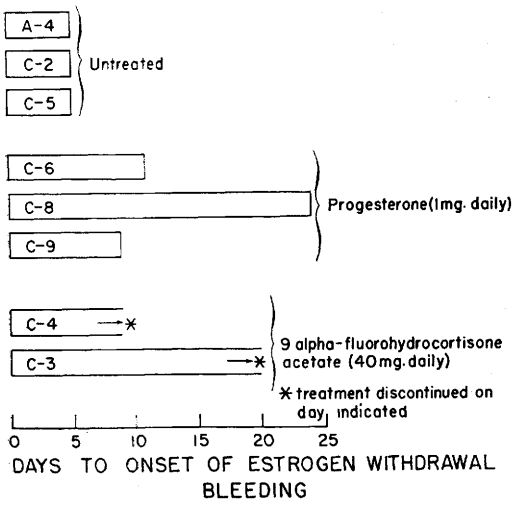

FIG. 1. Progestational activity of 9 alpha-fluorohydrocortisone acetate in the monkey.

Results obtained from experiments with estrogen-primed castrate female rhesus monkeys are shown in Fig. 1. Three untreated monkeys began estrogen withdrawal bleeding on the fifth day following the last injection of estradiol. The onset of bleeding was delayed from 9 to 24 days in 3 monkeys receiving 1.0 mg progesterone daily. Of 2 monkeys treated with a daily dose of 40 mg of 9 alpha-fluorohydrocortisone acetate, one continued for 9 and the other for 18 days without withdrawal bleeding. Treatment of these animals was discontinued to permit uterine biopsies. Histological examination of the biopsies presented a clearly defined progestational endometrium. In another animal, a daily dose of 20 mg of 9 alpha-fluoro-hydrocortisone acetate failed to delay estrogen withdrawal bleeding.

Our results demonstrated progestational activity of 9 alpha-chlorohydrocortisone, 9 alpha-fluorohydrocortisone and 9 alpha-fluoro-11-beta-hydroxyprogesterone in the rabbit. Hydrocortisone was shown to be inactive even at high levels. Delay of estrogen withdrawal bleeding in the monkey further demonstrates the progestational action of 9 alpha-fluorohydrocortisone. The lack of toxicity and any

unusual side effects of this halogenated corticosteroid in the monkey is in marked contrast with effects noted in the rabbit.

Discussion. Byrnes and Shipley(2) reported a lack of progestational activity for 11 beta-hydroxyprogesterone at a dose of 5 mg in the Corner-Allen assay. It is evident that the introduction of either a chlorine or a fluorine atom in the 9 alpha position imparts progestational activity in hydrocortisone and 11-beta hydroxyprogesterone both of which were progestationally inactive at comparable doses.† Junckmann(3) has reported that 17 alpha-hydroxyprogesterone, slightly active as a progestational compound, becomes active upon esterification. Huggins and Jensen(4) have recently described the potent depressant activity of 9 alpha-fluorohydrocortisone and 9 alpha-11-beta-hydroxyprogesterone on estrone-induced uterine growth in the hypophysectomized rat. Hydrocortisone and cortisone were considered to be weak inhibitors in the same test.

Summary. Progestational activity of 9 alpha-chlorohydrocortisone acetate, 9 alpha-fluorohydrocortisone acetate and 9 alpha-fluoro-11-beta-hydroxyprogesterone has been demonstrated using progestational proliferation of the rabbit endometrium (Clauberg test) as index of activity. Hydrocortisone acetate and cortisone acetate show a lack of activity in the same test. Progestational activity of 9 alpha-fluorohydrocortisone is further demonstrated by prevention of estrogen withdrawal bleeding and characteristic histological changes in the endometrium of the ovariectomized rhesus monkey pre-treated with estrogen.

† However, 11-beta-hydroxy progesterone has one-eighth the activity of 9 alpha-fluoro-11-beta-hydroxy progesterone in the Clauberg Test.

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Metabolism of the Lanthanons in the Rat.* (22175)

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For many years the behavior of the lanthanons in biological systems was regarded as a scientific curiosity. The name "lanthanon" has been suggested(1) for the group of elements of atomic numbers 57 to 71, lanthanum through lutetium. Until recently the commercial use of these elements was limited and their chemistry relatively obscure. With the development of the chain-reacting pile, and the discovery that certain light members of this group were produced in relatively large quantities in the fission process, knowledge of their metabolism† and radiotoxicity assumed greater importance. Certain members of this group can be reduced to the divalent state and others oxidized to the quadrivalent state; the principal valence state of the group as a whole, however, is plus three. Although the actual radius of an ion of a particular element depends principally upon its valency, the radii of the tervalent ions of the lanthanons show an interesting decrease with increasing atomic number. This phenomenon

* This work was performed under auspices of Atomic Energy Commission.

† There has been much dissension concerning the use of the word "metabolism" for elements not intimately involved in biological processes; this terminology, however, is in general use in tracer work of the type presented here.