tery rests (Fig. 1, F), is grasped in the jaws of the forceps and compressed firmly so as to constrict the vessel to the desired degree.

It has been found by experience that for female rats, weighing about 150 g, constriction of only one main renal artery, with the forceps set to leave a space of 0.014″ (or 0.355 mm) leads to development of hypertension in the majority of animals. The wall of the renal artery is actually compressed by this procedure to outside thickness of about 0.1 mm. Greater constriction, which leads to complete necrosis of the kidney, or inadequate constriction, is ineffective in raising the blood pressure. For female rats weighing around 200 g, a free space of 0.015″ is necessary, and for those weighing around 250 g, a free space of 0.016″ is most satisfactory. In the case of rapidly growing young male rats, a degree of constriction, adequate for a young female rat of equal weight may prove to be too tight and eventually lead to necrosis of the kidney, as the animal grows. This must be taken into consideration.

Summary. An instrument has been described which permits accurate control of degree of constriction of the main renal artery in small animals and facilitates production of hypertension in a large percentage of such animals.

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Metrotropic Activity of Some 21-Haloprogesterone Derivatives.* (25059)

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Recent interest in obtaining orally active progestational agents resulted in synthesis of many structural modifications of progesterone and 19-nortestosterone. Certain of these materials have been shown to be of value clinically. In the progesterone series, metrotropic activity has been reported for a series of 9-α and 12-α-halogenated derivatives of progesterone(1). 9α-Bromo-11-oxoprogesterone was effective clinically upon oral administration; its potency was rated as equal to that of 17α-hydroxyprogesterone acetate and about 2 to 3 times more potent than ethisterone(2,3). 9α-Fluoro-11β-hydroxyprogesterone and its 17α-hydroxy analogue were weak metrotropic agents in rabbits(4). These same agents were reported to be more active than progesterone as antagonists of estrone-induced uterine growth in rats(5). In a similar test 9α-fluoro-11β-hydroxy and 9α-fluoro-11-oxoprogesterone were about equal to progesterone(1). The present communication deals with progesterone-like activities and structure-function relationships of several 21-halogenated steroids.

Materials and methods. The following steroids were used: Progesterone, 21-fluoroprogesterone, 21-chloroprogesterone, 17α-hydroxyprogesterone, 17α-hydroxy-21-fluoroprogesterone, 17α-hydroxy-21-chloroprogesterone, 17α-acetoxyprogesterone, 17α-acetoxy-21-fluoroprogesterone, 17α-acetoxy-21-chloroprogesterone, 17α-acetoxy-21-bromoprogesterone, 17α-acetoxy-21-iodoprogesterone, 6α-methyl-17-acetoxy-progesterone, 6α-methyl-17-acetoxy-21-fluoroprogesterone and 6α-methyl-17-acetoxy-21-chloroprogesterone. Estimates of activity were obtained using the Clauberg assay, uterine carbonic anhydrase assay and the intra-uterine (McGinty) assay. *Clauberg Assay*(6). The test compound was administered subcutaneously or orally over 5 days to immature female rabbits previously primed for 6 days with 5 μg estradiol-17β. On day following last treatment, animals were sacrificed and

* The new steroids described in this study were prepared by Drs. C. G. Bergstrom, P. B. Sollman and R. M. Dodson, Division of Chemical Research, G. D. Searle & Co.

segments of uteri taken for histological inspection. On microscopic examination arborization of glandular epithelium was determined and graded from +1 to +4; +1 represented that type of uterus with no proliferation and +4 represented maximally stimulated epithelial glands. *Uterine carbonic anhydrase assay.* Uterine tissues weighing approximately 100 mg were removed from animals used in Clauberg assay. Tissues, which included both endometrium and myometrium, were homogenized using cold distilled water and centrifuged; the supernatant was analyzed for carbonic anhydrase using our modification of methods of Philpot and Philpot(7) and Keilen and Lutwak-Mann(8). Briefly, our method was as follows: 5 ml aliquots of supernatants were placed in reaction tubes along with 5 ml of a 0.0026 M $NaHCO_3$ solution, 10 drops bromothymol blue and 1 drop octyl alcohol; all solutions and subsequent reactions were carried out at 0-2°C. Blanks consisted of 10 ml 0.0026 M $NaHCO_3$ in addition to bromothymol blue and octyl alcohol. CO_2 was bubbled through solutions for exactly 2 minutes, the bubble rate regulated by pressure gauge and capillary tubing so that a color change in blank tubes occurred in approximately 75-80 seconds. At the end of 2 minute bubbling period 1 ml of a $NaCO_3$ $NaHCO_3$† solution was added and the reaction required to change the color of bromothymol blue from blue to blue-green was carefully timed. The end-point color was prepared using 10 ml buffer (Beckman #3501, pH 7) solution with 10 drops of the indicator. Units of activity were estimated with the following calculations, based on standard curves obtained with purified enzyme preparation (National Biochemical Corp.):

$$x = \frac{P}{100} - P \cdot X_{50} \cdot \frac{\text{Vol extract}}{\text{Vol ext. used}} \cdot \frac{100}{\text{Tissue wt}},$$

where x = weight of crystalline enzyme (or its equivalent activity in 100 mg tissue); X_{50} = amount of crystalline enzyme producing 50% reduction of reaction time; P = Per cent reduction in reaction time (RT)

$$= \frac{\text{RT blank} - \text{RT unknown}}{\text{RT blank}} \cdot 100.$$

McGinty assay(9). Ovariectomized estrone-primed rabbits were used. The compound, dissolved in corn oil, was administered locally in a one-inch segment of one horn of uterus; a similar segment of the contralateral horn of the uterus was treated with oil only. Three days later, animals were sacrificed and uterine segments removed and prepared for histological inspection as in the Clauberg technic.

Results. Agreement between Clauberg and uterine carbonic anhydrase assays is in keeping with earlier reports by others(10,11,12). Sensitivity and reproducibility of the enzyme test are about the same as with the Clauberg test even though no attempt was made to dissect the endometrial portion of the uterus away from the myometrium, as was done in the methods previously cited.

17α-Hydroxyprogesterone, inactive as a progestin in rabbits(13,14,15,16), guinea pigs (17) and humans(16) is highly active in mice (Hooker-Forbes Assay, 18). The marked activity found following 17α-acetylation of this compound (Table I) confirms that reported by others(3,12,13,14,15,19). Presence of 6α-methyl group in the progesterone molecule increased its potency 5-fold on subcutaneous administration (Table I). Orally, 6α-methylprogesterone was as effective as 17α-acetoxyprogesterone (Table II). The most potent metrotropic agent was 6α-methyl-17α-acetoxy-21-fluoroprogesterone, which was 50 times more potent than progesterone on subcutaneous administration. Orally, 6α-methyl-17α-acetoxy-fluoroprogesterone was 2 times more potent than its non-halogenated analogue and 10 times more potent than the standard, subcutaneously administered progesterone (Table II). This agent was also the most potent progestin of the present series in the McGinty assay, being about 50 times more potent than progesterone (Table III).

Systemically, 21-chloroprogestins were less potent than their C-21 fluoro-derivatives (Table I). The C-21 bromo- and iodo-substituted forms tested had only minimal activity, or were inactive, suggesting an inverse relationship between progestational activity and

† The reaction solution is prepared for each determination by adding 5 ml conc. $NaHCO_3$ to 25 ml conc. Na_2CO_3 and diluting to 100 ml with double distilled water.

TABLE I. Metrotropic Activity of C-21 Haloprogestins in Clauberg and Uterine Carbonic Anhydrase Assays Following Subcutaneous Administration.

Progesterone substitution			Clauberg assay			Carbonic anhydrase assay		
C-6	C-17	C-21	Daily dose (mg)	No. of animals	Relative activity*	Daily dose (mg)	No. of animals	Relative activity*
			.1	32	1	.1	32	1
		F	.05	4	2			
		Cl	1.	4	Inactive			
	OH		5.	4	"	5.	4	Inactive
	"	F	1.	4	"	1.	4	"
	"	Cl	1.	4	"	1.	4	"
	OAc		.01	8	10	.01	8	10
	"	F	.01	4	10			
	"	Cl	.05	4	2	.05	4	2
	"	Br	.1	4	1	.1	4	1
	"	I	1.	3	Inactive			
CH_3			.02	4	5	.02	4	5
"	OAc		.002	8	50	.003	8	30
"	"	F	.002	15	50	.002	14	50
"	"	Cl	.04	8	2	.04	8	2

* The lowest daily dose that produced a +3 endometrial response was arbitrarily chosen as the level at which potency comparisons were made in the Clauberg Assay. In the carbonic anhydrase assay comparisons were made at level that corresponded to 50 μg equivalents/100 mg wet wt of tissue.

atomic weight of the substituted halogen. In absence of adequate knowledge concerning metabolism of these substances, any explanation of structure-function relationships must be speculative. The increased oral effect of the 21-fluoro-progestins apparently results from decreased degradation in the gut or increased intestinal absorption since their parenteral activities are not different from those of their non-halogenated forms (Tables I and II). Conversely, the decrease in potency of the 21-chloro-, bromo-, and iodo-progestins when compared to the 21-fluoro-derivative may result from increased degradation of the molecule in the gastro-intestinal tract. Increased systemic inactivation can not be ruled out.

With the exception of 17α-acetoxy-21-fluoroprogesterone, similar structure-function relationships obtain following local instillation of these 21-haloprogestins (Table III). Based upon the relationship between metrotropic activity and atomic weight of the substituted halogen, the relatively low potency of 21-

TABLE II. Metrotropic Activity of C-21 Haloprogestins in Clauberg and Uterine Carbonic Anhydrase Assays Following Buccal Administration.

Progesterone substitution			Clauberg assay			Carbonic anhydrase assay		
C-6	C-17	C-21	Daily dose (mg)	No. of animals	Relative activity*	Daily dose (mg)	No. of animals	Relative activity*
			.1	32	1	.1	32	1
		F	1.	4	Inactive†			
		Cl						
	OH		5.	4	"	5.	4	Inactive
	"	F	1.	4	"	1.	4	"
	OAc		.5	3	.2	.5	3	.2
	"	F	.1	7	1.			
	"	Cl	.5	6	<.2	.5	6	<.2
	"	Br	1.	4	Inactive†	1.	4	Inactive
CH_3			.5	4	.2	.5	4	.2
"	OAc		.02	5	5.	.02	5	5.
"	"	F	.01	8	10.	.01	8	10.

* Subcut. administered progesterone was used as standard for comparison, since its oral activity is so low.

† Active, but not by standards of this study, since the compound did not produce a +3 response.

TABLE III. Metrotropic Activity of C-21 Haloprogestins in McGinty (Intrauterine) Assay.

Progesterone substitutions			Dose (μg)	No. of animals	Degree glandular development	Relative activity
C-6	C-17	C-21				
			.5	9	2.4*	1
		F	.1	6	2.3	5
		Cl	10.	4	2.0	.05
	OH		100.	4	1.0	Inactive
	"	F	100.	4	1.7	"
	"	Cl	100.	4	1.	"
	OAc		.5	11	2.1	1
	"	F	.5	8	2.2	1
	"	Cl	.05	6	2.1	10
	"	Br	.1	6	2.6	1–5
	"	I	1.	4	3.4	.5–1.†
CH_3			.1	6	2.4	5 †
"	OAc		.02	13	3.	25
"	"	F	.01	12	2.9	50
"	"	Cl	.05	8	2.	10

* McPhail scale.

† Preliminary data.

fluoro-acetoxy-progesterone does not fit into the expected potency pattern observed elsewhere.

Summary. The metrotropic activity of a series of C-21 haloprogestins was assayed in the Clauberg, uterine carbonic anhydrase, and intrauterine (McGinty) assays. C-21 Fluorination of both 17α-acetoxy-progesterone and 6α-methyl-17α-acetoxyprogesterone resulted in a marked increase in oral potency of both substances; subcutaneously, potency was unaltered by this structural modification. Upon local instillation of these 21-halogenated progesterone derivatives, decreased potency was observed as atomic weight of substituted halogen increased. 21-Fluoro-17-acetoxyprogesterone failed to increase progestational potency in the intrauterine assay thus differing from other 21-fluoroprogesterones. The most potent metrotropic agent tested was 6α-methyl-17α-acetoxy-21-fluoroprogesterone. Orally, it was twice as potent as its non-fluorinated analogue and 10 times more potent than subcutaneously administered progesterone. It was 50 times more potent than progesterone when both were given subcutaneously and about 50 times more potent than progesterone when given locally.

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