BIOLOGICAL ACTIVITY OF MEDROGESTONE: A NEW ORALLY ACTIVE PROGESTIN

C. REVESZ AND C. I. CHAPPEL†

Ayerst Research Laboratories, Montreal, Canada

(Received 12th February 1966)

Summary. A new synthetic orally active progestational agent ‘Medrogestone’ was investigated and its activity compared to progesterone and medroxyprogesterone acetate (MAP). Medrogestone fulfils the criteria of a pure gestagen, it has a spectrum of biological activity similar to that of progesterone but unlike progesterone, it is orally active. Medrogestone is free of undesirable side effects in experimental animals. It is being investigated clinically as an oral progestin (Carter, Faucher & Greenblatt, 1964).

INTRODUCTION

The lack of oral effectiveness of progesterone inspired the search during recent years for progestational compounds which would be orally active. A number of such compounds have been synthesized, tested in animals and are now widely used in menstrual irregularities, habitual, threatened abortion, cycle regulation, treatment of infertility and/or in combination with oestrogens as contraceptives. However, many of these substances in addition to their progestational activity produce certain side-effects such as androgenicity, masculinization of female foetuses and adrenal atrophy. The desirability of an orally active progestin which would be free from these objectionable properties led to the synthesis of a series of derivatives of 17-methylprogesterone (Deghenghi & Gaudry, 1961; Deghenghi, Revesz & Gaudry, 1963) and their testing as progestational agents. The biological activity of 6-methyl-6-dehydro-17-methylprogesterone, which appeared to be the most promising member of this series, forms the subject of this paper. It has the following structural formula:

* Generic name of 6-methyl-6-dehydro-17-methylprogesterone. Trade name in U.S.A. is Colprone, also known as AY-62022.

† Present address: Bio-Research Laboratories, Pointe-Claire, Quebec, Canada.

METHODS

Progestational activity

McPhail's (1934) modification of the Clauberg test was employed using progesterone and medroxyprogesterone acetate (MAP) as reference compounds.

Immature female rabbits were primed with a total dose of 15 μ g oestrone given over a period of 6 days. From the 7th to the 12th day of the experiment the test compound was given daily, subcutaneously (in 0.2 ml of sesame oil) or orally (in 2 ml of sesame oil). Twenty-four hours after the last dose was given the animals were killed and sections removed from the proximal, middle and distal portions of each uterine horn. Specimens were fixed in Bouin's solution, sectioned at 5 μ and stained with Harris's haematoxylin and eosin stain. The sections were evaluated according to McPhail's grading system.

Duration of action of progestational activity

The duration of effect of a single dose in the above test was determined by the method of Junkmann (1954). Progesterone, 17-hydroxyprogesterone caproate and MAP were used as reference compounds.

Immature female rabbits were primed with oestrone as previously described. On the 7th day the compound was given in a single high dose subcutaneously. On the following days 0.025 μ g of oestrone was then given daily subcutaneously. Groups of five animals were killed 3, 7, 10, 13 and 17 days after receiving the test compound. Uterine sections were evaluated as above.

Local progestational activity

In this test McGinty, Anderson & McCullough's (1939) method was used with progesterone and MAP as reference compounds.

Immature female rabbits were primed as described in the Clauberg test. On the 7th day of the experiment the rabbits were anaesthetized (Nembutal 30 mg/kg i.v.) and the uteri were exposed. Ligatures were placed at each end of the upper 3 to 4 cm segment of each uterine horn. The upper ligatures were tied tightly and the lower ones left loose without disturbance of the blood supply to the tissue. The test compound (dissolved in 0.1 ml of sesame oil) was introduced into one of the isolated segments through an incision below the lower ligature. As the contents of the syringe were injected, the lower ligature was tightened. The same volume of sesame oil was injected into the other horn as a control. The rabbits were killed 72 hr later and sections of the isolated segment taken for histological examination.

Deciduoma test

The method of Elton & Edgren (1958) was used, with progesterone and MAP as reference compounds.

Female hooded rats of the Long-Evans strain (140 to 160 g) were ovariectomized, allowed to recover for a week and then primed daily, for 4 days, with 5 μ g of oestrone subcutaneously. The test compound was then given subcutaneously daily for the following 9 days. On the 5th day of treatment with the test compound, the left horn of the uterus was exposed and 2.0 mg of histamine

dihydrochloride (in 0.05 ml distilled water) was injected into the lumen, while the right horn served as control. The animals were killed the day after the last subcutaneous injection. The uteri were dissected and the right and left horns weighed separately.

Maintenance of pregnancy test in rats

The effect of Medrogestone in maintaining pregnancy was determined according to the method described by Madjerek, de Visser, van der Vies & Overbeek (1960), with progesterone and MAP as reference compounds.

Effect of multiple doses. Groups of six female hooded rats of the Long-Evans strain were placed in cages which contained two mature males per cage. Vaginal smears were taken daily. The day spermatozoa were found in the smears was counted as Day 1 of the pregnancy and the animal was removed from the cage. On the 9th day of pregnancy the rats were ovariectomized and the number of implantations present in the uteri recorded. The test compound was given subcutaneously once daily, or orally twice daily, from the 9th to the 20th day of pregnancy. On the 21st day of pregnancy the uteri were removed through an abdominal incision and the living foetuses counted and weighed. The number of dead foetuses was also recorded.

Effect of a single dose. The procedure and reference compounds were the same as when multiple doses were given except that after ovariectomy on the 9th day of pregnancy a single dose of the test compound was administered subcutaneously in 0.5 ml of sesame oil.

The number of live foetuses found in each animal, divided by the number of implantations at the time of ovariectomy, multiplied by 100, was used as an index of the ability of a compound to maintain pregnancy.

Ovulation inhibition in rats

Ovulation was induced by the method of Wilson & Zarrow (1962). Norethynodrel, Enovid, Medrogestone and Medrogestone plus Premarin® were tested.

Twenty-four-day-old female hooded rats were injected subcutaneously with 30 units of PMSG (pregnant mare serum gonadotrophin). Exactly 56 hr later, they were injected s.c. with 10 units of HCG (human chorionic gonadotrophin).

The rats were killed and autopsied 24 hr after the HCG was given. The number of ova expelled from the oviduct was counted under the low power of a light microscope (Rowlands, 1942).

The test compounds were given subcutaneously in 0.1 ml of sesame oil with the PMSG, 24 hr before the HCG and with the HCG. The ED₅₀ for ovulation inhibition was the daily dose of the test compound which reduced the number of ova by 50% as compared with the controls.

Prevention of pregnancy test

Groups of five adult female hooded rats were injected with Medrogestone for 1 week before they were caged with sexually mature males. Treatment of the females was continued for 2 weeks after which the males were removed from the cages. Vaginal smears were taken during the experiment in order to

detect spermatozoa. The females were checked for pregnancy 1 week after the males were removed.

Gonadotrophin suppressant activity

This activity was determined in parabiotic rats using the methods of Bunster & Meyer (1933) and Biddulph, Meyer & Grumbeck (1940). Medrogestone was tested alone and in combination with Premarin®. Oestrone was used as the standard.

Pairs of 38- to 39-day-old hooded female litter-mates were united by surgical attachment of the lateral skin and abdominal muscles. One of the partners was spayed and given the test compound subcutaneously in sesame oil daily for 10 days. The animals were killed and autopsied on the morning of the 11th day of the experiment.

Percentage ovarian inhibition was calculated according to Miyake (1961):

$$\frac{100 (V-C)}{(V-V_1)},$$

where V = average ovarian weights of the intact partner united with the vehicle-injected spayed female;

C = average ovarian weights of the intact partner united with the test compound injected spayed female; and

V_1 = average weights of the intact partner united with the vehicle-injected intact female.

Androgenic and anabolic activity

The Herschberger, Shipley & Meyer (1953) assay was used with testosterone propionate, progesterone and MAP as reference compounds.

Twenty-one- to 23-day-old male rats (40 to 45 g) were castrated and given the test compound subcutaneously for 7 days, beginning on the day of castration. The animals were killed the day after the last injection and the weight of the levator ani, ventral prostate and seminal vesicles was recorded. Initial and final body weights were also determined.

Anti-androgenic effects

Some progestational agents have been reported to have strong anti-androgenic effects. The compounds were assayed for this activity in castrated rats as described by Lerner, Bianchi & Borman (1960). Progesterone and MAP were the reference compounds.

Twenty-one- to 23-day-old male hooded rats were castrated. Beginning on the day of castration the test compound alone and in combination with testosterone propionate (T.P.) was given subcutaneously daily for 7 days. The animals were killed the day after the last injection and the levator ani muscle, ventral prostate and the seminal vesicles were removed and weighed. Body weight increases were determined.

The effect of Medrogestone on the sex organs of intact male rats was also determined.

Effect on the developing foetus

The effect of Medrogestone on the genitalia and reproductive system of female offspring of mothers treated during pregnancy was determined as described by Revesz, Chappel & Gaudry (1960). Changes in the genitalia and reproductive system of male offspring were determined by the method of Hamada, Neuman & Junkmann (1963).

Test compounds were administered subcutaneously (in sesame oil), from the 15th to the 20th day of pregnancy, to female hooded rats of the Long-Evans strain. The pups were removed by Caesarean section on the 22nd day and were placed with foster mothers. On the day of birth the anogenital distance was measured and the pups weighed. Animals which survived to the 20th day were autopsied and the reproductive system examined.

Oestrogenic activity

This assay was performed with oestrone as the reference compound in a modified Allen & Doisy (1923) method.

Female hooded rats (150 g body weight) were ovariectomized. Three weeks later the rats were primed with oestrone subcutaneously ($2 \times 7.5 \mu\text{g}$) on two consecutive days. Vaginal smears were taken in the afternoon of the following day and in the morning of the next day. Rats with cornified epithelial cells in the vaginal smears were given the test compound subcutaneously daily for 3 days, 1 week after priming. On the 4th-day morning and afternoon, and the 5th-day morning, vaginal smears were again taken. The ED_{50} is expressed as the total dose at which 50% of the animals had cornified epithelial cells in the vaginal smears.

Effect on endocrine glands

Spayed female rats were treated orally or subcutaneously with the test compound for 14 days. The animals were killed on the 15th day of the experiment and the weight and histological appearance of the adrenals, pituitary, thyroid and thymus recorded. Progesterone and MAP were the reference compounds.

Effect on water and electrolyte excretion

Both normal and saline-loaded adrenalectomized rats were tested according to Llaurodo (1961). Anti-aldosterone properties were also examined.

Glycogen deposition test

Glycogen deposition in Medrogestone-treated animals was determined in fasted adrenalectomized rats as described by Olsen, Thayer & Kopp (1944). Single injections of the test compound were given to one group of animals 7 hr before the liver was removed. In a second group of animals consecutive injections were administered 24 and 7 hr before the removal of the liver. Hydrocortisone alcohol was used as reference compound.

Anti-inflammatory properties

Cotton pellet granuloma test. A single dose of the test compound was applied to carrageenin-soaked pellet irritants which were implanted subcutaneously in

adrenalectomized rats as described by Bush & Alexander (1960). Hydrocortisone was used as the reference compound.

Croton oil granuloma pouch test. Rats bearing a croton oil granuloma pouch were prepared by the method of Selye (1953), and the test compound was injected directly into the pouch (Robert & Nezamis, 1957). MAP and hydrocortisone were used as reference compounds.

TABLE 1
PROGESTATIONAL ACTIVITY IN OESTROGEN PRIMED RABBITS

Compound	No. of rabbits	Mean McPhail's score				
		0.005*	0.01	0.025	0.05	0.1
Medrogestone, oral	3		+1.8			
	8			+2.7		
	4				+3.1	
	3					+3.8
MAP, oral	5		+1.5			
	4				+3.5	
	4					+4
Progesterone, s.c.	3				+3.3	
	3					+4
Medrogestone, s.c.	6	+0.5				
	4		+1.9			
	4			+3.6		
MAP, s.c.	6	+3.6				
	5		+3.8			
	5			+3.5		

* All doses in mg.

RESULTS

Progestational activity

The progestational activity of a daily oral dose of 0.01 to 0.1 mg of Medrogestone and MAP were equal as shown in Table 1. With subcutaneous administration, Medrogestone was approximately four times as potent as progesterone but less potent than MAP.

Duration of action of progestational activity

As shown in Table 2, when 10 mg of Medrogestone was given there was activity 17 days after a single subcutaneous dose. The activity found at that time was similar to that found with the same dose of 17-hydroxyprogesterone caproate but less than with MAP. Progesterone did not exhibit long-acting properties at the 10 mg dose level.

Local progestational activity

At 10 µg dose level Medrogestone showed a marked local progestational effect similar to progesterone and MAP.

Deciduoma test

The results in Table 3 indicate that a daily dose of 1 mg of Medrogestone given subcutaneously for 9 days had a stronger deciduomagenic effect than progesterone at 1 mg or MAP at 2 mg dose.

Maintenance of pregnancy in rats

Multiple doses of Medrogestone maintained pregnancy in ovariectomized rats. Better results were produced with 5 mg Medrogestone subcutaneously

TABLE 2
DURATION OF ACTION OF PROGESTATIONAL ACTIVITY

Compound	Single dose (mg/rabbit)	Route	Mean McPhail's score				
			3*	7	10	13	17
Progesterone	10.0	s.c.	+3	+2	+1	+1	
17 α -Hydroxyprogesterone caproate	10.0	s.c.	+4	+4	+2.1	+1	+2.3
Medrogestone	10.0	s.c.	+4	+4	+2.6	+2	+2
MAP	10.0	s.c.		+4	+4	+3	+3

* Days after single dose.

TABLE 3
DECIDUOMA TEST IN RATS

Compound	No. of rats	Daily dose (mg/rat)	Weight of the uterine horn (mg)	
			Right (control)	Left (treated)
Sesame oil	10	0.2 ml	69.0 $\pm$ 7.0	97.0 $\pm$ 7.0
Progesterone	8	0.5	57.0 $\pm$ 1.9	90.0 $\pm$ 9.4
	8	0.8	62.0 $\pm$ 2.7	191.0 $\pm$ 28.3
	10	1.0	63.0 $\pm$ 4.0	183.0 $\pm$ 45.0
	10	2.0	81.0 $\pm$ 5.0	351.0 $\pm$ 7.0
	9	1.0	65.0 $\pm$ 5.0	924.0 $\pm$ 112.0
Medrogestone	9	1.0	65.0 $\pm$ 5.0	924.0 $\pm$ 112.0
MAP	10	2.0	90.0 $\pm$ 1.9	465.0 $\pm$ 70.0

than with the same dose of progesterone (Table 4). When 10 mg was given orally, Medrogestone was slightly more active than MAP.

Single subcutaneous doses of 5 to 10 mg of Medrogestone and MAP given at the time of ovariectomy were equally effective in maintaining pregnancy (Table 5). Progesterone showed the same effect but only at extremely high doses.

Ovulation inhibition

Medrogestone was effective in inhibiting ovulation in rats. The subcutaneous ED₅₀ of Medrogestone alone was found to be 1.5 mg (Table 6). The corresponding dose of norethynodrel was 3.1 mg. The addition of 17 α -ethinyl oestradiol-3-methylether (mestranol) to norethynodrel decreased the ED₅₀ with subcutaneous administration to 0.25 mg. When given orally norethynodrel was more

effective than Medrogestone, the respective ED_{50} doses being 2.8 and 4.8 mg. However, the addition of 10 μ g of Premarin decreased the oral ED_{50} of Medrogestone to 2.1 mg and 20 μ g of Premarin decreased the ED_{50} of Medrogestone to 1.2 mg.

TABLE 4
MAINTENANCE OF PREGNANCY TEST IN OVARIECTOMIZED RATS WITH MULTIPLE DOSES

Compound	Daily dose (mg/rat) and route	No. of spayed pregnant rats	No. of rats holding pregnancy to term with surviving foetuses	No. of implantations on the day of spaying	No. of live foetuses at autopsy (21 days)	No. of dead foetuses at autopsy (21 days)	% of surviving foetuses
Sesame oil	0.2 ml s.c.	5	—	46	—	—	—
Progesterone	5.0 s.c.	4	1	42	7	2	17
	10.0 s.c.	6	5	75	20	18	67
Medrogestone	1.0 s.c.	3	3	29	17	5	59
	2.0 s.c.	9	6	84	46	7	54.7
	5.0 s.c.	4	4	40	40	—	100
	10.0 oral	10	5	124	32	—	25.8
MAP	10.0 oral	18	4	208	30	—	14.4

TABLE 5
MAINTENANCE OF PREGNANCY TEST IN OVARIECTOMIZED RATS WITH A SINGLE INJECTION

Compound	Single dose s.c. (mg/rat)	No. of treated pregnant rats	No. of live births
			No. of implantations at ovariectomy $\times 100$
Controls			
Sesame oil	0.2 ml	2	0
Progesterone	50.0	3	0
	100.0	4	48
	150.0	7	52
	200.0	7	76
Medrogestone	5.0	4	25.6
	7.5	6	22.7
	10.0	8	60.8
	12.0	5	84.3
MAP	5.0	4	20.4
	7.5	4	12.2
	10.0	8	80

Prevention of pregnancy

Medrogestone at daily subcutaneous doses of 1.0 and 0.5 mg prevented pregnancy in all the animals, while 0.25 mg daily prevented pregnancy in 80% of the animals. The combination of 0.25 mg Medrogestone with 4 μ g Premarin prevented pregnancy in 100%. Premarin alone did not prevent pregnancy in the rat at this dose level.

Gonadotrophin suppressant activity

A combination of 2 and 5 mg of Medrogestone and 1 μ g Premarin given orally suppressed ovarian weight increase by nearly 100%. Premarin alone, at

this dose level had no effect (Table 7). Subcutaneously, at the dose levels tested, the addition of Premarin did not increase the effectiveness of Medrogestone. Medrogestone alone, either s.c. or orally, had little gonadotrophin suppressant activity.

TABLE 6
OVULATION INHIBITION IN RATS

Compounds	Dose	No. of rats	Mean No. of ova/rat	ED ₅₀
Sesame oil Norethynodrel	0.1 ml s.c.	7	54.2 ± 2.2	} 3.1 mg
	0.5 mg s.c.	5	44.6 ± 2.7	
	1.0 mg s.c.	5	35.4 ± 2.7	
	2.0 mg s.c.	5	29.4 ± 4.6	
	4.0 mg s.c.	5	25.6 ± 2.5	
Sesame oil Enovid	0.1 ml s.c.	12	53.9 ± 1.6	} 0.25 mg
	0.125 mg s.c.	5	39.4 ± 1.9	
	0.25 mg s.c.	5	26.2 ± 2.3	
	0.5 mg s.c.	5	15.2 ± 2.5	
	1.0 mg s.c.	5	13.6 ± 2.3	
	2.0 mg s.c.	5	10.0 ± 1.2	
Sesame oil Enovid	0.5 ml oral	5	50.2 ± 4.2	} 2.8 mg
	1.0 mg oral	5	39.6 ± 5.0	
	2.0 mg oral	5	29.4 ± 5.0	
	4.0 mg oral	5	22.0 ± 0.8	
Sesame oil Medrogestone	0.2 ml s.c.	21	42.3 ± 0.8	} 1.5 mg
	0.63 mg s.c.	17	30.5 ± 2.0	
	1.25 mg s.c.	17	22.8 ± 1.8	
	2.5 mg s.c.	17	14.7 ± 1.4	
Sesame oil Medrogestone	0.2 ml oral	20	42.0 ± 0.9	} 4.1 mg
	1.25 mg oral	17	34.0 ± 2.6	
	2.5 mg oral	17	26.7 ± 2.2	
	5.0 mg oral	17	18.3 ± 1.3	
	10.0 mg oral	5	11.8 ± 1.9	
Sesame oil Premarin Medrogestone + Premarin (10 µg)	0.2 ml oral	5	44.3 ± 3.8	} 2.1 mg
	10.0 mg oral	5	39.2 ± 3.1	
	1.25 mg oral	5	27.2 ± 3.8	
	2.5 mg oral	5	20.4 ± 2.1	
	5.0 mg oral	5	12.4 ± 2.7	
Sesame oil Premarin Medrogestone + Premarin (20 µg)	0.2 ml oral	5	47.0 ± 3.3	} 1.2 mg
	20.0 µg oral	5	38.2 ± 1.7	
	0.32 mg oral	5	35.2 ± 1.7	
	0.63 mg oral	5	28.6 ± 3.9	
	1.25 mg oral	5	22.2 ± 1.7	
	2.5 mg oral	5	15.4 ± 2.5	
	5.0 mg oral	5	11.6 ± 1.4	

Androgenic and anabolic activity in immature male rats

The results presented in Table 8 indicate that Medrogestone in daily s.c. or oral doses up to 5.0 mg did not produce any androgenic effect. The increase in prostate weight produced by progesterone was not found after treatment with Medrogestone. MAP given subcutaneously at daily doses of 0.5 and 2.0 mg showed marked androgenicity.

Anti-androgenic test

In this test the ability of the test compounds to counteract the effect of 0.03 mg of testosterone propionate (T.P.) given concomitantly was determined. Table 9 summarizes the results for progesterone, Medrogestone and MAP. Progesterone at a dose level of 25 mg exhibited anti-androgenic properties.

TABLE 7
GONADOTROPHIN SUPPRESSANT ACTIVITY

Compound	Daily dose	No. of pairs	Spayed uterus weight (mg)	Intact uterus weight (mg)	Ovaries weight (mg)	Ovarian inhibition (%)
Intact controls (sesame oil)	0.2 ml s.c.	4	—	135.6 ± 33.8	32.5 ± 4.6	—
Castrated controls (sesame oil)	0.2 ml s.c.	5	71.1 ± 9.2	206.7 ± 11.8	141.1 ± 22.5	0
Premarin	0.01 µg s.c.	5	56 ± 3.6	179.6 ± 14.4	103 ± 43.0	35
Premarin	0.2 µg s.c.	4	50.1 ± 4.2	87 ± 17.8	21.5 ± 3.6	108
Medrogestone	1.0 mg s.c.	4	59.2 ± 2.8	131 ± 19.2	82.5 ± 21.8	54
Medrogestone	2.0 mg s.c.	4	66 ± 11.0	157.7 ± 14.6	88 ± 25.0	51
Medrogestone + Premarin	1.0 mg s.c. 0.01 µg s.c.	5	69.2 ± 4.2	254.2 ± 32.5	118 ± 10.1	21
Medrogestone + Premarin	2.0 mg s.c. 0.01 µg s.c.	5	52.4 ± 5.5	147 ± 22.8	75 ± 13.9	61
Medrogestone + Premarin	2.0 mg s.c. 0.2 µg s.c.	5	79.8 ± 5.3	104.4 ± 19.8	14.4 ± 1.4	114
Premarin	1 µg oral	3	72 ± 2.8	182 ± 12.8	144 ± 30.4	0
Medrogestone	2.0 mg oral	4	53 ± 6.2	174 ± 24.4	120.5 ± 15.6	19
Medrogestone	5.0 mg oral	4	63 ± 10.4	197 ± 22.0	126 ± 24.4	14
Medrogestone + Premarin	2.0 mg oral 1 µg oral	4	73 ± 16.2	82 ± 19.8	40 ± 13.0	93
Medrogestone + Premarin	5.0 mg oral 1 µg oral	4	79 ± 3.4	163 ± 3.8	49.5 ± 7.4	84

Medrogestone had marked anti-androgenic effects at daily s.c. doses of 0.5, 2.0 and 5.0 mg. The anti-androgenic effect was slightly less pronounced when Medrogestone was given orally at dose levels of 2.0 and 5.0 mg. MAP showed only slight anti-androgenic effects with a daily s.c. dose of 2.0 mg only on the prostatic weight.

Table 10 shows the results in intact animals. At daily doses of 2.0 and 5.0 mg given subcutaneously there was a significant decrease in the weight of the ventral prostate, seminal vesicles and levator ani.

Effect on developing foetuses

In female offspring of mothers given daily subcutaneous doses up to 10 mg of Medrogestone during pregnancy no changes were observed at birth in the anogenital distance nor was any masculinizing effect detectable at 20 days of

age. In contrast, the anogenital distance was increased at birth and the external and internal genitalia were masculinized at 20 days of age in female rats from mothers treated daily during pregnancy with subcutaneous doses of 0.5 mg of MAP. No masculinization occurred at daily subcutaneous doses up to 100 mg of progesterone.

In male offspring of mothers given up to 10 mg of Medrogestone during pregnancy no change in the anogenital distance was observed at birth. However, as seen in Table 11, hypospadias and cryptorchidism were observed in male offspring at 20 days of age. This anti-masculinizing effect was observed when 10 and 20 mg daily doses were given to the mother, while at a 5 mg-dose level (25 mg/kg) the males of only one litter out of seven were affected. At the 2.5 mg-dose levels of Medrogestone no changes were observed.

TABLE 8
ANDROGENIC AND ANABOLIC ACTIVITY

Compound	No. of rats	Daily dose (mg/rat)	Ventral prostate (mg)	Seminal vesicles (mg)	Levator ani (mg)	Average body weight increase (g)
Sesame oil	165	0.2 ml s.c.	8.5 ± 0.7	9.8 ± 0.1	17.1 ± 1.0	27.1
Testosterone	243	0.03 s.c.	43.9 ± 1.9	50.3 ± 2.6	24.4 ± 1.3	28.9
propionate	12	0.5 s.c.	105.0 ± 2.6	175.5 ± 5.0	46.4 ± 1.2	30.0
Progesterone	12	2.0 s.c.	35.3 ± 2.1	17.0 ± 0.9	15.2 ± 0.6	27.6
	10	5.0 s.c.	29.9 ± 2.4	13.2 ± 0.6	14.8 ± 1.3	27.0
	10	25.0 s.c.	28.8 ± 2.8	14.9 ± 1.8	14.0 ± 1.6	24.0
Medrogestone	10	2.0 s.c.	8.4 ± 0.5	10.0 ± 0.7	15.0 ± 2.8	21.7
	9	5.0 s.c.	11.3 ± 0.9	12.7 ± 0.8	18.7 ± 0.8	31.7
	10	2.0 oral	10.3 ± 0.5	10.7 ± 0.5	15.2 ± 0.7	22.1
	10	5.0 oral	10.6 ± 0.4	11.5 ± 0.3	16.5 ± 0.8	27.9
MAP	12	0.5 s.c.	18.4 ± 1.0	20.8 ± 0.8	22.6 ± 0.6	30.0
	12	2.0 s.c.	40.0 ± 1.2	28.1 ± 0.7	24.4 ± 0.6	36.0

Oestrogenic activity

Medrogestone showed no oestrogenic effect at doses of 5 mg. The ED₅₀ of oestrone was 1.6 µg in our test.

Effect on endocrine glands

No change in organ weight or histological appearance occurred in ovariectomized rats given 2 mg of Medrogestone s.c. (Table 12). Progesterone decreased the adrenal and thymus weights at extremely high doses, while MAP produced the same changes at low doses. The histological changes in the adrenals found with MAP were the same as those described by Logothetopoulos, Sharma & Kraicer (1961).

Effect on water and electrolyte metabolism

In saline-loaded adrenalectomized animals, Medrogestone in s.c. doses of 2.0 and 3.0 mg did not produce definite changes in water and electrolyte excretion. At a dose of 4.0 mg, however, it showed some tendency to reduce

urine volume and electrolyte output. In intact rats, repeated daily injections of 1.0 mg of Medrogestone caused a small but significant increase in the total sodium and potassium excretion.

TABLE 9
ANTI-ANDROGENIC TEST

Compound	No. of rats	Dose (mg/day)	Ventral prostate (mg)	Seminal vesicles (mg)	Levator ani (mg)	Body weight increase (g)
Sesame oil	165	0.2 ml s.c.	8.5 ± 0.7	9.8 ± 0.7	17.1 ± 1.0	27.1
T.P.	243	0.03 s.c.	43.9 ± 1.9	50.3 ± 2.6	24.4 ± 1.3	28.9
T.P. + Progesterone	10	0.03 s.c. 2.0 s.c.	50.8 ± 2.7	44.6 ± 1.7	21.3 ± 0.8	32.0
T.P. + Progesterone	10	0.03 s.c. 5.0 s.c.	49.0 ± 2.1	39.7 ± 1.3	17.9 ± 0.8	31.0
T.P. + Progesterone	10	0.03 s.c. 25.0 s.c.	29.0 ± 1.2	16.7 ± 1.2	14.7 ± 1.8	27.0
T.P. + Medrogestone	10	0.03 s.c. 2.0 s.c.	20.8 ± 2.3	14.8 ± 1.0	20.3 ± 1.6	33.6
T.P. + Medrogestone	10	0.03 s.c. 5.0 s.c.	17.4 ± 1.0	12.3 ± 0.4	19.3 ± 1.3	32.1
Controls	7	0.2 ml oral	7.8 ± 0.4	9.6 ± 0.7	12.5 ± 1.6	21.3
T.P. + Medrogestone	9	0.03 s.c. 2.0 oral	26.9 ± 2.4	21.9 ± 1.4	15.6 ± 1.5	27.3
T.P. + Medrogestone	6	0.03 s.c. 5.0 oral	19.6 ± 1.2	16.2 ± 1.0	13.8 ± 1.0	25.1
T.P. + MAP	10	0.03 s.c. 0.5 s.c.	42.9 ± 2.2	53.0 ± 2.1	31.4 ± 1.0	31.0
T.P. + MAP	10	0.03 s.c. 2.0 s.c.	34.8 ± 1.6	47.9 ± 1.7	31.0 ± 2.2	26.0

TABLE 10
EFFECT OF 7 DAYS' TREATMENT IN INTACT MALE RATS

Compound	Dose s.c. (mg/day)	No. of rats	Ventral prostate (mg)	Seminal vesicles (mg)	Levator ani (mg)	Body weight increase (g)
Sesame oil	0.2 ml	5	91.2 ± 6.4	84.4 ± 8.0	68.4 ± 6.2	32.0
Medrogestone	2.0	5	61.0 ± 6.0	41.6 ± 3.7	34.8 ± 3.4	28.4
	5.0	5	59.9 ± 8.0	33.8 ± 2.6	42.1 ± 4.0	26.6

In adrenalectomized saline-loaded rats given *dl*-aldosterone acetate, Medrogestone showed some anti-aldosterone properties at the 2.0 mg dose level.

Glycogen deposition test

Medrogestone did not increase liver glycogenesis at total doses of 1.0 and 2.0 mg s.c.

Anti-inflammatory properties

Cotton pellet granuloma test. In this test Medrogestone did not exhibit anti-inflammatory or thymolytic properties at doses of 0.5, 1.0 and 2.0 mg/pellet.

Croton oil granuloma pouch assay. The capacity of Medrogestone to inhibit the

TABLE 11
EFFECT OF MEDROGESTONE ON OFFSPRING WHEN ADMINISTERED DURING PREGNANCY

No. of litters	Daily dose s.c. (mg/rat)	Normal females	No. of males			
			Normal	Hypospadias	Cryptorchidism	Hypospadias and cryptorchidism
1	20.0	3	4	—	—	—
1	20.0	4	6	—	—	—
1	20.0	4	1	4	—	2
1	20.0	7	1	1	—	—
1	20.0	4	—	5	—	—
1	20.0	3	—	7	1	—
1	20.0	6	1	—	—	—
1	20.0	6	1	3	—	2
8		37	14	20	1	4
1	10.0	6	2	2	2	1
1	10.0	6	3	1	—	—
1	10.0	3	—	5	—	—
1	10.0	2	2	3	—	—
1	10.0	2	2	6	—	—
1	10.0	6	3	1	—	—
1	10.0	7	3	1	—	—
1	10.0	5	3	4	—	—
8		37	18	23	2	1
1	5.0	6	6	—	—	—
1	5.0	7	6	—	—	—
1	5.0	12	7	—	—	—
1	5.0	4	2	—	—	—
1	5.0	1	2	—	—	—
1	5.0	4	2	—	—	—
1	5.0	7	1	5	—	—
7		41	26	5	—	—
1	2.5	3	7	—	—	—
1	2.5	9	5	—	—	—
1	2.5	8	4	—	—	—
1	2.5	7	6	—	—	—
1	2.5	5	7	—	—	—
1	2.5	6	2	—	—	—
6		38	31	—	—	—

formation of the inflammatory exudate in this test was very low when compared with MAP or hydrocortisone.

DISCUSSION

The results of these tests suggests that Medrogestone has a number of unique properties which have been confirmed in the clinic. Medrogestone meets the requirements for an orally active, purely progestational agent. It is active in

the Clauberg and McGinty tests, has a prolonged effect when given subcutaneously and is able to maintain pregnancy. In all these tests it compares favourably with MAP. However, in terms of secondary effects, MAP and Medrogestone are quite different. MAP has androgenic effect in castrated male rats and causes masculinization of female foetuses, whereas Medrogestone is completely negative in these tests. The masculinizing effect of MAP is shown when 0.5 mg is given daily to pregnant rats. This androgenic effect has only been demonstrated, however, in experimental animals and has not been reported in man. Medrogestone is not androgenic, but has a marked anti-androgenic activity in the

TABLE 12
EFFECT ON ENDOCRINE GLANDS

Compound	Daily dose	Route	No. of rats	Body weight (g)		Adrenals (mg)	Thymus (mg)	Thyroids (mg)	Pituitary (mg)
				Initial	Final				
Control—Sesame oil	0.2 ml	s.c.	8	97.0 ± 3.6	144.0 ± 8	40.7 ± 2.5	508.0 ± 40	12.2 ± 0.5	11.0 ± 0.4
Medrogestone	0.5 mg	s.c.	8	102.0 ± 2.3	168.0 ± 3	39.5 ± 1.7	419.0 ± 24	12.4 ± 0.6	10.5 ± 0.4
	1.0 mg	s.c.	8	100.0 ± 2.4	175.0 ± 5	38.3 ± 2.5	470.0 ± 41	13.5 ± 6.7	11.0 ± 0.6
	2.0 mg	s.c.	8	100.0 ± 4.3	173.0 ± 3.7	38.0 ± 2.0	414.0 ± 38	12.4 ± 0.6	10.8 ± 0.4
Progesterone	1.0 mg	s.c.	10	89.0 ± 2.3	157.0 ± 3	44.0 ± 3	557.0 ± 38	13.3 ± 0.4	10.3 ± 0.3
	15.0 mg	s.c.	10	86.0 ± 2	162.0 ± 4	24.3 ± 2	486.0 ± 38	13.8 ± 0.6	11.2 ± 0.5
MAP	0.5 mg	s.c.	10	106.0 ± 2	172.0 ± 4	20.0 ± 1.4	319.0 ± 19	12.5 ± 11	9.8 ± 0.5
	1.0 mg	s.c.	10	81.2 ± 1.6	126.0 ± 4.3	13.8 ± 3	266.3 ± 24	12.0 ± 0.7	8.7 ± 0.5

castrated male rat test. This property is evident in its effect on male offspring when high doses (above 5 mg daily) are given to the mother during pregnancy. At a 5 mg daily dose it caused feminization of the male offspring in only one of seven litters.

This anti-androgenic effect might prove clinically useful in cases where a suppression of increased endogenous androgens would be advantageous, i.e. benign prostatic hypertrophy, Stein-Leventhal syndrome, hirsutism, etc. MAP has a strong depressant effect on the adrenal gland, causing adrenal atrophy in ovariectomized female rats. In contrast, Medrogestone did not cause adrenal atrophy at the same dose levels.

Clinical experience with MAP is relatively recent, considering the time required to accumulate clinical evidence in the endocrine field. It is, therefore, difficult to assess with certainty the importance of the secondary effects of this compound. However, from a pharmacological view-point the freedom from secondary effects in Medrogestone-treated animals offers definite advantages.

ACKNOWLEDGMENTS

The authors wish to thank Dr J. Guy Rochefort for the anti-inflammatory tests, and Mrs Marie Wehner and Mr A. Legault for their technical assistance.

REFERENCES

- ALLEN, E. & DOISY, E. A. (1923) An ovarian hormone; preliminary report of its localization, extraction and partial purification, and action in test animals. *J. Am. med. Assoc.* **81**, 819.
- BIDDULPH, C., MEYER, R. K. & GUMBRECK, L. G. (1940) The influence of estriol, estradiol and progesterone on the secretion of gonadotrophic hormones in parabiotic rats. *Endocrinology*, **26**, 280.
- BUNSTER, E. & MEYER, R. K. (1933) An improved method of parabiosis. *Anat. Rec.* **57**, 339.
- BUSH, T. E. & ALEXANDER, R. W. (1960) An improved method for the assay of anti-inflammatory substances in rats. *Acta endocr., Copenh.* **35**, 268.
- CARTER, W. F., FAUCHER, G. L. & GREENBLATT, R. B. (1964) Evaluation of a new progestational agent, 6,17 α -dimethyl-6-dehydropregesterone. *Am. J. Obstet. Gynec.* **89**, 635.
- DEGHENGI, R. & GAUDRY, R. (1961) Sterically controlled Grignard reactions. A new simple route to 17 α -methylated steroid analogs. *J. Am. chem. Soc.* **83**, 4668.
- DEGHENGI, R., REVESZ, C. & GAUDRY, R. (1963) New synthesis and structure activity relationship in the 17-alkylated progesterone series. *J. med. Chem.* **6**, 301.
- ELTON, R. L. & EDREN, R. A. (1958) Biological actions of 17 α -(2-methyl)-19-nortestosterone, an orally active progestational agent. *Endocrinology*, **63**, 464.
- HAMADA, H., NEUMANN, F. & JUNKMANN, K. (1963) Intrauterine antimaskuline Beeinflussung von Rattenfeteten durch ein stark gestagen wirksames Steroid. *Acta endocr., Copenh.*, **44**, 380.
- HERSCHBERGER, L. G., SHIPLEY, E. G. & MEYER, R. K. (1953) Myotrophic activity of 19-nortestosterone and other steroids determined by modified levator ani muscle method. *Proc. Soc. exp. Biol. Med.* **83**, 175.
- JUNKMANN, K. (1954) Über protrohhierbarkeit wirksame gestagene. *Arch. exp. Path. Pharmac.* **223**, 244.
- LENER, L. J., BIANCHI, A. & BORMAN, A. (1960) Anorprogesterone, an androgen antagonist. *Proc. Soc. exp. Biol. Med.* **103**, 172.
- LLAURADO, J. G. (1961) The effects of some 21-methyl-substituted corticoids on inflammation, liver glycogen and electrolyte-regulating activity in the rat. *Acta endocr., Copenh.* **38**, 137.
- LOGOTHETOPOULOS, J., SHARMA, B. B. & KRAICER, J. (1961) Effects produced in rats by the administration of 6 α -methyl-17 α -hydroxypregesterone acetate from birth to maturity. *Endocrinology*, **68**, 417.
- MADJEREK, Z., DE VISSER, J., VAN DER VIES, J. & OVERBEEK, G. A. (1960) Allylestrenol, a pregnancy maintaining oral gestagen. *Acta endocr., Copenh.* **35**, 8.
- MCGINTY, D. A., ANDERSON, L. P. & MCCULLOUGH, N. B. (1939) Effect of local application of progesterone on the rabbit uterus. *Endocrinology*, **24**, 829.
- MCPHAIL, M. K. (1934) The assay of progestin. *J. Physiol.* **83**, 145.
- MIYAKE, T. (1961). Inhibitory effect of various steroids on gonadotrophin hypersecretion in parabiotic mice. *Endocrinology*, **69**, 534.
- OLSEN, R. E., THAYER, S. A. & KOPP, L. J. (1944) The glycogenic activity of certain crystalline steroids on the adrenal cortex when administered singly and with corticol extract to fasted, normal and adrenalectomized rats. *Endocrinology*, **35**, 464.
- REVESZ, C., CHAPPEL, C. I. & GAUDRY, R. (1960) Masculinization of female fetuses in the rat by progestational compounds. *Endocrinology*, **66**, 140.
- ROBERT, A. & NEZAMIS, J. E. (1957) The granuloma pouch as a routine assay for antiphlogistic compounds. *Acta endocr., Copenh.* **25**, 105.
- ROWLANDS, I. W. (1942) Collection of eggs from the fallopian tube of a rat. *Nature, Lond.* **150**, 267.
- SELYE, H. (1953) The diseases of adaptation. Introductory remarks. *Recent Prog. Horm. Res.* **8**, 117.
- WILSON, E. D. & ZARROW, M. X. (1962) Comparison of superovulation in the immature mouse and rat. *J. Reprod. Fert.* **3**, 148.