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By

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It is well established that administration of progesterone causes a significant increase in body weight in female rats (3, 4, 9, 17, 20, 21, 35) and mice (6, 8), which resembles the increase in maternal weight during pregnancy or pseudopregnancy (7, 18, 26).

In the course of experiments on synchronization of oestrus in this laboratory it has been observed that: i) in beef cattle a considerable stimulation of weight gain followed treatment with chlormadinone acetate (CAP) (39); ii) in rats body weight gain appeared to be greater after intramuscular treatment with CAP than in previous control periods (36). Increased weight gain in rats treated with CAP has also been reported by Hervey & Hervey (18) and the authors (37)*.

The following experiments were therefore undertaken to ascertain the effects of treatment with CAP, by various forms and routes, on body weight gain in female rats. The weight gains of rats injected with CAP have principally been compared with those of control and progesterone-treated rats; in some experiments pseudopregnant and oral CAP-treated rats were also included.

Materials and Methods

Adult, nulliparous (multiparous in experiment V) Wistar rats bred in our laboratory were used. They were fed a pellet diet, "Bulet B" (Zenkoren), intended for piglets, with a crude protein content of 20%. This was given *ad lib.* and supplemented with fresh green vegetables once a week. Vaginal smears were examined every day at 9–10 a.m., from about three cycles before the start to the end of each experiment; only rats with regular 4-day cycles were used.

* A part of the present report (Experiment IV) has been submitted to the Journal of Endocrinology as a short communication (37). The data are contained in this report in detail on approval of the editor of the Journal so as to compare with the results of our other experiments and to discuss altogether.

Table 1. Treatment groups in each experiment.

Experiment number (medium or form)	Treated control	Non-treated control	Orally treated control	CAP			Progesterone 5mg×10(s.c.)	Pseudo-pregnancy
				5mg×10(s.c.)	Oral 10mg×10	Spayed female 5mg×10		
I:propylene glycol	+			+			+	
II:propylene glycol	+		+	+	+		+	
III:sesame oil	+			+	+		+	+
IV:suspension	+	+		+			+	
V-1:micro-suspension	+	+		(5mg×5) + (25mg×1) +		(5mg×5) +	(5mg×5) +	
V-2:micro-suspension	+			+			+	

Treatment was begun at a random time in the oestrous cycle.

The groups included in each experiment are summarized in table 1. "Treated control" rats received daily injections of the vehicle. Nontreated controls were also included in experiments IV and V-1. In experiments I-III chlormadinone acetate (CAP, 17α-acetoxy-6-chloropregna-4,6-diene-3,20-dione, Teikoku Hormone Mfg. Co., Tokyo) and progesterone (Teikoku Hormone Mfg. Co. and Iwaki Chem. Co.) were given as 10 mg/ml solutions in propylene glycol or sesame oil, the steroids being first dissolved in a minimal volume of acetone. Some of the CAP crystallized out of this solution after a time, and the preparation had to be warmed to about 60°C to obtain a clear solution before injection. In experiments IV and V, CAP and progesterone were given as 10 mg/ml suspensions; in experiment IV this was made by a Potter homogenizer in 20% Tween 80 (v/v), and in experiment V a microsuspension was supplied by Teikoku Hormone Mfg. Co.. The size of particle in the former suspension was not measured but as small as the particle could be passed through the needle-gauge (No. 23) of syringe. That of the latter was in average 30 (5–50)μ in diameter.

Doses of 5 mg of the steroids were injected subcutaneously in the abdominal region daily for 10 days. The needle was passed through the peritoneum and then back into the subcutaneous tissue, in order to prevent loss by external leakage, having in mind the rather large volume of the injections. For oral treatment 10 mg CAP was given by stomach tube. Pseudopregnancy was induced by electrical stimulation of the cervix uteri on two days of pro-oestrus and oestrus (34). In the spayed rats ovariectomy had been perfomed about two months earlier.

Weighing was begun 8 or 10 days before the start of injections, and was carried out on every 5th day from the start (day 0) until day 30. In experiments IV and

V-2 weighing was continued to day 70 and day 50 respectively on every 10th day after day 30. In experiments IV and V each reading was in fact the mean of two weighings on consecutive days (the nominal and the previous day) to minimize random fluctuations.

Comparisons of the changes in body weight found were made by analysis of variance or by "t" test, or in some instances by Duncan's multiple range test. The gains during individual periods were compared as well as the gains over whole experiments. In addition in experiment IV the regression coefficients of weight on time over days 0–20 were calculated and compared.

Results

A. *Change in body weight*

a) *Experiment I.*

As shown in figure 1, there were significant differences between both steroid-treated groups and the control group. Since the patterns of increase in body weight on CAP and on progesterone appeared different, the increases in the various periods were individually compared; the results are also shown in table 2.

The increase caused by progesterone occurred early on (day 0 to 15), whereas the effect of CAP appeared rather late but lasted longer (days 0 to 20 or 25). In

Table 2. Changes of body weight in rats treated (s.c.) with chlormadinone acetate and progesterone in propylene glycol (Experiment I).

	−8	0	5	10	15	20	25	30
Treated cont. (10[a])	186±3.5[b]	196±4.1	196±4.8	197±4.9	205±5.3	213±5.3	215±5.5	221±4.8
CAP 5mg×10 (7)	183±5.5	197±4.7	204±4.3	211±3.9	228±4.1	239±4.5	243±3.6	243±4.5
Progesterone 5mg×10 (10)	180±3.7	196±5.1	210±4.9	220±6.4	233±5.0	213±6.3	239±6.6	246±7.5

Mean of gain during various periods.

	−8~0	0~10	10~20	20~30	0~15	10~25	0~20	10~30	0~30
i) Treated cont. (10)	10	2	16	8	9	17	18	24	26
ii) CAP 5mg×10 (7)	15	13	28	4	31	33	41	32	45
iii) Progesterone 5mg×10 (10)	16	24	11	15	37	19	35	26	50

Significance of the differences (*, $P<0.05$; **, $P<0.01$).[c]

i~iii	–	**	**	*	**	**	**	–	**
ii vs. iii	–	*	**	*	–	*	–	–	–

a) Number in bracket indicates rats used.
b) Mean±S.E.
c) Tested by the analysis of variance or t-test: i~iii means the comparison of 3 groups, and ii vs. iii, that of 2 groups.

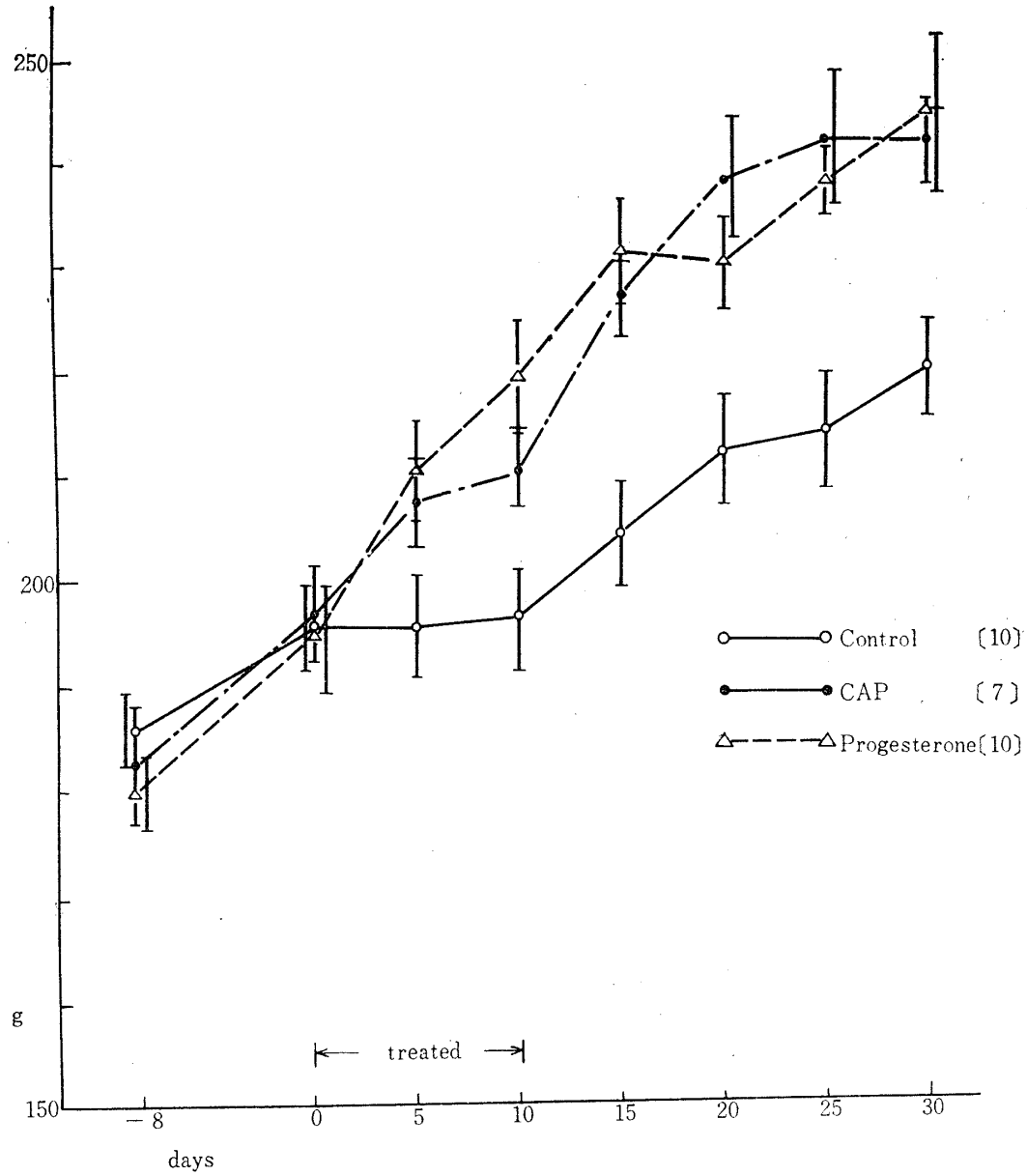

Fig. 1. Changes of body weight in rats injected (s.c.) with chlormadinone acetate and progesterone in propylene glycol (Experiment I).
Bars indicate S.E. of Mean, and at earlier dates indicate from left to right, control, CAP and progesterone, respectively.
Figures in bracket mean the number of rats used.

the CAP-treated group weight gain during the first 10 days seemed to be significantly less than on progesterone ($P<0.05$). The gains in weight by days 20 and 30 were not different between the two groups. At 30 days the progesterone group, whose weight had levelled off, appeared to increase again.

The control rats showed a marked inhibition of growth during the period of treatment.

b) *Experiment II.*

The results of this experiment are shown in table 3 and figure 2. Smaller rats than the previous experiment were used, and groups were included which received oral CAP treatment, and oral vehicle. No significant difference was found between the group given CAP orally and its control group. The two control groups, treated subcutaneously and orally with the vehicle, showed significantly different weight gains only for the periods 0–15 and 20–30 days ($P<0.05$).

CAP and progesterone-treated groups were compared with both the injected control group and the orally treated control group, (the latter was regarded as in

Table 3. Changes of body weight in rats treated (s.e.) with chlormadinone acetate and progesterone in propylene glycol (Experiment II).

	−10	0	5	10	15	20	25	30
s.c.;								
Treated control (7) [a]	161±2.6 [b]	187±3.0	192±4.1	198±3.6	206±3.2	216±3.0	228±2.9	232±3.4
CAP 5mg×10 (7)	164±2.3	189±3.3	192±5.0	201±4.5	214±5.1	231±6.0	242±5.1	248±5.8
Progesterone 5mg×10 (5)	168±3.0	190±5.1	200±4.3	213±4.0	222±3.3	227±3.6	238±4.9	247±4.3
oral;								
Control (6)	164±3.4	192±3.6	199±3.1	207±3.2	218±2.9	223±2.2	237±2.7	246±2.4
CAP 10mg×10 (5)	158±2.2	185±2.3	192±4.9	203±6.4	212±3.3	221±5.0	232±5.2	240±4.1

Mean of gain during various periods.

Groups	−10∼0	0∼10	10∼20	20∼30	0∼15	10∼25	0∼20	10∼30	0∼30
subcutaneous									
i) Treated cont. (7)	26	11	18	16	19	30	29	34	45
ii) CAP 5mg×10 (7)	25	12	31	17	25	42	43	47	59
iii) Progesterone 5mg×10 (5)	23	22	14	20	32	25	37	34	57
oral									
iv) Control (6)	28	15	17	22	26	30	32	39	54
v) CAP 10mg×10(5)	27	18	18	19	26	29	36	37	54

Significance of the difference (*, $P<0.05$; **, $P<0.01$) [c].

i∼v	−	− [d]	**	−	−	* [d]	*	**	−
i∼iv	−	*	**	−	−	− [d]	*	−	−
i∼iii	−	**	**	−	*	**	**	*	*
ii∼iv	−	*	**	−	−	**	−	−	−
i vs. iv	−	−	−	*	*	−	−	−	−
ii vs. iii	−	*	**	−	−	*	*	−	−
ii vs. iv	−	−	**	*	−	*	−	−	−

a) Number in brackets indicates rats used.
b) Mean±S.E.
c) See the footnote of Table 2.
d) Difference approaches significant at a higher level (−→*, *→**).

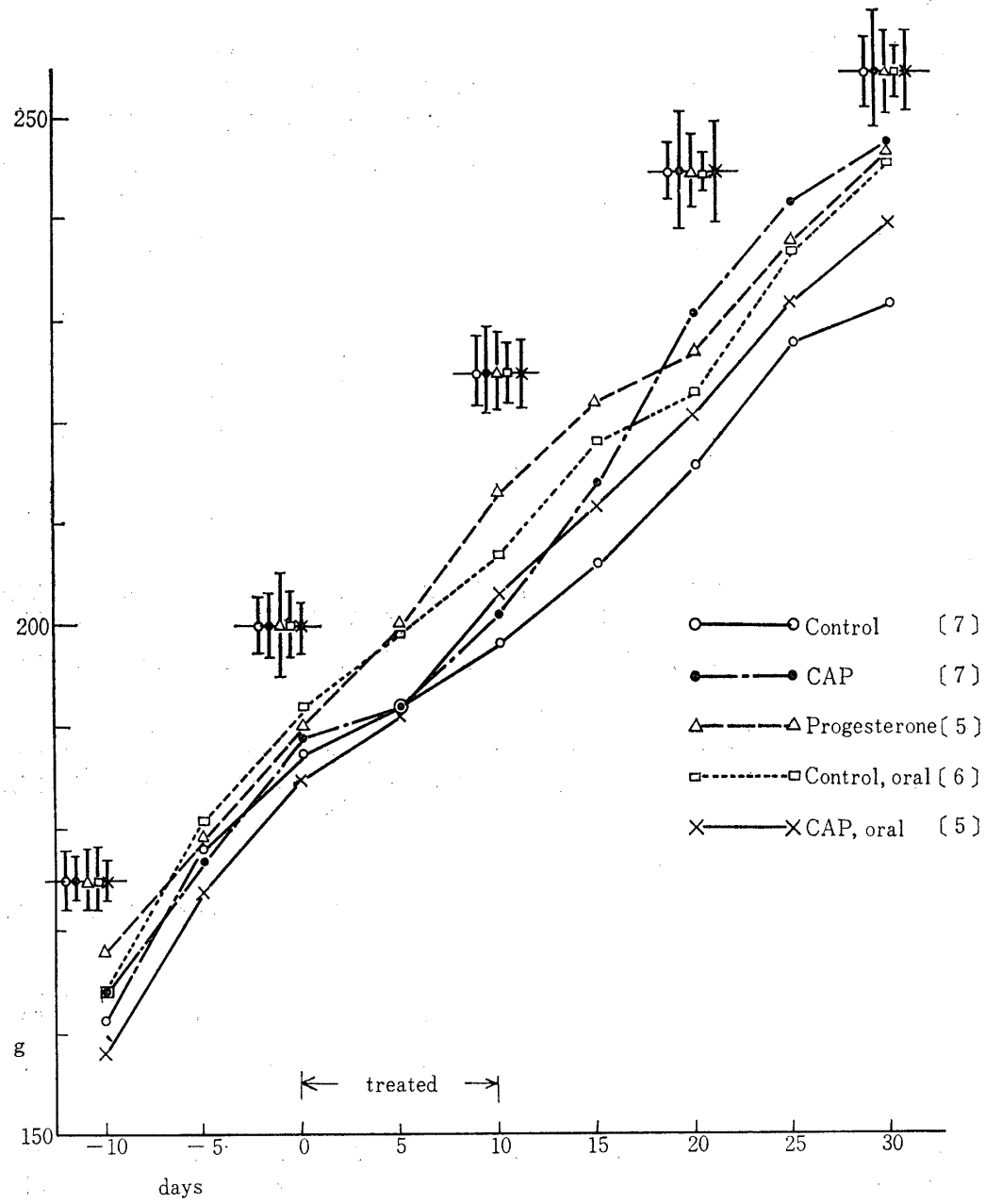

Fig. 2. Changes of body weight in rats injected (s.c.) with chlormadinone acetate and progesterone, and treated CAP orally (Experiment II).
Bars indicate S.E. of Mean and from left to right, control, CAP, progesterone, oral control and oral CAP.
Figures in bracket mean the number of rats used.

effect a non-treated group). The characteristic patterns of weight gain on CAP and on progesterone were, on the whole, observed again: *i.e.* the effect of CAP was not apparent until day 10, and occurred over the period days 10–20 or 25, while the effect of progesterone was most marked over the period days 0–10 or 15, (judged by comparison with the injected controls). When the CAP group was compared with the orally treated controls the same tendency could be seen.

Retardation of growth in injected controls was again seen, but was less marked than in experiment I. The final weights of the two steroid-treated groups and the orally treated controls showed no differences at day 30.

c) *Experiment III.*

To avoid the growth-inhibiting effect of propylene glycon seen in experiments I and II, sesame oil was substituted as vehicle; the rats were also heavier than in experiments I, II and IV. As seen in table 4 and figure 3, the stimulation of weight gain by CAP again appeared after a delay, in contrast to the progesterone-treated and pseudopregnant groups. The weights of the latter two groups were similar throughout the experiment, except that the progesterone-treated group reached a slightly heavier weight at day 10 ($P<0.05$). As in experiments I and II the

Table 4. Changes of body weight in rats treated (s.c.) with chlormadinone acetate and progesterone in sesame oil (Experiment III).

	−10	0	5	10	15	20	25	30
Treated control (9) [a]	210±3.9 [b]	219±3.8	222±4.1	232±4.3	230±3.8	232±3.5	234±3.9	239±4.5
CAP 5mg×10 (9)	206±2.1	212±2.8	220±3.3	230±3.8	238±4.7	242±4.7	240±4.6	240±4.7
Progesterone 5mg×10 (9)	209±3.7	213±3.8	229±4.2	243±4.6	239±4.5	238±5.2	239±4.9	241±5.0
Pseudopregnancy (6)	208±5.4	214±6.0	227±6.9	237±6.7	237±5.4	235±6.1	237±6.1	244±7.2

Mean of gain during various periods.

Groups	−10∼0	0∼10	10∼20	20∼30	0∼15	10∼25	0∼20	10∼30	0∼30
i) Treated control (9)	9	13	0	7	12	2	13	7	20
ii) CAP 5mg×10 (9)	6	18	12	−2	26	10	30	10	28
iii) Progesterone 5mg×10 (9)	5	30	−5	4	26	−4	25	−2	28
iv) Pseudo-pregnancy (6)	6	23	−2	10	23	0	21	7	31

Significance of the difference (*, $P<0.05$; **, $P<0.01$) [c].

Analysis of variance									
i∼iv	−	**	**	**	**	**	**	**	− [d]
i∼iii	−	**	**	**	**	**	**	**	− [d]
i vs. iv	−	**	−	−	**	−	*	− [b]	*
iii vs. iv	−	*	−	−	−	*	−	*	−
Duncan's test among groups		iii∼i	ii∼iii	i∼ii	ii, iii∼i	ii∼iii	ii∼i	ii∼iii	ii, iii ∼i
i∼iii		iii∼ii	ii∼i			ii∼i	iii∼i		

a) Number in brackets indicates rats used.
b) Mean±S.E.
c) See the footnote of Table 2.
d) Difference approaches significant at the level of 5%

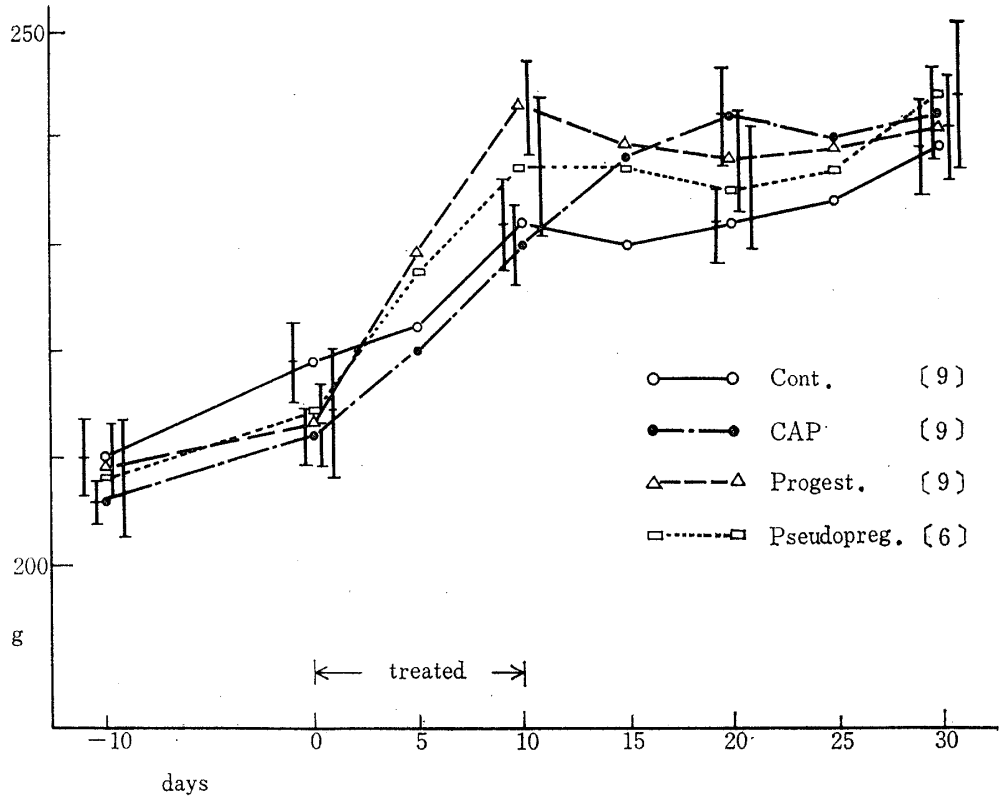

Fig. 3. Changes of body weight in rats injected (s.c.) with chlormadinone acetate and progesterone in sesame oil, and of pseudopregnancy (Experiment III).
S.E. of Mean is given separately, and from left to right, control, CAP, progesterone, and pseudopregnancy.
Figures in bracket mean the number of rats used.

period of most rapid weight gain was seen in the progesterone-treated group during the first 10 days, and in the CAP-treated group during the period 10–20 days. All groups reached similar weights at day 30.

d) *Experiment IV.*

The results are shown in table 5 and figure 4. Comparisons of weight gain are given up to day 50, although measurements were continued until day 70 to follow the levelling-off process. Differences between injected and non-treated controls were not significant except for the treatment period, when the gain by the injected controls was again reduced. The injected controls caught up later on. As in previous experiments the period of markedly increased weight gain in the progesterone-treated group was from day 0 to 15 or 20, whereas in the CAP-treated group it lasted to day 30 or 40. In this experiment, however, the difference between CAP and progesterone was not significant for the periods 0–10, 10–20 or 0–20 days, but it was significant for days 5–15 ($P<0.01$).

In the CAP-treated group weight gain was steep until day 30 and was then slower; in the progesterone-treated group the effect stopped after 15 days. In

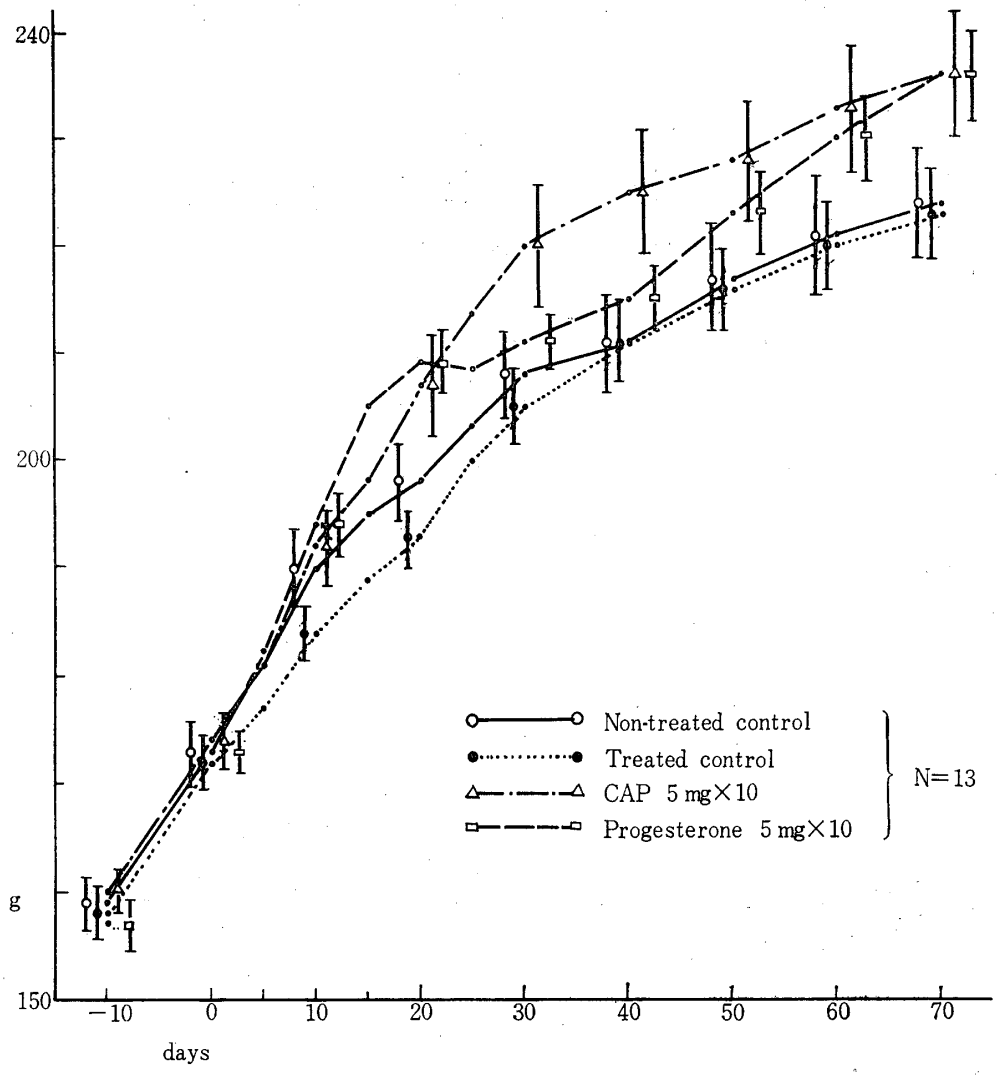

Fig. 4. Changes of body weight in rats treated (s.c.) with chlormadinone acetate and progesterone in suspension (Experiment IV).
S.E. of Mean indicates in the same way with Figure 3.
From day 0 to 30 weights are plotted on every 5th day, but S.E. on every 10th day.

both steroid-treated groups, however, there was a further increase in the last experimental period. The suspension preparations used may account for the prolonged effects in this experiment (also refer to the suppression of oestrus recurrence, p. 179). At day 70 the mean body weights of the two steroid-treated groups were equal, and heavier than the two control groups, which also had similar weights to each other.

The regression coefficients over the first 20 days in this experiment were 1.28, 1.08, 1.66 and 1.90 g/day, respectively, for non-treated control, injected control, CAP-treated and progesterone-treated groups; the differences were significant between all groups ($P<0.01$).

Table 5. Changes of body weight in rats treated (s.c.) with chlormadinone

	−10	0	5	10
Non-treated control (13)[a]	159±2.5	173±3.0	181±3.5	190±3.5
Treated control (13)	158±2.4	172±2.6	177±2.6	184±2.7
CAP 5mg×5 (13)	160±2.1	174±2.6	181±2.7	192±3.4
Progesterone 5mg×10 (13)	157±2.4	173±2.1	182±2.6	194±3.0

Mean of gain during various periods.

	−10∼0	0∼10	5∼15	10∼20	20∼30	30∼40	40∼50	0∼20
i) Non-treated control (13)	14	17	14	8	10	4	5	25
ii) Treated control (13)	14	13	12	9	12	6	5	22
iii) CAP 5mg×10 (13)	14	19	17	15	13	5	3	33
iv) Progesterone 5mg×10 (13)	16	21	23	15	2	4	8	36

Signfiicance of the differences (* $P<0.05$; **, $P<0.01$).

Analysis of variance	−	**	**	**	**	−	*	**
Duncan's test		iv-ii	iv-ii iv-i iv-iii	iv-i iv-ii iii-i iii-ii	iii-iv ii-iv i-iv		iv-iii	iv-ii iv-i iii-ii

a) Number in brackets indicates rats used. b) Mean±S.E.

e) *Experiment V.*

The rats used in this experiment were fully grown, 2 to 3-parous, and heavier than those in the other experiments. The experiment consisted of two parts: in the first phase, four groups were the same as in experiment IV except that treatment lasted only for five days, and two additional groups were included. One of these received a single dose of 25 mg CAP; the other was ovariectomized, and received 5 mg/day CAP for 5 days. In the second phase (experiment V-2) 11 of the 13 rats used in certain groups in experiment V-1 were treated again as follows: non-treated controls, CAP 5 mg×5 and CAP 25 mg×1 groups of experiment V-1 were, respectively, used as injected control, CAP 5 mg×10 and progesterone 5 mg×10 groups in experiment V-2. Day 30 of experiment V-1 became day −10 of experiment V-2.

The results are shown in table 6 and figure 5. In experiment V-1 rats were allotted so that the average weights of the groups were equal at day −10; for unknown reasons the weight changes from day −10 to day 0 were unequal (table 6–1), although the mean weights at both days −10 and 0 were not signifiicantly

acetate and progesterone in suspension (Experiment IV).

15	20	30	40	50	60	70
195±3.5	198±3.7	208±4.2	211±4.6	217±5.2	221±5.4	224±5.3
189±2.7	193±2.8[a]	205±3.4	211±3.8	216±3.7	220±4.1	223±4.3
198±4.1	207±4.7[a]	220±5.7	225±5.8	228±5.7	233±6.1	236±6.0
205±3.3	209±2.9	211±2.5	215±3.3	223±3.8	230±4.1	236±4.3

10~30	20~40	30~50	0~30	10~40	20~50	0~40	10~50	0~50
18	14	9	35	22	19	39	27	44
20	17	11	33	26	22	39	31	44
28	18	8	46	33	22	52	36	55
18	6	12	39	21	14	42	30	51

**	**	—	**	*	*	*	—	— c)
iii-iv iii-i	iii-iv ii-iv		iii-ii iii-ii	iii-iv iii-i	ii-iv iii-iv	iii-ii iii-i iii-iv		iii-ii iii-i

c) difference approaches significant at the level of 5%.

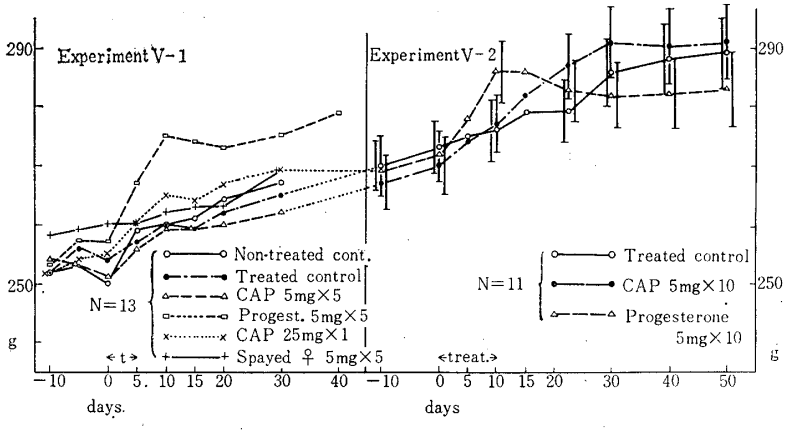

Fig. 5. The changes of body weight in rats treated (s.c.) with chlormadinone acetate and progesterone in microsuspension (Experiment V-1 and -2).
See text (p. 176) as regards the relation of groups in both experiments. Day 30 of experiment 1 corresponds to day −10 of experiment 2.

different. With 5 days of treatment, only progesterone gave a noticeable increase in weight by day 10, and this was followed by a slight decrease. CAP in either multiple or single dose did not give a significant increase. Furthermore, in the

Table 6-1. Change of body weight in multiparous rats treated (s.c.) with chlormadinone acetate and progesterone in microsuspension (Experiment V-1).

	−10	0	5	10	15	20	30
	b)						
Non-treated control (13) a)	252±5.3	250±5.3	259±5.3	260±5.1	261±5.0	264±5.4	267±5.4
Treated control (13)	252±4.8	254±4.9	257±4.9	260±5.0	259±5.0	262±5.1	265±6.4
CAP 5mg×5 (13)	254±5.4	251±5.8	256±5.8	259±6.0	259±6.1	260±6.9	262±6.8
Progesterone 5mg×5 (13)	253±5.2	257±4.8	267±4.8	277±3.9	274±4.4	273±4.2	275±4.6
CAP 25mg×1 (13)	252±5.3	255±5.9	260±5.9	265±6.0	264±4.9	267±4.7	269±5.1
Spayed female 5mg×5 (8)	258±5.6	260±5.6	260±5.6	262±5.0	263±5.5	263±5.8	269±6.0

Mean of gain during various periods and significance of the difference (*, $P<0.05$; **, $P<0.01$) c).

	−10∼0	0∼10	10∼20	20∼30	0∼20	0∼30
i) Non-treated control (13)	−2	10	4	3	14	17
ii) Treated control (13)	2	6	2	3	8	11
iii) CAP 5mg×5 (13)	−3	8	1	2	9	11
iv) Progesterone 5mg×5 (13)	4	20	−4	2	16	18
v) CAP 25mg×1 (13)	3	10	2	2	12	14
vi) Spayed females CAP 5mg×5 (8)	2	2	1	6	3	9
Analysis of variance						
i∼vi	*	**	**	—	**	*
i∼v	*	**	**	—	—	— d)
Duncan's test among groups i∼v	iv-iii iv-i v-iii v-i ii-iii	iv-ii iv-iii iv-v iv-ii	i-iv ii-iv v-iv			

a) Number in brackets indicates rats used.
b) Mean±S.E.
c) See the footnote of Table 2.
d) Difference approaches significant at a higher level.

CAP-treated groups in this experiment only a slight effect on the oestrous cycle was observed: vaginal dioestrus was prolonged in some rats, only by a few days, whereas progesterone treatment suppressed vaginal oestrus for a mean of 10.8 days (see below).

In experiment V-2 progesterone gave a clear increase in weight gain during the 10 days of treatment, then there was a slight decrease followed by levelling off. On CAP weight increased slowly but continuously up to day 20 or 30; after day 30 the weight was constant until day 50, and from day 30 it was similar to the

Table 6-2. (Experiment V-2).

	−10	0	5	10	15	20	30	40	50
Treated control (11)[a]	270±4.5[b]	273±4.7	275±5.0	276±5.3	279±5.5	279±5.6	286±5.8	288±6.1	289±5.9
CAP (11) 5mg×10	267±5.3	270±4.4	274±5.1	277±5.4	282±5.5	287±5.9	291±6.5	290±6.2	291±6.4
Progesterone 5mg×10 (11)	269±4.5	272±4.9	278±5.9	286±6.1	286±6.0	283±5.3	282±5.7	282±6.0	283±6.4

The mean of gain during various periods and significance of the difference (*, $P<0.05$; **, $P<0.01$)[c]

	−10 ~0	0~10	5~15	10~ 20	20~ 30	30~ 40	0~20	10~ 30	20~ 40	0~30	10~ 40	0~40
i) Treated control (11)	3	3	4	3	7	2	6	10	9	13	12	15
ii) CAP (11)	3	7	8	10	4	−1	17	14	3	21	13	20
iii) Progest. (11)	3	14	8	−3	−1	0	11	−4	−1	10	−4	10
Analysis of variance	−	**	−[d]	**	**	−	*	**	**	*	**	−
Duncan's test		iii-i	ii-i	ii-iii ii-i i-iii	i-iii		ii-i	ii-iii	i-iii	ii-iii	ii-iii i-iii	

a) Number in brackets indicates rats used.
b) Mean±S.E.
c) See the footnote of Table 2.
d) Difference approaches significant at the level of 5%.

weight of the control group. The pattern of weight change in this experiment was thus similar to that in experiment III.

B. *Recurrence of oestrus*

The vaginal smear usually became that of dioestrus within two days of the start of treatment with steroids. The time of recurrence of oestrus in days after the cessation of treatment is given in table 7. Since there was no difference in this respect between experiments I and II, the results of these experiments have been combined

The time to recurrence of oestrus after cessation of treatment with CAP was two to three times as long as after progesterone treatment. The differences between the experiments may be related to the preparations used. The times seem to show the following relationships: for CAP, oil<propylene glycol=microsuspension≦suspension; for progesterone, oil=propylene glycol<microsuspension=suspension.

Table 7. Oestrus-recurrence in days after the end of the treatment

	CAP (s.c.)	Progesterone (s.c.)	
I	17.7±4.3 (7)	7.4±1.7 (10)	[oral CAP]
II	22.0±2.3 (7)	6.2±0.8 (5)	3.8±0.8 (5)
I+II	19.9±4.1 (14)	7.0±1.5 (15)	[pseudopregnancy]
III	14.0±1.4 (8)	7.1±2.4 (9)	15.8±1.2 (6)
IV	23.7±7.4 (13)	13.6±5.3 (13)	
V-1	–	10.8±1.9 (13)	
V-2	21.6±3.4 (11)	12.3±2.5 (11)	

Mean±S.D. Number in bracket indicate rats used.

Statistical significance of differences (*, $P>0.05$; **, $P<0.01$).

	CAP	Progesterone
I vs. II	–	–
I+II vs. III	**	–
I+II vs. IV	*	**
III vs. IV	**	**
I+II vs. V-2	–	**
IV vs. V-2	–	–
III vs, V-2	**	**

Discussion

Although the conditions of the experiments varied in respect of body weight at start and of the preparations given, the weight change caused by CAP showed a consistent pattern. Increased weight gain was evident for a longer period than when progesterone was given, but occurred at a slower rate.

Propylene glycol was used as solvent in experiments I and II because it gives more rapid absorption from a subcutaneous injection site than does oil. It later became evident that this solvent itself depressed weight gain, and it could be surmised steroids might protect against the toxicity of the solvent. In experiment III, however, when sesame oil was used as solvent, the weight gain due to steroid treatment was essentially the same as in experiments I and II. When the two steroids were given as suspension in experiment IV, the weight gain was broadly similar to that seen in the earlier experiments, but it was more prolonged. The mode of action of CAP may, however, have been different, for in all experiments the onset of weight gain was delayed until after the 10th day, and thereafter was slow but sustained. Hervey and Hervey have also noticed the dose-response of CAP was less at various doses than that of progesterone (18).

The mean daily gain of weight on time (increase in g/days of interval) for the periods of rapid weight gain are shown in table 8. The values obtained were erratic, perhaps because of non-uniform conditions in the experiments. Therefore the net gains were calculated, as the differences between the daily gains for treated and for appropriate control groups. The net daily gain on CAP treatment then

Table 8. Daily gain (g) of chlormadinone acetate and progesterone treated groups at the fast-growing period with the corresponding control values.

Exp. No.	CAP (10-20th day)	Progesterone (0-10th day)	Treated control[1]		Non-treated control[1]		Net gain[3]	
			(a)	(b)	(a)	(b)	CAP	progesterone
I	2.8	2.5	1.6	0.1			1.2	2.4
II	3.0	2.3	1.8	1.1	(1.6	1.5)[2]	1.2	1.2
III	1.2	3.0	0.0	1.3			1.2	1.7
IV	1.5 (0-10th day)	2.1	0.9	1.2	0.8	1.7	0.6	0.9
V-1	0.8	2.0	0.5	0.6	1.0	1.0	0.3	1.4
V-2	1.0	1.4	0.3	0.3			0.7	1.1

1) a and b indicate the corresponding values for CAP and progesterone during the same periods.
2) Orally treated with propylene glycol group.
3) Net gains express the values: CAP or progesterone minus the corresponding treated controls.

became 1.2 g/day for experiments I-III, and half this in experiment IV and V-2. For progesterone treatment, the net daily gain was largest in experiment I and least in experiment IV (table 8). These figures would seem to suggest that: i) in fast-growing stage the effect of progesterone was usually greater than that of CAP; ii) the large net daily gain of the progesterone-treated group in experiment I might have been due to the marked interference with growth seen in the propylene glycol-injected controls; iii) the lower gains found for both CAP and progesterone in suspension in experiment IV may have been associated with a more prolonged action.

It is not clear why in experiment V-1 treatment with CAP for 5 days did not cause either significant gain of weight or suppression of vaginal oestrus, whereas treatment with progesterone caused almost as much effect as did 10 days' treatment in experiment V-2.

The period of rapid weight gain produced by treatment with either CAP or progesterone was followed by a short period of failure to gain or by a slight loss of weight. This contrasted with the marked losses of weight reported previously to follow immediately on withdrawal of progesterone in mice (6, 8) and rats (17, 20).

Although more thorough investigation is needed, the period of increased weight gain appears to correspond to the period of suppression of oestrus for both steroids, suggesting a correlation with the duration of steroid action. Dewar noticed in mice that the stage of falling body weight was related to the time of recurrence of oestrus, after progesterone treatment (6) and after pseudopregnancy (7). It can thus be tentatively suggested that CAP given subcutaneously has a longer action than progesterone, and especially so when given as suspension.

Changes in body composition and in food intake were not investigated in the

present experiment. Galletti & Klopper (9), Dewar (8) and Hervey & Hervey (17, 19, 21) demonstrated gains of water and of fat on progesterone treatment. The two latter authors and Bourdel *et al.* (5) also found an increase in lean tissue or protein. Hervey (20) carried out an interesting experiment to determine the cause of the weight increase with progesterone, and considered that reduction in voluntary exertion might make a substantial contribution. Dewar (8) showed that retention of water and protein still occurred when food intake was restricted, while body fat was lost. It is of interest to note that Hervey & Hervey (18) have recently found only an increase in fat in rats treated with CAP compared with progesterone. This again suggests that the mechanism of action may differ somewhat from that of progesterone.

In addition, there are many works which showed that the existence of functional ovaries is thought to be necessary (9, 13, 14, 15, 16, 22), though the opposite results is also presented (4). In the present report spayed females treated CAP did not show any significant response. Hervey and Hervey suggested a possible mechanism of action of progesterone on body weight; dependence on oestrogen withdrawal (13, 19, 22).

It is also interesting to compare the weight change caused by progestagen treatment in humans or cattle with that of rats. In contraceptive trilas in humans, in which an oestrogen was administered in combination with gestagens, weight gains were seen in a considerable proportion of patients given 19-nor-testosterone compounds by mouth (11, 25, 38). In rats, however, these gestagens have been reported to cause loss of body weight (23, 32, 33). With CAP, Rice-Wray (29) and Balin & Wan (1) did not report weight gain; Goldzieher *et al.* (10), however, found weight gains in a small percentage of cases in sequential therapy of oestrogen and gestagen. The effect of gestagens on weight in man is thus somewhat obscure.

In the field of animal husbandry, the growth-promoting actions of oestrogen or of oestrogen and progesterone in combination are well established (27). Little is known, however, about the effect of progesterone on weight gain in ruminants. O'Mary *et al.* (28) and Henneman *et al.* (12) did not find a significant effect of progesterone on the rate of weight gain in lambs. Takeuchi *et al.* (39), however, observed significant weight gain in cattle when 20 mg/day of CAP was given orally for 15 days in order to synchronize oestrous cycles. The average weight of 7 treated cows was 32 kg above a constant level of pre-treatment period at 18 days after the end of the treatment period; faster weight gain persisted in the period after treatment. Only slight changes in weight were also seen in untreated control, dry or lactating cows. Recently further evidence has accumulated: i) in young bulls and gilts norethinolone enanthate and CAP seemed to increase body weight (24); ii) Raum *et al.* (30, 31) reported that CAP increased growth rate in heifers but not in steers; iii) Bloss *et al.* (2) demonstrated that melengesterol acetate also stimulated growth in heifers.

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Summary

The effects of adminstration of chlormadinone acetate on the body weight of adult female rats have been investigated, and compared with the effects of progesterone. Five mg per day of each steroid was given for 10 days, in solution in propylene glycol or sesame oil and in suspension.

As the period of rapid weight gain seemed to occur at different times for the two steroids, statistical comparisons were made for periods of various intervals, and for regression coefficients during the period of most rapid weight gain and over the period of 20 days from the start of treatment. When propylene glycol, or sesame oil was used as solvent, the weight gain caused by CAP administration occurred, though delayed, over a longer period than that of progesterone, *i.e.* for 15 to 20 days, whereas the weight gain on progesterone ended within 10 days. The similar patterns of gain were obtained with the steroids with microsuspension. When suspensions were used, both steroids maintained their effects for longer but the effect of CAP sustained longer than that of progesterone. The period of steep increase of body weight was followed by a period of no change or of slower gain. The duration of increase of body weight seemed to be correlated with that of suppression of oestrus.

The results are discussed in relation both possible mode of action and to weight gain on progestagen medication in humans and to the effects on weight of progestagen treatment of farm ruminants.

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