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THE PHYSIOLOGY OF PREGNANCY IN THE RAT.—I. THE PROLONGATION AND INTERRUPTION OF PREGNANCY. By Annie M. Hain. From the Department of Animal Genetics, University of Edinburgh. (With eight tables.)

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THE PHYSIOLOGY OF PREGNANCY IN THE RAT.—I. THE PROLONGATION AND INTERRUPTION OF PREGNANCY.[1]

By ANNIE M. HAIN. From the Department of Animal Genetics, University of Edinburgh. (With eight Tables.)

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THE part played by the anterior pituitary in the maintenance of pregnancy has recently formed the subject of study, in the course of which the effects upon the pregnant rat of the following methods of treatment were examined:—

(1) The implantation of anterior lobe tissue.
The injection of:
(2) An alkaline extract of anterior pituitary.
(3) An extract of human pregnancy urine.
(4) A preparation of anterior pituitary rich in growth hormone.

ENGLE and MERMOD (1928) reported an interruption of pregnancy in all but the latest stages following the implantation into rats and mice of small amounts of anterior lobe tissue; the interruption was associated with œstrous conditions induced in the ovaries and uterus of the animal treated. It has been demonstrated that follicular maturation is not, however, an invariable result of pituitary implantation, and that luteinisation and a prolonged pseudopregnancy (rabbit) can follow the implantation (ROBSON, 1931; PHILLIP, 1931).

A similar twofold effect was noted by ZONDEK and ASCHHEIM (1928) in experiments with human pregnancy urine, and it was accordingly held that the substance in pregnancy urine having this effect is anterior pituitary hormone.

As TEEL (1926) showed that pregnancy may be prolonged by the formation and persistence of functioning luteal tissue, it was thought that this result should be obtained equally by the implantation of untreated tissue and the injection of concentrated pregnancy urine extracts as by an alkaline extract of pituitary.[2] Such experiments are reported in the present communication. The presence in the corpus

[1] Much of this work formed the basis of a thesis submitted for Ph.D. (Edinburgh), December 1931. The author is indebted to Mr P. G. MARSHALL and to Dr R. E. ILLINGWORTH for the preparation of the extracts of human pregnancy urine and purified growth hormone respectively.

[2] Since completing this portion of the investigation the author found that EVANS and SIMPSON made a similar observation in 1929; this work confirms and amplifies their results.

luteum of a substance which directly or indirectly inhibits uterine contractions was demonstrated by KNAUS (1929) and confirmed by ROBSON and ILLINGWORTH (1931); the latter produced evidence which suggests that the proliferative and inhibitory actions of the corpus luteum might be due to different factors. Recent researches by REYNOLDS (1932) indicate that a substance present in human pregnancy urine affects uterine motility independently of the ovary, "yet when this organ is present it probably contributes to the effect."

The fourth series of tests was made with a preparation of growth hormone extracted from the anterior pituitary after the prescription of VAN DYKE (1930), and held by him to be non-gonadotropic; tests also were made with the residue from this preparation, which, in combination with the growth hormone, produces a typical ovarian reaction.

METHODS.

1. *Experimental Animals.*

For these experiments 132 pregnant rats were employed, exclusive of controls. They were all albino rats of the Edinburgh-Wistar stock, weighing from 150 to 180 grams and between three and five months old; the diet was maintained on a sound vitamin basis. The animals were weighed daily throughout pregnancy and vaginal smears examined by the usual method. Seventy-nine pregnant rats were used as controls. The gestation period, dated from the appearance of vaginal plug, is estimated at twenty-one and a half days (LONG and EVANS, 1922), *i.e.* parturition takes place on the 22nd day. However, as instances occurred among the controls where pregnancy lasted till the 23rd day, deviations from the normal of a single day in the experimental animals have not been attributed to treatment. Absorption occurred in only one control animal. The average weight of the fœtus at birth has been taken as 5·25 grams. From the 21st day of pregnancy the cages were examined for litters three or four times daily; the final survey was made about 10 p.m. The vaginal plug sometimes persisted for 3 to 8 days; one case of 16 days' duration was observed. A serious error in estimating the length of pregnancy would arise if a plug were estimated as "at most 24 hours old," which is the maximum reported by LONG and EVANS (1922).

2. *Preparation of Material.*

A. Implantation of anterior lobe tissue. Bovine pituitary glands were received in a frozen state and were prepared for implantation within eight hours of killing. The anterior lobes were cleaned by being placed in 40 per cent. alcohol for 10 minutes and were then cut very finely: portions from several glands were mixed before implantation so as to equalise the chances of active material being employed. A dorsal incision was made and, with the exception of one group

of animals, a single intraperitoneal graft was implanted; its weight was calculated on the basis of one ox-pituitary weighing 1·5 grams.

B. Extract of human pregnancy urine. This extract was prepared in the Macaulay Laboratory by the methods described by WIESNER and MARSHALL (1931); both barium-alcohol and phosphotungstic acid extracts were used. The extract was injected in the dilution of 10 c.c. of water per yield of approximately 2 litres of pregnancy urine.[1] The earlier experiments show that there is considerable variation in the strength of the extract, due largely to variations in the yield of hormone; moreover, the weight of the yield (in powder form) was not an invariable indication of the strength of the active principle obtained. In a later stage of the experiment, material extracted from several consignments of urine was combined to ensure more strictly comparable results; this material was used in 23 animals. Before this course was adopted it was ascertained that the extract would not deteriorate even though kept for more than six weeks.

C. Alkaline extract of anterior pituitary. Bovine pituitary glands were extracted by the method described by TEEL (1926); the supernatant fluid was kept frozen between injections. One c.c. of extract is equivalent to 1 gram of the tissue.

D. Growth hormone of anterior pituitary. An alkaline extract was treated with Na_2SO_4 as prescribed by VAN DYKE and WALLEN-LAWRENCE (1930); 1 c.c. growth hormone was equal to 1 gram tissue. The inactive faction was injected in the proportion of 3·25 grams tissue to 1 c.c. of extract.

RESULTS.

Implantation of Bovine Anterior Lobe Tissue (11 *Rats*).

TABLE I.—EFFECT OF IMPLANTATION OF ANTERIOR LOBE TISSUE INTO PREGNANT RATS.

Total amount of tissue.	No. of rats.	Time of implantation.	Effect upon pregnancy.
GROUP I.			
Half ox ant. lobe	4	7th and 9th days. " " " " 6th and 8th days. 5th and 7th days of pregnancy.	Prolongation of pregnancy for 1 to 2 days.
GROUP II.			
Whole ox ant. lobe	5	13th, 13th, 11th, 9th, and 4th days of pregnancy.	3—Prolongation of pregnancy for 4 to 6 days. 2—Absorption of fœtuses.
GROUP III.			
Small piece ox ant. lobe.	2	4th day of pregnancy.	No effect.

[1] An exception was made in one group of nine animals to be specified.

Group I.—The four pregnant rats in Group I. (Table I.) received implants equal to half an ox-pituitary (administered in two operations). In no case was there an interruption of pregnancy. Although live young were born on the 23rd and 24th days and the average weight of the fœtuses was normal, abnormalities occurred which were most likely due to the treatment, and indicated that gestation was slightly prolonged.

(1) Protracted parturition occurred in one animal; three dead fœtuses were born at 6 p.m. on the 23rd day and were followed by three others at 10 a.m. on the 24th day, of which two were alive. According to LONG and EVANS (1922), parturition normally lasts not longer than four and a half hours. KING (1911) met only two instances of protracted parturition, and only one such was found in our control animals.

(2) In two cases the maximum weight of the mother was reached before normal term, and remained almost stationary for 30 hours. In the control pregnant rats it was found that there is an increase in weight up to the time of parturition.

(3) Necropsy in the remaining animal on the 12th day showed that development had been retarded in the initial stages; embryonic growth was equivalent to an 8th-day pregnancy.

Group II.—This consisted of five pregnant rats, in each of which anterior lobe tissue equal to one whole ox-pituitary was implanted intraperitoneally at one operation. The rats were in a state of partial collapse for some days following the implantation and refused food. They were kept as warm as possible, and were given hot milk and brandy twice daily: one rat required to be hand-fed daily throughout pregnancy. Pregnancy was considerably prolonged in three cases, and absorption occurred after hæmorrhage in two in which implants were made on the 4th and 13th days respectively. The facts are, briefly, as follows:—

(1) Parturition occurred on the 28th day in two rats, and from the uterus of the third rat fœtuses were removed on the 26th day —*i.e.* a prolongation of 6 and 4 days respectively.

(2) In all cases the fœtuses were dead and partially decomposed, and, with the exception of those of the rat which was ill throughout pregnancy, they were above the average weight — 6 grams (average of 5), 7·5 grams (average of 2; others were half-eaten).

(3) Where prolongation occurred, the mother attained her maximum weight either the day before or the day after normal term, and the weight remained stationary for 3 or 4 days and then fell slightly. One animal gained 15 grams after normal term, which is accounted for by the excessive weight of the fœtuses (7·5 grams at birth).

Group III.—Whereas the interruption of pregnancy followed repeated implantation of small amounts of anterior lobe tissue in the experiments reported by ENGLE and MERMOD (1928), in those now described it is manifest that the bovine anterior pituitary may cause a

considerable prolongation of pregnancy, the extent of which is roughly proportional to the size of the implant. It was found that, below a certain size, a single implant has probably no effect. Portions from six glands of a total volume of approximately 9 to 16 cub. mm. were implanted in two rats on the 4th day of pregnancy. Normal litters (average weight 5 grams) were born alive on the 23rd day; the placental sign was slight and occurred at the normal time.

Injection of Extract of Human Pregnancy Urine (78 *Rats*).

For this experiment the rats were divided into six groups. Groups I. to IV. may be described in the first place.

Group I.—Twelve animals received small daily doses over a long period.

Group II.—Two animals were injected from the appearance of placental sign onwards.

Group III.—Eight animals received the same amount of extract but at various stages of pregnancy.

Group IV.—Twenty-three animals were injected with the same extract in different amounts at parallel stages of pregnancy.

Group I.—In all rats in this group pregnancy was interrupted: in three rats before the ova were embedded, mating occurring repeatedly during the period of injection; in the remainder after placental sign had been observed. As there had been very little increase in weight, the animals were killed on the 15th day. In each case the uterus and vagina were full of blood and distended; the ovaries were a mass of corpora atretica, presenting the "mulberry" appearance described by Evans (1923); as many as 25 were counted in one ovary (rat 803/2). Histological examination showed the lumen of the uterus to be full of red blood-cells and leucocytes; the appearance of the uterine mucosa with its high cells and considerable glandular development indicated a high degree of secretory activity. The nuclei of the stroma were crowded and the muscle-layer thick. The conditions in the normal rat at the same stage of pregnancy (15th day) as described by Allen (1931) provide a marked contrast.

The ovaries were very strongly luteinised. No difference in structure could be detected between the corpora lutea of normal pregnant rats and those of the animals in which absorption took place, except that the ovaries of the former were perhaps more vascular than those of the latter. The vagina was in all cases typical of the pregnant animal (mucification).

One rat in this group was retained and injections continued for 17 days. Hæmorrhage occurred from the 11th to the 18th day, and again on the 27th day. She mated four times during the period: 10th,

15th, and 17th days after the first plug. Daily smears from the 17th day onwards showed no cornified cells in the vagina, yet on the 27th day she again mated. At necropsy the same day the vagina and uterus were found to be full of blood and the lumen distended; there were two blood-points in one ovary and many corpora atretica.

TABLE II.—EFFECTS OF INJECTION OF VARYING AMOUNTS OF EXTRACT OF HUMAN PREGNANCY URINE INTO PREGNANT RATS.

(Concentration: yield of 2 litres urine to 10 c.c. H_2O.)

Daily dose.	Day of pregnancy.	No. of injections.	No. of rats.	Effect on pregnancy.
GROUP I.	From:			
0·1 c.c.	3rd day	13	4	Pregnancy interrupted.
0·2 ,,	3rd ,,	13	3	
0·3 ,, [1]	7th ,,	9	5	
GROUP II.				
0·2 c.c.[1]	From placental sign	9	2	Prolongation of pregnancy.
GROUP III.	From:			
0·2 c.c.	3rd and 4th days	7	2	1—Prolongation 4 days. 1—Absorption after hæmorrhage.
0·2 ,,	6th day	4	1	Absorption after hæmorrhage.
0·2 ,,	7th, 9th, and 10th days	5	5	Prolongation 4 to 5 days.
GROUP IV.				
0·4 c.c.	7th day	5	4	Absorption after hæmorrhage.
0·1 ,,	7th ,,	5	3	
0·4 ,,	11th ,,	5	4	Prolongation 4 to 5 days.
0·1 ,,	11th ,,	5	3	1—Prolongation 4 to 5 days. 2—Absorption after hæmorrhage.
0·4 ,,	16th ,,	5	3	Prolongation 5 to 7 days.
0·1 ,,	16th ,,	5	3	
0·4 ,,	3rd ,,	5	3	1—Absorption after hæmorrhage. 2—Normal pregnancy.[2]

N.B.—One and the same extract was used throughout Group IV.

Group II.—The same sample of extract as was used for five rats in Group I. was given to two rats from the first appearance of the placental sign onwards for a period of 9 days. The date of mating was not known. They increased in weight continuously for 14 days, gaining 55 and 50 grams respectively during this period. A drop of 15 grams was noted two days after the maximum was reached and the uterus was then examined. Abnormally large fœtuses were seen in both horns, and there appeared to be much blood of dark colour in the lumen and within the

[1] The same preparation of extract was used in these cases.
[2] Double injection on the 1st day.

amnion. Parturition did not take place; absorption occurred in one and death in the other. It is to be noted, however, that if the first appearance of placental sign is taken as occurring on the 13th day (Long and Evans, 1920) pregnancy lasted 29 days; if it is placed earlier, say the 10th day,[1] pregnancy lasted 26 days—*i.e.* there was an extension of the gestation period of 4 days or possibly of 7 days.

Table III.

(*a*) Results in Relation to Stage of Pregnancy at which Injections were commenced.

Effect.	Injections.		
	1st to 7th day.	8th to 14th day.	15th to 22nd day.
Prolongation . . .	2	11	6
Absorption . . .	22	2	..
Normal	2	..	..

(*b*) Effect in Relation to Dose.

Effect.	Dose.			
	2·0 to 2·7 c.c.	1·3 to 1·8 c.c.	0·8 to 1·0 c.c.	0·5 c.c.
Prolongation .	7	3	5	4
Absorption . .	13	5	1	5
Normal . .	2	..	..	..

Group III.—In six rats in this group pregnancy was prolonged either 4 or 5 days (*i.e.* to the 26th or 27th day), with the accompanying features described in Group IV.; in two rats in which absorption occurred, the maximum weight was reached on the 14th and 22nd days respectively.

Group IV.—In order that strictly comparable results might be obtained it was decided to examine the effects of the same sample of extract in both large and small amounts when given at the same stages of pregnancy. Accordingly, for this group, the yield of 8 to 10 litres of urine was combined, giving approximately 40 c.c. of extract. Injections were spread over a month, and were commenced on the 7th, 11th, and 16th days of pregnancy; 23 animals were used in the group, which falls into two sections:

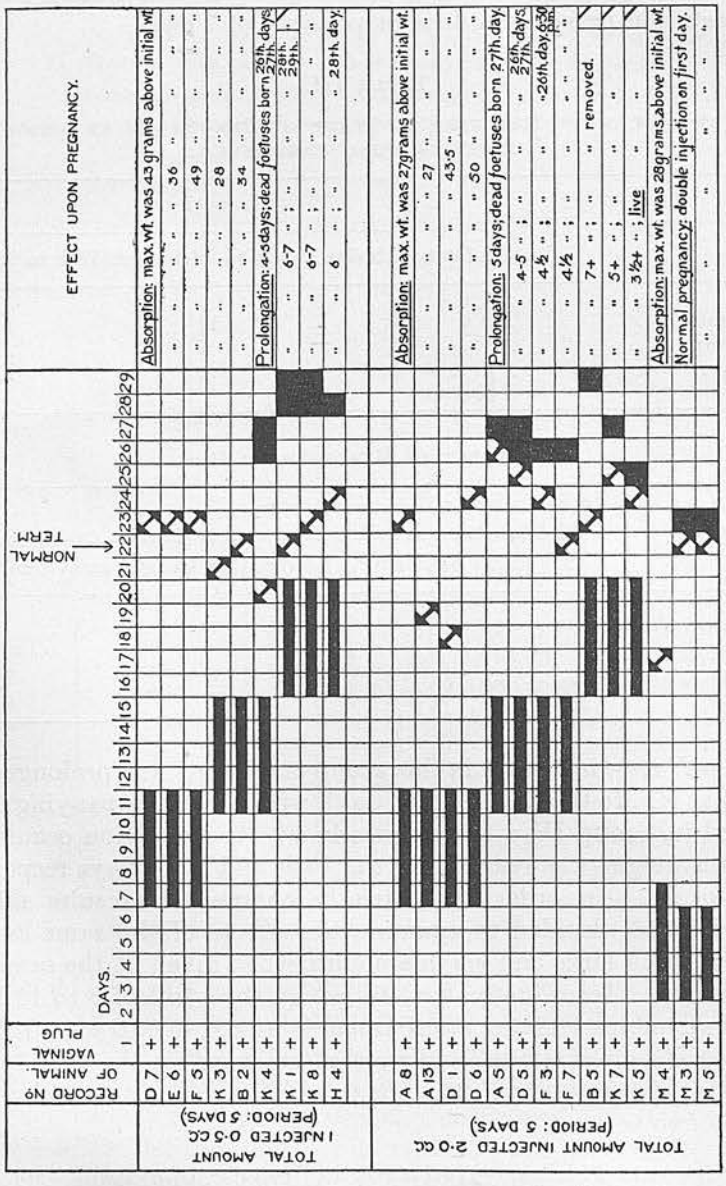
A, Nine rats which received a total of 0·5 c.c. of extract.
B, Fourteen rats which received a total of 2·0 c.c. of extract.

[1] Placental sign was observed as early as the 10th and 11th days in several control as well as in injected animals.

Absorption occurred as follows:—

All rats injected from the 7th day of pregnancy, irrespective of the amount injected (7 rats). Two rats injected with the small amount from the 11th day of pregnancy.

TABLE IV.—EFFECTS OF INJECTION OF EXTRACT OF HUMAN PREGNANCY URINE IN 23 PREGNANT RATS (see Table II., Group IV.).



In several instances the weight remained stationary for 3 to 4 days after the maximum was reached and then fell gradually. Excessive hæmorrhage accompanied the late stages of absorption.

Prolongation occurred as follows:—

All rats injected with the larger amount from the 11th day.
One rat injected with the smaller amount from the 11th day.
All rats injected from the 16th day, irrespective of the amount injected (6 rats).

The prolongation was of the same extent in both A and B groups, *i.e.* whether a total of 2·0 c.c. of extract was given or only 0·5 c.c. In rats from which the fœtuses were removed by abdominal section on the 27th and 29th days the amniotic membranes were found to be septic, the uterine lumen contained venous blood, and the fœtuses were dead. When they were removed at 5.30 p.m. on the 25th day they were alive, but proved to be not viable.

The same features as were observed in the implantation experiment and in Group III. of the urine extract experiment were noticeable here:

(1) Where pregnancy was prolonged for more than three days the litters were still-born.
(2) The mother usually continued to gain weight till the 23rd or 24th day, after which the weight remained stationary for 4 to 5 days.
(3) Fœtuses were abnormally large; 6·8, 6·25, 6·92, 6·7, and 5·7 grams being the averages of litters which totalled over 100 fœtuses.
(4) The placentæ were frequently *sub*-normal in size, but instances were observed where they were abnormally large—the average of one set in this group was 0·600 gram.
(5) Parturition was protracted in four rats in Group IV. and in one in Group III.
(6) The ovaries of rats killed or operated upon in prolonged pregnancy were large and a mass of atretic bodies.[1]
(7) Hæmorrhage occurred at two periods, mid- and late pregnancy; in late pregnancy it was severe.

It might be concluded from the data given in the preceding paragraphs that injection in the early stages of pregnancy invariably causes pregnancy to be interrupted, yet in two rats in which a double injection (a total of 0·8 c.c.) was given on the first day (in this case the third day after insemination) pregnancy followed a normal course and live litters were born. In one rat in which the injections were made at the same stage and in the usual way, absorption occurred.

As the ovaries of animals in which pregnancy was artificially prolonged were generally characterised by marked luteinisation, an effort was made to ascertain if prolongation could be obtained in the absence of the ovarian effect. The problem was approached in the following way:—

(1) A potent extract of pregnancy urine was boiled prior to injection, in order to destroy the luteinising principle (Wiesner and Marshall, 1931).

[1] In contrast to those described on p. 259.

(2) Bilateral oöphorectomy was performed following injection and the period noted during which pregnancy continued. In these groups 24 and 9 rats respectively were employed; controls were used that there might be no question as to the potency of the material. A third method of approaching the problem is described later under "growth hormone."

TABLE V.—CONCENTRATED EXTRACT OF HUMAN PREGNANCY URINE.

(Total amount injected, 1·75 to 2·0 c.c. Period: 2 days.)

Rat.	Days injected.	Max. wt. reached.	Parturition.	Effect on pregnancy.
Control J_1	14th, 15th	24th day	26th day	Prolong. 4 days.
,, M_2	,, ,,	22nd ,,	24th ,, (dead)	,, 2 + days; fœtuses removed 8 hours after bilateral oöphorectomy.
	Same Extract boiled 2 Minutes.			
H_1	3rd, 4th	14th day	..	Absorption after bleeding.
D_5	5th, 6th	22nd ,,	23rd day	Normal.
J_3	,, ,,	24th ,,	25th ,,	Prolongation 3½ days; alive but not viable.
H_4	11th, 12th	23rd ,,	25th ,,	Prolongation 3 days; do.
N_2	13th, 14th	22nd ,,	23rd ,,	Normal.
E_5	,, ,,	19th ,,	29th ,,	Prolongation 7 + days; removed by abdominal section.
N_4	,, ,,	24th ,,	29th ,,	Prolongation 7 + days; born after bilateral oöphorectomy 27th day.
	Same Extract boiled 5 Minutes.			
H_4	3rd, 4th	..	..	Ovulation and mating occurred on 5th day; pregnancy resulted.
B_3	7th, 8th, 9th	23rd day	24th day	Normal.
F_1	,, ,, ,,	22nd ,,	23rd ,,	,,
A_4	12th, 13th, 14th	21st ,,	Absorption	Partial abortion 19th and 21st days.
A_2	15th, 16th	15th ,,	,,	*N.B.*—Total amount injected 1·6 c.c.
H_2	,, ,,	23rd ,,	23rd day	Normal.

From an examination of Table V. it is seen that boiling a highly concentrated extract of human pregnancy urine for 2 minutes does not destroy the factor which prolongs pregnancy. The same extract boiled for 5 minutes did not prolong pregnancy, but a tendency to absorption was present. When 2·0 c.c. of the latter extract were injected in immature mice no blood spots were produced, but full cornification of the vagina and distension of the uterus with fluid resulted. Two features were noted in the rats: Rat M_2 was ill on the 24th day of pregnancy and

it was accordingly decided to examine the ovaries. These were found to be a mass of large follicles with no trace of luteal tissue; they were ligatured and removed. Eight hours later the rat was dead, and 8 fœtuses, weighing 43 grams, were removed from the uterus. In Rat N_4 pregnancy had been prolonged for 5 days when both ovaries were removed. She did not litter until 2 days later, *i.e.* the 29th day of pregnancy.

In the experiments so far described the extracts used were of a uniform strength, viz. the equivalent of 10 mg. of dry substance per c.c. The amount that produced hæmorrhagic follicles in the ovaries of immature mice, weighing 9 to 11 grams, was 0·2 mg. In 9 rats a preparation, further purified by the barium method, was used, the m.u. of which was 0·05 mg. The equivalent of 4 m.u. did not prolong pregnancy, but 12 m.u. had this effect. When this extract was boiled for 3 minutes and 8 m.u. given, it was without effect on the pregnant animal.

The control animal in Table VI. is the most striking example of prolongation reported. The maximum weight of Rat B_2 was $51\frac{1}{2}$ grams above her initial weight, and was attained on the 23rd day of pregnancy. It remained almost stationary for 4 days and then slowly fell. When laparotomy was performed on the 29th day 12 corpora lutea were counted in the right ovary and 8 in the left; no follicles could be seen. On the 32nd day half a dead fœtus was found in the box at 5.45 p.m. No further births were noted till the 35th day, when other partly eaten fœtuses were found: there had been a drop in weight of 22 grams overnight. The mother was quite healthy, and was again pregnant a fortnight later.

The same extract as was given to B_2 was injected into eight pregnant rats, in five of which the ovaries were removed shortly before, at, and after normal term respectively. In two such rats pregnancy continued for 4 and 5 days after the operation (until the 26th and 27th days), and in three others for 2 and 3 days respectively. Rat $B_2^{(1)}$ is remarkable in that growth continued after oöphorectomy at normal term, 5 grams being gained between term and parturition; the fœtuses were healthy, and one weighed 6·7 grams. The ovaries of this rat and of Rat K_2 were a mass of large follicles when removed, some measuring 2·0 mm. and 2·2 mm., and others 1·6 mm.

When the ovaries were removed on the 12th and 14th days of pregnancy respectively, the maternal weight rose 5 to 6 grams after the initial drop due to the operation. At this figure it remained stationary for several days. In Rat K_4 a drop of 11 grams occurred during the night between the 22nd and 23rd day, after which the weight remained at this level and no further discharge was observed from the vagina. It is possible that fœtal elements were expelled at this stage and eaten. In Rat P_2 the ovaries were removed on the 16th day; after remaining

TABLE VI.—CONCENTRATED PREGNANCY URINE EXTRACT 0·1 GRAM IN 10 C.C. H_2O FOLLOWED BY OÖPHORECTOMY.

Total amount injected.		Days. 1–9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	Effect upon pregnancy.
3·0 c.c.	B_2	Control								—	—	—				+									■			■	Prolongation 10 to 13 days.
2·4 c.c.	B_1							—	—						0^+	Pl.	−11	■											„ 3 days; 1 fœtus alive.
	J_1							—	—						0^+				■	(dead)								„ 4 days; 8 dead fœtuses removed.
4·0 c.c.	P_2						—	—	0^+	—	—			■		■	■												Protracted parturition; underdeveloped fœtuses.
	K_4				—	—	0^+	—	—					//		−11													Absorption; weight almost stationary for several days.
	B_6		—	—	0^+	—	—						//															
3·6 c.c.	H_2				—	—		—	—				+	0	−14	■													Littered 55 hours after oöphorectomy.
1·6 c.c.	K_2									—	—	—	—				0		■										„ 46 „ „ „ *
3·0 c.c.	B_2(1)										—	—	—		0				+	■									„ 112 „ „ „ *

+ Attained maximum weight.
// Last day of hæmorrhage from vagina.
−11, −14, grams drop in weight.
0 Bilateral oöphorectomy.
* Ovaries were a mass of follicles when removed.
— Injection. ■ Fœtuses born or removed.

stationary for three days the weight slowly fell. On the evening of the 21st day an under-developed fœtus was born, and on the following morning others could be felt on handling. No more young were found until the morning of the 23rd day, when portions of another under-developed fœtus were removed from the cage. On the 24th day a small lump was still felt on handling but no other young were born.

Finally 7 oöphorectomised mice were injected with urine extract, but macroscopical examination gave no evidence of a direct effect of the extract on the uterus.

Alkaline Extract of Anterior Pituitary (30 *Rats*).

TEEL (1926) had injected 6, 12, 15, and 20 c.c. in his experiments, but it has been found that 2·0 c.c. or even less, when injected over a period of six days from the 11th, 12th, or 13th day of pregnancy, has an effect upon pregnancy in every way comparable with the maximum dose given. Pregnancy was prolonged 4, 5, and 6 days and litters were still-born. The prolongation of pregnancy was associated with all the features already described in connexion with the same phenomenon in the two previous experiments, and notably among these, marked luteinisation of the ovaries. Ovaries weighing 0·091 grams were encountered. The placentæ were frequently abnormally large, *e.g.* 1·202 and 1·097 grams; in one rat such a placenta was found adherent to the muscle-layer of the uterus when it was cut away on the 26th day.

TABLE VII.—ALKALINE EXTRACT OF PITUITARY.

	No. of rats.	Daily dose.	Days of pregnancy.	Prolongation.
			From :	
	4	1·0 c.c. for 6 days	13th	5 to 7 days.
	6	0·3 „ „ 6 „	11th, 12th, 13th	2, 4, 5, 6 days.
	4	1·0 „ „ 4 and 6 days	1st	2 to 2½ days.
	1	0·3 c.c. for 6 days	6th	2 to 2½ „ (live litters).
Extract one month old	3	1·0 „ „ 5 „	3rd	„ „
Filtered extract .	3	0·4 c.c. and 0·8 c.c. for 4 days	3rd	„ „
Late pregnancy . .	1	3·0 c.c. in 24 hours	20th	4 to 5 days
Feeding experiment .	5	2 and 3 c.c. for 4 days	2nd, 7th, 11th, 15th	No effect.
Dilute extract . .	3	Total 0·1 to 0·4 c.c.	3rd, 4th, 12th	„ „

As was noted by TEEL, where the alkaline extract is injected from the first to the sixth day, pregnancy is only slightly prolonged—2 to 2½ days—and live litters are obtained.

Certain incidental observations are worth mentioning:

An extract which had already caused a prolongation of pregnancy of from 4 to 6 days was again tested after being kept frozen for a month

and was found to have retained its potency. Filtration through a Seitz filter did not deprive the extract of its effectiveness; but in this group the fœtuses were considerably under the average (3·8 and 4 grams). The mother generally attained her maximum weight before normal term, and this weight remained stationary for a considerable period before parturition—36 hours, 72 hours (litter of 10, of which 7 were dead), 30 hours, 65 hours.

As COURRIER and KEHL (1929) had induced follicular formation in cats by injecting small amounts of alkaline extract, the effect of various dilutions was examined. There was no effect on the pregnant rat where as little as 0·025 c.c. was given daily for four days. Injection was commenced on the 3rd, 4th, and 13th days of pregnancy.

A test made in late pregnancy seems of interest. Rat F_2 was given a total of 3·0 c.c. with the effects upon pregnancy as described:

20th day, 8 p.m., 1·0 c.c.
21st ,, 10 a.m., 1·0 ,,
,, ,, 6 p.m., 1·0 ,,

23rd day, 10 a.m., a live fœtus was born.
26th ,, 12 noon, a dead fœtus weighing 5 grams.
27th ,, rat killed under ether; three more fœtuses were in the uterus, which was septic; weights 5, 7, and 5 grams. The ovaries contained several well-vascularised corpora lutea.

Five rats were used for a feeding experiment. From 8 c.c. to 12 c.c. of extract administered orally to each animal over a period of four days had no effect on pregnancy.

Growth Hormone from the Anterior Pituitary (prepared after VAN DYKE and WALLEN-LAWRENCE (1930)).

This preparation is considered by VAN DYKE to be almost entirely free of any gonadotropic substance; accordingly its effect upon the pregnant animal was tested, as well as that of the "inactive" residue. For control purposes immature mice were injected with the same extracts as were used on the pregnant rats. It was found that, whereas 1·3 c.c. growth hormone had no macroscopic effect on the ovaries, uterus, or vagina of 3-week-old mice, 2·0 c.c. caused masses of hæmorrhagic follicles to form in the ovaries of such animals; this amount, injected into immature rats weighing 33 to 38 grams, was without macroscopic ovarian effect. The residue, after removal of the growth hormone, had no effect on the ovaries of immature mice when injected in amounts equal to 1·65 gram of tissue.[1]

[1] Although 1·3 c.c. of growth hormone was apparently without effect on the ovaries of immature mice, the same amount, with its equivalent of "inactive" residue dissolved therein, produced blood-points.

TABLE VIII.—GROWTH HORMONE (Prescription of VAN DYKE).
(2·0 c.c. = 2 grams anterior lobe tissue.)

Rat.	Days injected.	Maximum weight reached.	Parturition.	Effect on Pregnancy.
M_1	18th to 20th	22nd day	24th day (noon)	Prolongation 2 days; weight stationary for 60 hours.
H_1	15th to 17th	24th ,,	28th day (5 p.m.)	Prolongation 6½ days.
J_2	,,	22nd ,,	25th, 26th days	Prolongation 3 to 4 days. Protracted parturition.
D_1	11th, 12th	24th ,,	26th day (dead)	Prolongation 4 days; fœtuses removed at necropsy.
G_4	,, ,,	23rd ,,	Absorption	Prolongation (?) days; hæmorrhage until 27th day.
E_1	5th, 6th	24th ,,	24th day	Normal (?).
P_1	,,	22nd ,,	23rd ,,	Normal.

It is seen from Table VIII. that injection of the growth hormone on or after the 11th day of pregnancy causes a prolongation of the gestation period of from 3 to 6 days, with the accompanying features noted in the experiments previously described. Injection in the early stages (viz. 5th and 6th days) does not cause interruption of pregnancy.

The "inactive" residue, injected at the same three stages of pregnancy in six rats, was without effect; normal litters were born at term in all cases. The amount given to each rat was extracted from 6·5 grams of anterior lobe tissue.

DISCUSSION.

1. *The Prolongation of Pregnancy.*

The experiments described demonstrate that pregnancy can be prolonged by 4 to 10 days by any of the following treatments: (1) Implantation of anterior pituitary tissue; (2) an alkaline extract of that gland; (3) extracts of human pregnancy urine; and (4) a purified extract containing the growth hormone. The prolongation of pregnancy was associated with a failure of the birth mechanism, since full fœtal development was reached at normal term. In similar circumstances, TEEL (1926), finding the ovaries of such animals to be almost invariably highly luteinised, was of the opinion that prolongation was due to the persistence of the corpora lutea formed as the result of the treatment administered. LEVIN, KATZMAN, and DOISY (1931) were unable to offer an adequate explanation for prolongation, as "several large follicles were usually observed," as well as "a mass of corpora lutea," in the ovaries of animals in which parturition was delayed after injection of pregnancy urine extracts. They also comment on the fact that even

large quantities of follicular hormone do not cause pregnancy to be interrupted. When marked luteinisation of the ovaries was found in conjunction with prolongation, with every indication of functional tissue being present (as, *e.g.*, after recent injection), the possibility cannot be excluded that the inhibition of uterine motility ascribed to a secretion of the corpus luteum (KNAUS, 1929; ROBSON and ILLINGWORTH, 1931) plays a considerable part in the impairment of the birth mechanism, though this action of the corpus luteum has not yet been demonstrated in the rat.

There are, however, indications that another factor is concerned in the maintenance of pregnancy:

(1) Expulsion of a part of the uterine contents occurred in animals the ovaries of which were highly luteinised and in which no enlarged follicles were found. The same ovarian structure was, in other cases, associated with a continuance of pregnancy.

(2) The ovaries removed at biopsy in three rats (M_2, K_2, $B_2^{(1)}$) in which pregnancy was experimentally prolonged consisted of a mass of very large follicles and contained no corpora lutea. The fœtuses were alive at the time and pregnancy continued thereafter for some days. Sections of the uterus between placental sites showed the endometrial structure typical of normal pregnancy.

(3) Pregnancy continued for 3, 4, and 5 days in rats from which both ovaries had been removed at term after injection of an extract of human pregnancy urine, and under-developed fœtuses were born on the 21st and 23rd days of pregnancy after bilateral oöphorectomy on the 16th day.

The dependence of pregnancy on the ovaries has been convincingly demonstrated in a large number of animals, and more particularly in relation to the corpus luteum. In the rabbit, CORNER (1930) was able to maintain pregnancy by the injection of corpus luteun extract after removal of the ovaries as early as 18 hours after insemination; the fœtuses attained full development. In the experiments with extract of pregnancy urine, development does not appear to have advanced far after the ovaries were removed, but abortion did not speedily follow as is stated to occur after oöphorectomy in the untreated animal. JOHNSON and CHALLANS (1930) found abortion to occur in rats within 48 hours of the removal of the ovaries; and it is the writer's experience that abortion occurs in mice within 24 hours if oöphorectomy is performed on or after the 17th day; prior to this fœtuses are absorbed.

It is to be inferred that (*a*) an extra-ovarian factor acts upon the uterus, perhaps restraining uterine motility as suggested by REYNOLDS (1932), and so reinforcing the action of the corpus luteum; (*b*) that mere degeneration of the corpus luteum and renewal of follicular secretion is insufficient to bring about parturition. It is uncertain whether the secretion of the posterior pituitary plays an important

part in parturition in the rat, since, according to the observations of SMITH (1932), the removal of this organ has no effect on pregnancy or on its termination in this animal.

The prolongation of pregnancy with an extract containing growth hormone is suggestive that the factor acting upon the uterus may be hypophyseal. But, in spite of the opinion of VAN DYKE and WALLEN-LAWRENCE regarding the constitution of their extract, it cannot be concluded that this factor is not gonadotropic, for SCHOCKAERT (1931) reports an effect on testicular development in the male duck. Moreover, in our own controls, when growth hormone from as much as 2 grams of anterior pituitary was injected, blood-points formed in the ovaries of immature *mice*, although it was without macroscopic effect on the ovaries of immature *rats*. This amount caused a prolongation of pregnancy in rats, as described. On the basis of the rat unit being equivalent to 4 m.u. (LAQUEUR and DE JONGH (1928)), the effect on the pregnant rat was obtained at a lower level than that at which the immature mouse ovary reacted. The 4 : 1 ratio has, however, been disputed by BECKER and others (1931), and by COWARD and BURN (1927); it is therefore worthy of note that even 4 m.u.[1] of concentrated pregnancy urine extract did not prolong pregnancy in the rat. Further experiments are necessary to determine whether the factor present in human pregnancy urine which has a direct action upon the uterus is of hypophyseal origin, and these are being carried out. The growth hormone appears to be precluded, as its existence in human pregnancy urine is denied (EVANS and SIMPSON, 1928). It is interesting to note that, according to ALLAN and WILES (1932), pregnancy continues for from 2 to 11 days after hypophysectomy in the cat.

2. *The Interruption of Pregnancy.*

Whereas in the experiments by ENGLE and MERMOD (1928) the interruption of pregnancy following implantation of anterior pituitary tissue was held to be due to the œstrous condition induced in the ovaries and uterus, in the majority of the animals injected with urine extract in which absorption occurred such conditions were conspicuously absent: the existence of masses of corpora lutea, the absence of all traces of cornification in the vagina, and the gross anatomical character of the uterus make it exceedingly doubtful if the secretion of the œstrous hormone (alpha) could account for the effect. LEVIN, KATZMAN, and DOISY (1931) report similar conditions as attending interruption after injection of pregnancy urine extracts in rats, and COURRIER (1928) in the rabbit, after injection of alpha.

At the present moment the interference with pregnancy cannot be

[1] A mouse unit is here taken to be the amount which produces blood-points in the ovaries of immature mice weighing 8 to 10 grams.

explained by reference to any known action of gonadotropic hormones. It is unlikely that the effect is due to toxic substances, as fœtuses above the average weight were born after injection at a later date. REYNOLDS (1932) suggests a possible explanation when he describes a markedly increased motility as sometimes preceding uterine quiescence after injection of a pregnancy urine extract in the rabbit.

It is evident that pregnancy is readily interrupted during the early stages of embryonic development; for this reason the divergent effects obtained as the result of administration of the pregnancy urine extract at different stages of pregnancy are not to be explained on the basis of quantity (Table III.); the amounts which in early pregnancy caused absorption, uniformly prolonged pregnancy when injected at a later stage. It is to be noted, however, that neither the alkaline extract of pituitary nor the "purified" growth hormone interrupted pregnancy at any stage.

In examining the points raised in this discussion it is manifest that few conclusions of a definite character can be drawn regarding the mechanism responsible for the prolongation and the interruption of pregnancy respectively. It seems, however, that future studies will have to look to some organ other than the corpus luteum—and perhaps to factors other than gonadotropic hormones—if the physiology of pregnancy and parturition is to be further elucidated. Whereas the investigations of previous authors have shown that the corpus luteum is necessary for the development of the fœtus, it seems now that its secretion alone does not necessarily secure the progress of pregnancy; on the other hand, lutein secretion may be interfered with (at least in the latter part of pregnancy) without disturbance of pregnancy. It is hoped that experiments which are in progress may yield information about these (apparently) extra-ovarian factors.

SUMMARY.

1. The gestation period in the rat can be prolonged by from 4 to 10 days by (*a*) administration of anterior pituitary tissue; (*b*) extract of human pregnancy urine; (*c*) alkaline extract of pituitary; (*d*) extract containing "purified" growth hormone.

2. Pregnancy is apt to be interrupted when injection of the urine extract is made in the early stages.

3. The evidence adduced suggests that the pregnant condition is not maintained solely by the corpus luteum, and that parturition is not due merely to degeneration of that body.

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THE PHYSIOLOGY OF PREGNANCY IN THE RAT. FURTHER DATA BEARING ON THE PROLONGATION OF PREGNANCY, WITH A STUDY OF THE EFFECTS OF OÖPHORECTOMY DURING PREGNANCY.

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The author showed in a previous paper [Hain, 1932 *b*] that the period of gestation in rats could be prolonged by the injection of (*a*) œstrin-free extracts of human pregnancy urine, (*b*) alkaline extracts of anterior pituitary, and (*c*) purified growth hormone (phyone) prepared by the method of Van Dyke and Wallen-Lawrence [1930]. These materials differ in their hormonic content in that extracts of human pregnancy urine are rich in gonadotropic substances and contain no growth hormone [Evans and Simpson, 1928], whereas phyone is held to contain little if any gonadotropic substances. These results suggested that the prolongation of pregnancy might be due to some unidentified hormone of the anterior pituitary. The experiments described below were undertaken to amplify the results already described, and to determine whether the presence of the ovaries was essential for the prolongation of pregnancy.

I. Methods.

Experimental Animals.—Altogether 64 pregnant albino rats of the Edinburgh Wistar Stock were employed: for the most part these were primigravid and between 3 and 5 months old. They were weighed on the day of mating and at intervals until the twelfth day, from which time weights were recorded daily until parturition; after oöphorectomy, however, each rat was weighed thrice daily: at 9 a.m., 5 p.m., and 8.30 p.m., and the cages examined at each occasion and also at midday. In the albino rat parturition occurs normally between the 22nd and 23rd day; the average weight of a new-born fœtus of this stock is 5·25 grm., and of a full-term placenta 0·417 grm. [Hain, 1932 *a*].

Oöphorectomy.—Care was taken to remove the ovary intact and to exclude the tip of the uterine cornu from the ligature which was placed round the uterine end of the oviduct. The administration of anæsthesia

and operation occupied 10 to 15 minutes. Although a careful examination was made at the time of operation to ensure entire removal of the ovaries, all rats so used were killed within 20 days after parturition, when a further examination was made for any trace of ovarian tissue.

Extracts.—(1) The alkaline extract of anterior pituitary was similar to that used in the previous experiment [Hain, 1932 *b*]; 1 c.c. was equal to 1 grm. of tissue.

(2) Phyone, *i.e.* the purified growth hormone of the anterior pituitary prepared by Van Dyke and Wallen-Lawrence. In the 1932 experiment a preparation based on the prescription of these workers was employed, but since it was found impossible to use the high centrifugalisation which was deemed by them to be necessary (even 35,000 R.P.M. were inadequate), it was feared that the prolongation of pregnancy obtained might not follow injection of the pure extract. At the request of Dr Wallen-Lawrence supplies of phyone have kindly been supplied by Dr Klein from the Wilson Laboratories, Chicago. 1 c.c. of phyone was equal to 0·8 grm. of anterior pituitary tissue.

II. The Effects of Oöphorectomy.

Twenty-one Pregnant Rats.—The ovaries were removed from 14 controls on the 16th and 17th days of pregnancy and at normal term in order that data might be available of the continuance of pregnancy in the uninjected rat after oöphorectomy. It was thus possible to ascertain to what extent, if any, anterior pituitary extracts were able to modify the duration of pregnancy and to affect fœtal development in the absence of the ovaries. Control operations were performed at normal term to determine the effect of trauma (Table I.).

The results demonstrate that pregnancy is not speedily terminated by removal of the ovaries on the 16th day, but persists till the 20th to 21st day, and that a degree of fœtal and placental development is attained considerably beyond that reached at the time of oöphorectomy: a fœtus weighing 3·0 grm. was recovered, and portions of partly eaten fœtuses were almost term size; placentæ removed from the uterus on the 22nd, 23rd, and 24th days of pregnancy (rats S6, S5, S4) weighed 200, 300, 400, and 500 mg. each—11 in all. The drop in weight at parturition was between 12 and 18 grm.

Oöphorectomy on the 17th day was followed by abortion at the same stage of pregnancy, and parturition was protracted. As was to be expected, there was greater fœtal growth than in the 16th-day group: thus there were 9 fœtuses weighing >3 grm. and 3 of >4 grm. Not only was individual growth greater, but a larger number of fœtuses participated in this development. This is reflected in the number of fœtuses aborted: 5, 12, 5, and 6; and in the drop in weight at parturition: 44, 42, 25, and 35 grm. The most noteworthy fact, however,

is that one fœtus was born alive (to V6) during the night of the 20th to 21st day.

Following oöphorectomy on the 18th day of pregnancy rat X7 aborted undergrown, stillborn fœtuses at term (22nd day) and again two days later.

Contrary to expectation, oöphorectomy at normal term did not precipitate parturition, but appears definitely to have delayed it, so that gestation was prolonged for 2 to 2½ days.[1] Parturition was protracted, and lasted between 10 and 20 hours; in all litters, with one exception, some fœtuses were born alive—in one case on the 25th day (rat S9). Generally throughout this group either the placentæ or the fœtuses were above the average size.

For control purposes laparotomy and manipulation were performed on seven rats at normal term: four were unilaterally oöphorectomised, and in the remainder both ovaries were merely raised. Four of the seven rats had live litters during the night of the 22nd day or by 9 a.m. on the 23rd day; of the others, two littered during the night of the 23rd and one on the 24th day; the weight of the latter remained stationary for 51 hours prior to littering. It appears, therefore, that operative interference a few hours before parturition is due may have the effect of delaying parturition in some cases, more especially when both ovaries have been manipulated. In no case, however, was parturition prolonged to the extent observed after bilateral oöphorectomy at the same stage of pregnancy, and the results obtained in that group cannot therefore be ascribed entirely to operative trauma.

A. *Alkaline Extract of Anterior Pituitary* (23 pregnant rats).

This extract was shown in a previous paper [Hain, 1932 *b*] to prolong pregnancy between 5 and 7 days. Two control rats were injected to confirm the activity of the sample of extracts used in the present experiment. One animal (Table II., rat A7) reacted abnormally, and showed premature but prolonged parturition; in the other animal (Table II., rat A10) pregnancy was prolonged 5 days, which was the average previously obtained.

The remaining 21 rats were oöphorectomised,

(*a*) at term (Table II.),
(*b*) on the 16th day of pregnancy } Table III.,
(*c*) on the 13th day of pregnancy }

following the injection of 2·5 to 3 c.c. of alkaline extract.

(a) *Oöphorectomy at Term after Injection* (Table II.).—Pregnancy was prolonged, but the prolongation was not equal to that obtained with intact injected animals [Hain, 1932 *b*), and did not exceed that in the uninjected rat oöphorectomised at the same stage of pregnancy.

[1] In the case of rat X2 only 3 out of 12 fœtuses were born on the 23rd day.

When injection was resumed after removal of the ovaries, the duration of pregnancy was not augmented. It is of interest to note that, again, fœtuses were born *alive* 2 and 3 days after oöphorectomy at term.

(b) *Alkaline Extract of Anterior Pituitary+Oöphorectomy on 16th Day* (Table III.).—Although there is one instance of the continuance of pregnancy for 7 days after removal of the ovaries on the 16th day (rat C5), the average of 5 days does not show an increase over that in the uninjected animal (Table I.). As in the latter, the fœtuses born were undergrown, also a considerable amount of absorption took place after oöphorectomy at this stage, as is indicated by the maternal weight which remained stationary for several days, as well as by the small number of fœtuses born. When oöphorectomy was followed by a resumption of injections (Table III. B), the maternal weight was considerably greater than in those cases when injection ceased at operation. That this was partly due to an augmentation of fœtal growth is demonstrated by the drop in weight (19, 27, and 16 grm.) which occurred between the 20th and 21st days, at which time, presumably, littering took place. In spite of repeated examination of the cages it was exceedingly difficult to secure fœtuses for weighing or measuring, as they were generally eaten by the mother at birth; it is not known, therefore, whether the increase in weight noted was due to an increase in the weight of individual fœtuses or to a diminution of absorption resulting in a larger number of fœtuses developing. Owing to this difficulty ventral laparotomy was performed on rat P9 (Table III. B) on the 19th day of pregnancy. Large fœtuses were then seen in the uterus, each measuring 23 mm. long in its curled-up position in the uterus. Parturition occurred during the night and the fœtuses were eaten. The crown-rump length of a fœtus on the 19th day is given by Donaldson [1924] as 22·7 mm.; those of rat P9 must have exceeded this figure and were perhaps normal for the Edinburgh Wistar Stock at the same stage, the newborn fœtus of which has a higher average weight than Donaldson's stock (5·25 *versus* 4·63 grm.). It should be noted that injection was continued until a marked drop in weight indicated abortion, and in this way the suggestion cannot be made that the loss in weight was attributable to the stoppage of injection of an extract rich in growth hormone.

It was hoped that the injection of very large amounts of alkaline extract of anterior pituitary after oöphorectomy might permit pregnancy to continue to term, and accordingly 5 to 7 c.c. were injected after the date of operation (Table III. C). The increase in maternal weight which followed was actually much less than when a smaller amount was administered; yet the uterus of one rat in this group contained a fœtus almost as large as the maximum obtained following oöphorectomy at this stage of pregnancy, viz. 3·25 grm. as against 3·6 grm. (rats T5 and C5 respectively).

(c) Table III. D illustrates that in the injected rat the pregnant condition is not terminated (*i.e.* the uterus is not evacuated) even when oöphorectomy is performed on the 13th day of pregnancy, since placentæ of almost term size were found in the uteri of two rats; 300 and 250 mg. on the 19th and 17th days. The average weight of a 13th-day placenta is not available for comparison, but that of the 15th day is 187 mg. $\pm$6·5 [Hain, 1932 *a*]. Absorption was irregular in that 8 and 9 other placentæ found in the same two rats were diminutive and atrophic.

In four rats in which oöphorectomy was performed in mid-pregnancy after the injection of alkaline extract of anterior pituitary, the ovaries were found to contain blood points or hæmorrhagic follicles. In one (rat B5, which was accidentally drowned later) 10 follicles of medium size and 1 measuring 3 mm. were counted in one ovary together with 4 old corpora lutea, and one very vascular one. The other ovary contained corpora lutea, 3 blood points, and several small follicles.

B. *Purified Growth Hormone of Anterior Pituitary: Phyone*

(12 pregnant rats, also unmated rats and mice as controls).

In order that the effects of the American preparation of phyone might be compared with those obtained when the extract prepared here was used, the equivalent of the same amount of anterior pituitary tissue was injected in both experiments: 2·5 c.c. of phyone were equal to 2·0 c.c. of Dr Illingworth's preparation [Hain, 1932 *b*]. Of 4 rats so injected (Table IV.) pregnancy was definitely prolonged in one: rat P4 for 5 to 7 days. In this animal prolongation was associated with all the features previously recorded: protracted parturition and still-born fœtuses of abnormally large size. Five days after parturition she again mated, and littered on the 23rd day. It is perhaps significant that the two rats which littered at the normal time had large litters, viz. 12 and 13.

An effort was made to secure additional examples of prolongation following the administration of phyone: accordingly, two rats were injected with a total of 4·0 c.c. Pregnancy was prolonged for 7 to 8 days in rat S7, but occurred within the normal limit in rat S8, which had a litter of 14. Laparotomy was performed on rat S7 on the 26th day, that the ovaries might be examined. The left ovary contained apparently normal corpora lutea, and no follicles were seen; in the right ovary were several diminutive corpora lutea resembling beads, and 4 or 5 large follicles, one of which was of considerable size. Both ovaries were larger than those removed in normal pregnancy at term. Pregnancy was not terminated till three days later.

The results in the group in which oöphorectomy at term followed injection of phyone (Table IV.) were similar to those obtained when alkaline extract of pituitary was employed, but the effect was less

marked; the fœtuses and placentæ were larger than the average. Two of the three rats oöphorectomised on the 16th day gave birth on the 21st day to undergrown fœtuses weighing 1·5 to 3·0 grm.

In a personal communication Dr Wallen-Lawrence states that "in all preparations (of phyone) which have been made and assayed in our own laboratory, these factors affecting the ovary have invariably been found absent when tested for in rats." The indications were, therefore, that the effect of phyone in prolonging pregnancy was due to an action either directly upon the uterus or, at anyrate, through some extra-ovarian channel. To investigate this point further, the same amount of phyone as was given to the pregnant rats was injected in each of the following in 5 doses (0·5 c.c. twice daily):—

2 immature rats aged 5 weeks,
4 ,, mice ,, 3 ,,
2 mature mice, the œstrous cycles of which had been found to be strictly regular during the five weeks prior to injection.

Vaginal smears continued to be taken throughout injection and for two cycles thereafter, and indicated that the normal cyclic activity of the ovaries was slightly modified by the injections. In all of the immature mice the vaginæ opened in the course of injection; in one both cornified and epithelial cells were found on the 5th day (weight of mouse $10\frac{1}{2}$ grm.), and histological examination confirmed shedding of the cornified layer; in another, "blood points" were found in both ovaries on the 6th day as well as many corpora atretica. No effect was observed macroscopically in the remaining two mice. It is to be noted that the typical ovarian reaction ("blood points") was much less pronounced than when Dr Illingworth's preparation was injected in mice of the same age. No "blood points" were produced in immature *rats* injected with phyone.

In the previous experiment in which extracts of pregnancy urine were injected into pregnant rats [Hain, 1932 *b*], it was found that an amount equal to four times the unit which produced "blood points" in immature mice failed to prolong pregnancy when injected into rats. As this difference seemed to merit further investigation, 8 pregnant rats were injected with a concentrated protein-free extract of pregnancy urine prepared by Mr Marshall of the Macaulay laboratory. The mouse unit was first ascertained, after which 4 rats received 1 m.u. each from the 16th to 18th days of pregnancy and the remainder 4 m.u. each from the 14th to 16th days. In none was pregnancy prolonged; all littered by 9 a.m. on the 23rd day, with the exception of Q8 which aborted on the 20th day 11 fœtuses, of which the combined weight was only 16 grm. This rat had been injected with 4 m.u.

Discussion.

1. *The Effect of Oöphorectomy on Pregnancy.*—It is now common knowledge that, in most species, removal of the ovaries, except in the latest stages of pregnancy, causes abortion. Man provides an outstanding exception in that oöphorectomy between the first and third months may be followed by a full-term pregnancy (33 instances are cited by Ask-Upmark [1926]). In the pregnant mare [Cole *et al.*, 1931] and the Platyrrhine monkey [Wislocki, 1930] there are indications that the corpus luteum does not play the important rôle in pregnancy which is attributed to it in the lower animals. In the rabbit oöphorectomy at the 16th and 21st days of pregnancy caused abortion 4 days later [McIlroy, 1912], and in those guinea-pigs in which removal of the ovaries interrupted pregnancy, abortion did not take place till several days after the operation [Herrick, 1928]. According to Johnson and Challans [1930] bilateral oöphorectomy in rats between the 6th and 21st days was followed by abortion in 48 hours.

(1) The experiments herein described show that in the rat abortion does not immediately follow removal of the ovaries on the 16th and 17th days, that considerable fœtal and placental growth takes place thereafter, and that living fœtuses may be born 4 to 5 days after oöphorectomy. Birth is premature (20th to 21st days), fœtal development is incomplete, and there is absorption. It follows therefore that (*a*) the removal of the inhibitory influence exerted upon the uterus by the corpus luteum is not sufficient to cause evacuation of the uterus.[1] (*b*) For the full development of the fœtus the ovaries are necessary throughout pregnancy in the rat. Of this, further evidence is afforded by the experimental findings of Haterius and Nelson [1929], as a normal pregnancy ensued after oöphorectomy in rats in which the operation was preceded by the transplantation of virgin ovaries.

(2) Oöphorectomy at normal term delays parturition for 48 hours, an effect which laparotomy showed was not attributable to trauma. Parturition can occur in the absence of the ovaries, but is protracted. Protracted parturition occurred also in those rats in which pregnancy was prolonged after injection of anterior pituitary extracts, and this suggests that, in the latter also, parturition may have occurred independently of ovarian action. The expulsion of fœtuses observed on the 19th to 20th and 20th to 21st days may possess some significance and be related to some uterine change which normally manifests itself at this stage of pregnancy, but, on the other hand, the circumstance may be fortuitous. It seems possible that, in the rat, the secretion of

[1] This function of the corpus luteum has not yet been demonstrated in the rat, but in the rabbit there are indications that the corpus luteum is not the sole source of inhibition of uterine contractions [Westman, 1926; Reynolds and Friedman, 1930; Reynolds, 1932].

œstrin in combination with the degeneration of the corpus luteum is a *sine qua non* to the *rapid* expulsion of the fœtus to ensure living births, and that parturition may be delayed and gestation prolonged either by suppression of the activity of the anterior pituitary [Pencharz and Long, 1931] or by removal of the ovaries. In the experiments performed on rats by Pencharz and Long, hypophysectomy between the 10th and 20th days of pregnancy was followed by a prolongation of gestation of 3 to 4 days, and in all cases the mother died without littering, unless operative measures were taken to remove the fœtuses. The explanation apparently lies in the fact that corpora lutea persist for weeks and even months after hypophysectomy [P. E. Smith, 1927 and 1930]. It is readily understood, therefore, that when any trace of anterior pituitary tissue was left in the rats operated upon by Pencharz and Long, parturition was normal. Yet since degeneration of the corpus luteum and the rise of follicular secretion were unable to effect parturition in the experiments here described, and as parturition can occur in the absence of the ovaries, it seems to be implied that a third factor plays a part in the birth mechanism. In view of the experiments of P. E. Smith [1932] it appears that we must look elsewhere than to the posterior pituitary for this factor, at least in the case of the rat.

2. *The Effect of Anterior Pituitary Extracts on Pregnancy in the Absence of the Ovaries.*—The possibility that the prolongation of pregnancy after the injection of growth hormone was due to direct action on the uterus was investigated for two reasons: (1) The ovarian condition associated with prolonged gestation both in this experiment and in that previously reported [Hain, 1932 *b*] was not uniform, in that prolongation was associated equally with pronounced and multiple luteinisation, and the disappearance of corpora lutea combined with the occurrence of large follicles measuring 2 mm. in diameter. (2) Teel [1929] reported a marked effect of the growth hormone upon the endometrium of a dog in which acromegaly was experimentally induced by growth extract. The ovaries contained numerous large follicles, but no corpora lutea. He therefore suggested that "the growth principle by itself may cause great enlargement of the genital system."

The experiments here reported do not indicate a direct action of the growth hormone upon the structure of the endometrium of the rat, as determined by the duration of pregnancy after oöphorectomy (*e.g.* at term), since this was not equal to that in the intact (injected) animal, and such prolongation as was observed found parallels in the control oöphorectomised rats. The effect upon fœtal and placental growth in one group of rats in which oöphorectomy was followed by further injection (Table III. B) did not occur in other but similar circumstances. It is clear that the extracts were unable to maintain pregnancy to its normal termination in the absence of the ovaries. At the same time

the results suggest that the anterior pituitary may have other forms of action on the ovary than those commonly described.

3. *Quantitative Considerations in the Action of Pregnancy Urine and Phyone in prolonging Pregnancy.*—Any interpretation of the results should be based on the assumption that the substances responsible for the prolongation of pregnancy are common to both extracts. It then follows that the growth hormone can be excluded. As to gonadotropic substances, it must be recognised that both extracts produced ovarian effects, namely, hæmorrhagic follicles.[1] It is commonly accepted that the substances which produce follicular maturation and luteinisation in the ovaries are also responsible for the formation of hæmorrhagic follicles. Whether this should prove to be correct or a separate substance be found responsible for the latter, the prolongation of pregnancy cannot be referred to a given concentration of gonadotropic hormones or of this substance, *as measured by the typical ovarian effect, hæmorrhagic follicles, or "blood points,"* in view of the following:—

The minimum amount of urine extract or of phyone which produces "blood points" in immature mice,	1 unit
To produce prolongation of pregnancy by pregnancy urine extracts there are needed . . .	>4 units
To produce ditto by phyone	1 unit

In view of these differential thresholds, it would seem that the "blood-point" factor cannot in itself account for the prolongation of pregnancy. Three interpretations are suggested:

(*a*) That it *is* responsible for prolonging pregnancy, but its action may be partly overridden by the presence of Rho factors in the pregnancy urine extract. When the more concentrated extracts are used, particularly after mid-pregnancy, this effect would less readily be experienced owing to the luteinising properties of such extracts.

(*b*) It is possible that the "blood-point" factor is only in part responsible for prolonging pregnancy, and that an unknown factor (x) present in larger amount in the anterior pituitary extract than in that of pregnancy urine, intensifies the prolonging action of the "blood-point" substance without increasing the "blood-point" effect.

(*c*) On the other hand, it is possible that this x factor, acting independently and alone, is responsible for the prolongation of pregnancy. In any case, the effect upon the gestation period cannot be accounted for by the different concentrations of growth and gonadotropic hormones, and we are forced to assume the existence either of an additional hormone or of a hitherto unknown property of the anterior pituitary secretions.

[1] Effects upon the sex apparatus following the injection of phyone have been reported by: Adams [1932], on *Triturus viridescens*; Schockaert [1931], on the drake; Leonard [1931] and Hertz *et al.* [1932], on the rabbit.

The lack of uniformity in the morphological condition of the ovaries associated with the prolongation of pregnancy, the argument put forward above, and the likelihood that hæmorrhagic follicles did not form in all pregnant rats in which pregnancy was prolonged (since they did not occur in the immature rat [1]) suggest the advisability of examining channels through which the prolongation of pregnancy may have been achieved.

In view of the experimental findings of Reynolds [1932], both with pregnancy urine and an alkaline extract of anterior pituitary, it appeared possible that the motility (rather than the structure) of the uterus was affected. However, since in the experiments herein described the full effect was achieved only in the presence of the ovaries, any action that there may have been *directly* on uterine motility can only have been partially responsible for the ultimate effect.

On the other hand, the immobility of the uterus might be due, not to direct action from without, but to the abnormal character of the ovarian stimuli. Since highly follicular ovaries were associated with live fœtuses in utero at, and even after, normal term following the injection of pregnancy urine extracts and, to a less degree, after the injection of phyone (rat S7), it would appear that the apparent uterine irreactivity was due to the suppression of some factor (presumably ovarian) essential to parturition, and that the action of the anterior pituitary was incomplete. A partial suppression of hypophyseal activity as the result of the administration of pituitary substances finds a parallel in the stultifying effect of the injection of œstrin on the ovaries [Allen, 1928 (monkeys); Kunde *et al.*, 1931 (rats); and Meyer *et al.*, 1931 (rats)]. Several workers hold that this action is an indirect one, and Moore and Price [1930] have advanced the theory that the reduced size of immature ovaries after the injection of œstrin is due to the inhibition of the anterior pituitary in its secretion of gonad-stimulating hormone. To draw such a parallel, therefore, involves a slight (but perhaps legitimate) extension of Moore and Price's theory.

Finally, the possibility that the extracts inhibited the release of some substance (perhaps extra-ovarian) which is essential to the birth mechanism, is not precluded.

General Result.—The experimental results indicate that an unknown principle of the anterior pituitary may cause pregnancy to be prolonged. This unidentified hormone acts either directly or indirectly through the ovaries, since it is unable to maintain pregnancy till normal term in their absence.

Conclusions.

1. Previous work showed that the gestation period in the rat could be prolonged by the injection of substances rich in gonadotropic hor-

[1] The relation of the rat unit to the mouse unit was discussed in the previous paper [Hain, 1932 *b*].

mones, but containing no growth hormone [Hain, 1932 *b*]. The results in this paper show that it can be prolonged by an extract of anterior pituitary rich in growth hormone (phyone), and having only a slight ovarian reaction.

2. The ovarian reaction was the same with both extracts and was of a typical character, namely, the production of hæmorrhagic follicles or "blood points." It is considered unlikely that the substance producing this effect was, by itself, responsible for the prolongation of pregnancy. The most probable alternative is the existence of a hitherto unknown hypophyseal factor which causes the prolongation of pregnancy. Channels through which it may act are briefly examined.

3. The combination of oöphorectomy with injections of the above-mentioned extracts shows that the presence of the ovaries is essential for the production of the full effects of the extracts in the prolongation of pregnancy.

4. Oöphorectomy interrupts pregnancy, but the action is not immediate, and removal of the corpus luteum does not cause evacuation of the uterus. Considerable fœtal and placental development takes place in the absence of the ovaries, but the ovaries are necessary for full-term development in the rat.

Parturition can occur in the absence of the ovaries, but in such event it is protracted. This feature was common also to the group in which anterior pituitary extract was injected, and it is suggested that in both cases parturition may have occurred without ovarian action. Since parturition is protracted after removal of the ovaries and is completely prevented in absence of the anterior pituitary, it seems probable that ovarian-hypophyseal action is essential to the *rapid* expulsion of the fœtus and to ensure live births; at the same time the existence of another factor in the birth mechanism is strongly indicated.

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OOPHORECTOMY	ANIMAL	DAYS 1-13	14	15	16	17	18	19	20	21	22 (NORMAL TERM)	23	24	25	26	GAIN IN WT. AFTER O GMS.	DROP IN WT. AT PARTURITION GMS.	PARTURITION.
16TH DAY	S4				O			+					K			18	18	2 PARTLY-EATEN FOETUSES—21ST DAY-8.30 PM OTHERS O'NIGHT. PLACTAE AT K - 300, 400, 500 MG.
	S5				O		+		?			K				TRACES	12	TRACES EVE 20TH DAY FOETUS 3 GMS. 21ST DAY. PLACTAE AT K - 200, 300, 400 MG.
	S6				O+				?		K					STAT.	14	TRACES 20TH AND 21ST DAYS, NIL FOUND. PLACTAE AT K - 200, 300 MG.
17TH DAY	V2					O			+							7	44	5 FOETUSES 1·4 TO 2 GMS.—20TH DAY 5 PM TO 9 A.M. 21ST DAY
	V3					O			+							15	42	12 " 1·9 TO 3·7 GMS— " " 8 PM TO " " 22ND DAY
	V6					O		+								13	25	5 " 2·0 TO 4·6 GMS— " " 9 A.M. TO " " 21ST DAY (ONE BORN ALIVE ON 21ST DAY)
	V8					O				+						20	35	6 " 3·6 TO 4·0 GMS.—21ST DAY 8.30 PM. TO 7 PM. 22ND DAY—TUMOR
18TH DAY	X7						O			+						4	30	4 " 2·5 TO 4·3 GMS—22ND DAY 10·15 A.M. AND 24TH DAY 9 & 11·30 A.M.
NORMAL TERM	V7										O+					–	83	12 " 5¼ TO 6 GMS.—24TH DAY 9 A.M. TO 9 A.M. 25TH DAY.
	S9										O+					–	84	9 " AVER. 7·6 GMS—24TH DAY 8.30 P.M. TO 9 A.M. 25TH DAY (3 BORN ALIVE ON 25TH DAY)
	T8										O+					–	66	8 " 5 TO 6·5 GMS.—24TH DAY 11.40 A.M. TO 9 A.M. 25TH DAY (ONE BORN ALIVE ON 24TH DAY)
	V5										O+					–	67	10 " 5·2 TO 6·3 GMS.—24TH DAY 2 P.M. TO 6 P.M. 25TH DAY (6 BORN ALIVE ON 24TH DAY)
	S8										O+					–	53	9 " AVER 4·5 GMS—24TH DAY 9 A.M. (ONE BORN ALIVE)
	X2										O+					–	87	12 " " 5·3 GMS—23RD DAY 9 A.M. TO 24TH DAY 9 A.M. (2 BORN ALIVE ON 24TH DAY)
UNILATERAL OÖPHORECTY. AT NORMAL TERM	Z1										L+					–	49	6 (ALIVE) BY 9 A.M. 23RD DAY
	B3										L+					–	34	6 (4 ALIVE) " " " "
	P5										L	+				–	24	4 (2 ALIVE) 23RD DAY 8·30 P.M. AND DURING NIGHT.
	P4										L+					–	49	7 (5 ALIVE) BY 9 A.M. 23RD DAY
LAPAROTOMY AT NORMAL TERM.	X4										L+					–	35	4 (1 ALIVE) 24TH DAY NOON. WT. STATIONARY 51 HOURS.
	X5										L+					–	25	4 (ALIVE) BY 9 A.M. 23RD DAY.
	X6									+	L					–	60	9 (DEAD) 23RD DAY 8.30 P.M. AND DURING NIGHT

TABLE I.—The Effect of Oöphorectomy on Pregnancy, with Seven Laparotomies as Controls.

— injections. ■ parturition or significant drop in weight. + max. wt. reached. O = oöphorectomy. K = killed. L = laparotomy.

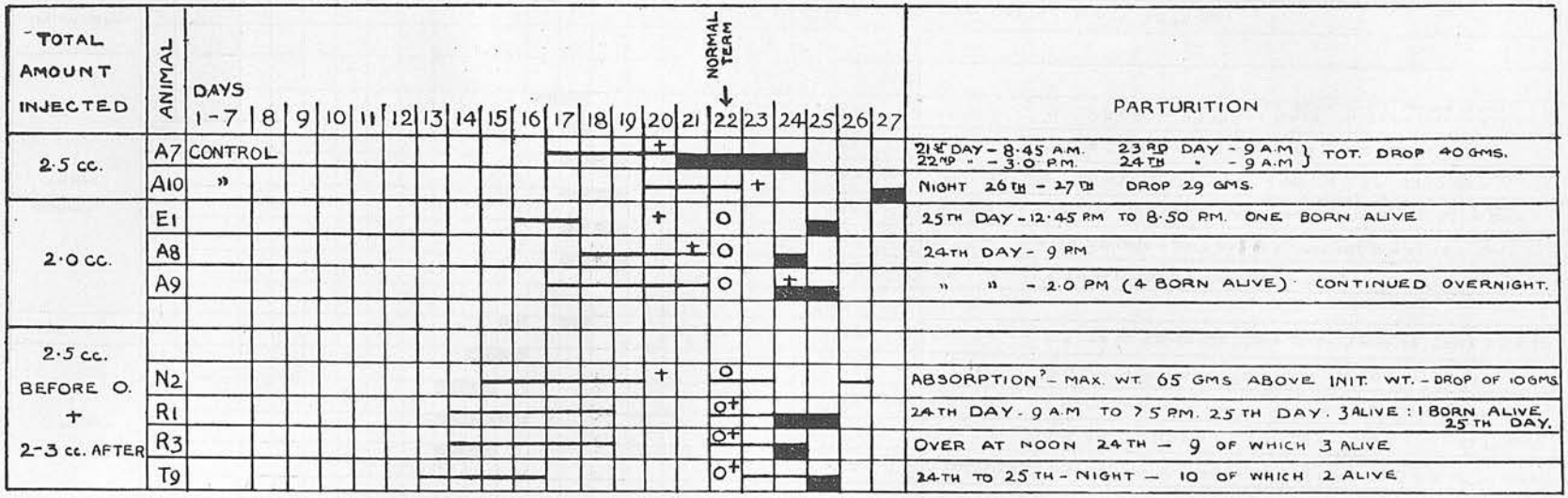

TABLE II.—The Effect of Alkaline Extract of Anterior Pituitary on the continuance of Pregnancy after Oöphorectomy at Normal Term.

TOTAL AMOUNT INJECTED	ANIMAL	DAYS 1-7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	SECTION	GAIN IN WT. AFTER O. GMS.	DROP IN WT. AT PARTURITION GMS.	PARTURITION
																NORMAL TERM ↓						
2·5 cc	M1										O			+					A	1	14	TRACES MORN 21ST DAY NIL FOUND
	C5									+	O					+		K		MAX. ATTAINED 15TH & 21ST DAY	KILLED	FOETUS REMOVED UT. 3·6 GMS. PLAC 1·6 GM
	D5										O+									NIL	12	2 TINY FOETUSES WT OF ONE 1·75 GM
2·4 cc. BEFORE O. + 2·0 cc AFTER																						
	P6										O		+			K			B	18½	(4)	PROBABLY ABSORPTION – UT. EMPTY
	P7										O				+	K				29½	19	NIGHT 20TH TO 21ST. DAY: PLAC. 400 MG
	P8										O				+	K				28½	27	" " " " " : PLAC 340 MG.
	P9										O			+		K				20	16	" 19TH TO 20TH DAY {FOETUSES SEEN AT VENT. LAPARTY
3·0 cc BEFORE O. + 5-7 cc. AFTER																						
	T3										O+								C	NIL	9	FOETUSES PALPATED; NIL FOUND: 3 PLAC. IN UT. – AVER. WT 230 MG
	T4										O		+							2	9	" " ; NIL FOUND
	T5										O				+		K			6	>6	" " FOETUS IN VAG. 5 P.M. 20TH DAY: " " UT. 3·25 GM.
	T6										O			+						9	13 {20TH – 21ST	" " FOETUS IN VAG. 5 P.M. 19TH. DAY: OTHERS 20TH – 21ST.
3·0 cc. BEFORE O. + 4·0 cc. AFTER																						
	T7							O						+K					D	28	–	NIL UT. CONTAINED PLACTA 1 = 300 MG.
	S1							O				+K								10½	–	NIL " " 1 = 250 MG. AND 8 MM. EMBRYO
	S2							O				+K								11½	–	NIL " " ATROPHIC.

TABLE III.—The Effect of Alkaline Extract of Anterior Pituitary on the continuance of Pregnancy after Oöphorectomy on the 16th and 13th Days of Pregnancy.

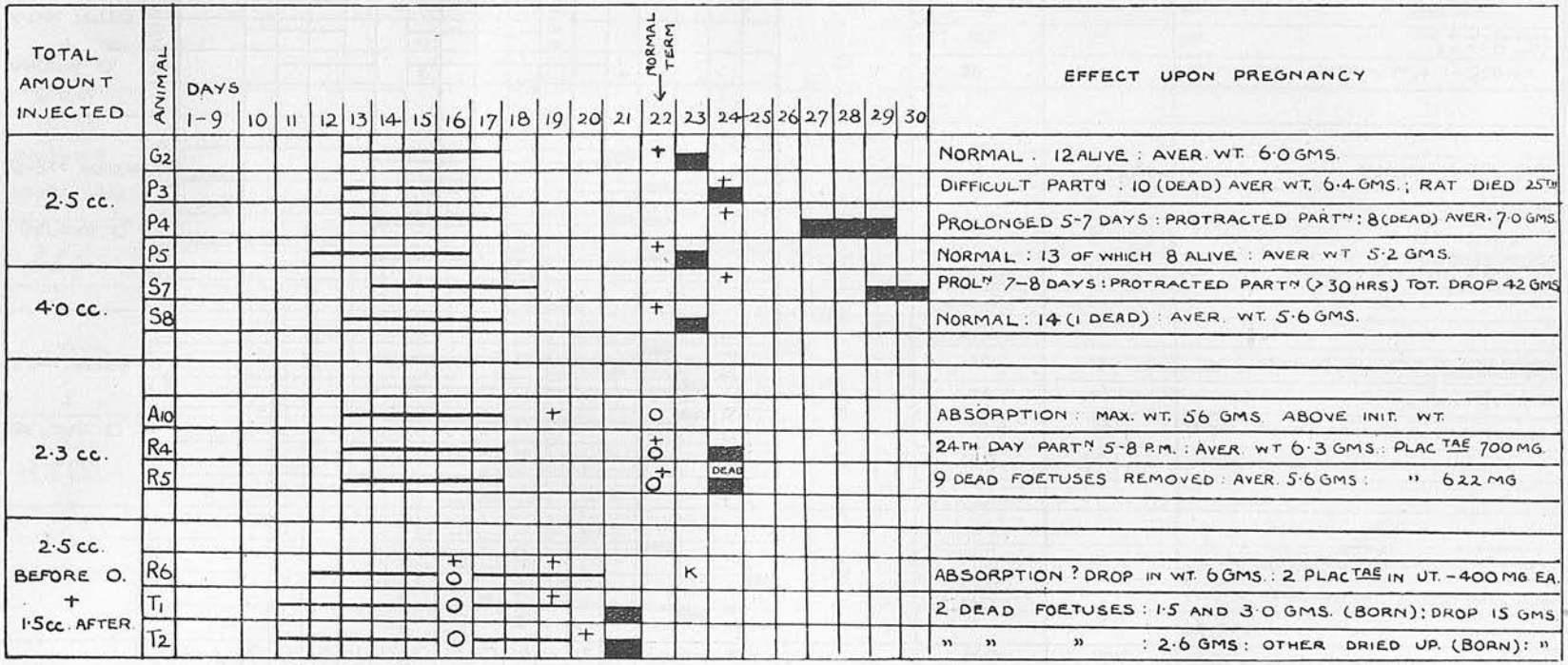

TABLE IV.—The Effect of Phyone (Purified Growth Hormone) on Pregnant Rats, with and without Oöphorectomy.

CONTENTS

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THE PHYSIOLOGY OF PREGNANCY IN THE RAT: AN HORMONAL INVESTIGATION INTO THE MECHANISM OF PARTURITION. EFFECT ON THE FEMALE RAT OF THE ANTE-NATAL ADMINISTRATION OF ŒSTRIN TO THE MOTHER.

By A. M. Hain (Carnegie Research Fellow). Institute of Animal Genetics, University of Edinburgh.

(*Received for publication 25th January* 1935.)

The two main theories as to the cause of the onset of labour have as their basis (1) the regression of the corpus luteum, (2) the activity of the œstrous hormone. According to one, the withdrawal of luteal activity releases the inhibition exerted throughout pregnancy upon the reactivity of the uterus to the secretion of the posterior pituitary [Knaus, 1930]. The other theory postulates the necessary co-operation of follicular secretion by means of its synergistic action in sensitising the uterine muscle to the secretion of the posterior pituitary. This action was first demonstrated by Miura [1926], and has since been confirmed by numerous workers, *e.g.* Parkes [1930], Robson [1933]. According to both theories changes in the ovary, uterus, and pituitary are involved.

The present investigation was prompted by certain considerations arising out of recent experimental results:—

(*a*) The removal of the ovaries from pregnant rats was not immediately followed by parturition, and their removal at normal term even delayed parturition; also fœtuses were expelled in the absence of ovarian secretion [Hain, 1934].

(*b*) Parturition can occur in the absence of the posterior pituitary [Smith, 1932, rat; Allan and Wiles, 1932, cat].

(*c*) In the rat, removal of the anterior pituitary after mid-pregnancy causes gestation to be prolonged, and the mother dies, unable to litter [Pencharz and Long, 1931; Selye, Collip, and Thomson, 1933].

The foregoing suggested that the effect upon pregnancy of œstrin not only in conjunction with oxytocin, but also in conjunction with anterior lobe secretion, might be further investigated.

The experiments recorded in the present paper deal with:—

I. The effect upon pregnant rats of œstrin (i) alone; (ii) in conjunction with oxytocin; (iii) in conjunction with anterior pituitary-like hormone.

II. (*a*) The action of the last two substances when administered in the absence of œstrin. (*b*) The effect of anterior pituitary-like hormone when followed either by œstrin or by oxytocin.

III. An investigation of the effects upon pregnancy of substances from parturient rats. Since lactation almost synchronises with parturition, the action of a hypophyseal preparation said to contain the lactation hormone was also examined.

IV. In order to study the normal act of parturition observations were made of a parturient rat throughout the expulsion of a single fœtus.

I. Material and Technique.

Experimental Animals.—302 pregnant rats, 153 immature and ovariectomised mice and rats, and 1 rabbit were used. All pregnant rats were weighed daily from the date of injection or implantation as a check on absorption or abortion, and whenever rats were treated before the appearance of placental sign the existence of pregnancy was first ascertained by means of laparotomy.

Standardisation of anterior pituitary or pregnancy urine extracts was performed by the injection of three weeks old mice and rats. Ovariectomised animals were not used until a month after operation.

Extracts.

(1) *Œstrin.*—Two crystalline preparations obtained from British Drug Houses Ltd. were used: Keto-hydroxy-œstrin and Tri-hydroxy-œstrin. In the earlier stages of the experiment these were injected in an oily solution, but subsequently 25 per cent. (and latterly 10 per cent.[1]) alcohol was substituted in the hope of obtaining a speedier effect. As the results were the same with both media, no differentiation is made in the text.

In some experiments 15–18 hourly injections were spread over 30–36 hours; but when 0·5 mg. and 1·0 mg. were administered injections were confined to 12 hours. By adopting this procedure (as to time limit and frequency of injection), it was hoped to secure more complete absorption [Marrian and Parkes, 1929].

(2) *Anterior Pituitary Extract.*—Ox anterior pituitaries were ground and extracted with a volume of 1 per cent. acetic acid equal to the volume of tissue used. Cornification in immature rats occurred within 48 hours of the first injection of a total of 1·2 c.c., and this amount was accordingly injected when anterior pituitary extract was employed on pregnant rats.

(3) *Human Pregnancy Urine.*—(i) As a source of anterior pituitary hormone, and in order to supplement supplies from this source, two extracts of pregnancy urine were utilised: (*a*) Concentrated, œstrin-

[1] As considerable skin irritation, depilation, and abrasion occurred when 25 per cent. alcohol was used, the weaker solution was substituted with no ill-effects.

free extracts prepared by Mr. P. G. Marshall [1931 and 1933]. On the basis of a unit being the minimum amount which caused hæmorrhagic follicles to form in the ovaries of immature mice, it was found in previous experiments [Hain, 1932] that a minimum of approximately 12 mouse units was required to cause pregnancy to be prolonged in the rat. (*b*) Antuitrin "S" (kindly supplied by Parke, Davis & Co.). When tested on 28–30 days old rats of our own stock, the unit, based on the occurrence of "blood spots," lay between 0·01 c.c. and 0·02 c.c. (ii) *Pregnandiol.*—The author is indebted to Dr. Butenandt for supplying 100 mg. crystals; these were dissolved in boiling alcohol, and an aqueous extract was prepared which gave negative results on 20 pregnant rats.

(4) *Posterior Pituitary Extract.*—The preparations of the posterior pituitary: "Pituitrin" and "Pitocin" (Parke, Davis & Co.) were used.

(5) *Prolactin.*—This extract was kindly supplied in powder form by Professor Riddle.

(6) *Implants.*—Parturient rats were killed when not more than two fœtuses had been expelled and the uterus and its contents were removed. The portions used were cut up finely and implanted into pregnant rats at a single operation under aseptic conditions. Rats from which the hypophyses were removed were killed on the afternoon of the day on which parturition was due (*i.e.* the 22nd day).

Results.

1. *Œstrin, with or without Anterior Pituitary Extracts.*

(i) *Œstrin.*—74 pregnant rats were injected with œstrin in amounts ranging from 0·04 to 1·0 mg. The results, summarised in Table I., show that

(*a*) Œstrin does not cause immediate abortion in rats (by "immediate" abortion is meant expulsion of the uterine contents 1–6 hours after the last injection, as defined by Parkes, 1930).

(*b*) The gestation period is sometimes prolonged in rats injected with œstrin. This is not necessarily due to the death of the fœtuses, as live fœtuses were born in one instance on the 25th day and in another on the 26th day.

(*c*) Fœtuses that are aborted are dead or non-viable and their development is below the average for the stage of gestation reached. This is most marked in the group injected with 1·0 mg. of œstrin. This adverse effect of œstrin on fœtal growth is occasionally experienced in litters carried to term, though to a less degree. Whenever œstrin had an adverse effect in the earlier stages of gestation, absorption took place; abortion did not occur before the 19th day, and has been termed "delayed," as occurring 3–4 days after injection. It was encountered after injections on the 16th, 17th, and 18th days of pregnancy.

(*d*) Generally amounts of œstrin which were injurious to the growth of the fœtus in the earlier stages of gestation were without effect when fœtal development was more advanced.

TABLE I.—EFFECT ON PREGNANT RATS OF MULTIPLE INJECTIONS OF ŒSTRIN.

(The numeral in brackets denotes the day of parturition; *cf.* normal, 22nd–23rd day.)

Amount injected.	No. of rats.	Days pregnant.	Results.
mg.			
0·04	1	18–19	1L
0·05	1	10–11	1D
0·0625	6	10–12*	1D, 5L
0·08	3	18–21	3L
	2	12–13	1A, 1D
0·1	2	15–16	2L
	1	10–11	1A
0·125	6	10–12	2A, 3L, 1P (27)
0·2	1	18–19	1L
	2	15–16	1DA, 1D
	1	10–11	1A
0·25	9	16–17	3DA, 5L, 1D
0·45	2	18–21	1DA, 1L
0·5	12	15–16	2DA, 5L, 5P (25†–28)
	4	18–19	1L, 3D
1·0	6	16	4DA, 1D, 1P (23, 26, 28)
	11	17	4DA, 1A, 1‡L, 4D, 1P (27, 28)
	4	18–20	4‡L
	74	..	15DA, 6A, 32L, 13D, 8P

* Injections covered 12 hours only. † Two born alive on 26th day.
‡ Modified females.

DA = Delayed Abortion. A = Absorption.
L = Live Litter. D = Dead Litter (term).
P = Prolonged Pregnancy.

(*e*) A remarkable effect[1] was observed in five litters, the members of which lived, and thus permitted a careful examination to be made. All the females of litters born to two rats injected respectively on the 17th and 18th day of pregnancy, and to three rats injected on the 20th day with 1·0 mg. of œstrin, had a modified corpus urethræ; not only is the corpus deeply cleft but within the fissure there is exposed a clitoris-like structure. This cleft, which runs along the line of the raphe between the "urethral prominence" and the introitus vaginæ, is apparently the result of absorption of a portion of tissue which developmentally formed the anterior wall of the urogenital sinus. Consequently the urethra opens immediately in front of the vaginal opening, the distance between

[1] See Plates I. and II.

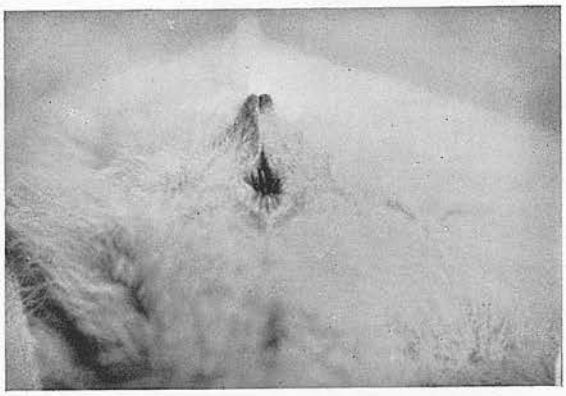
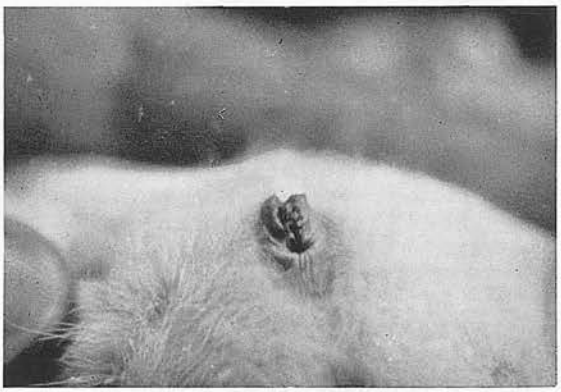
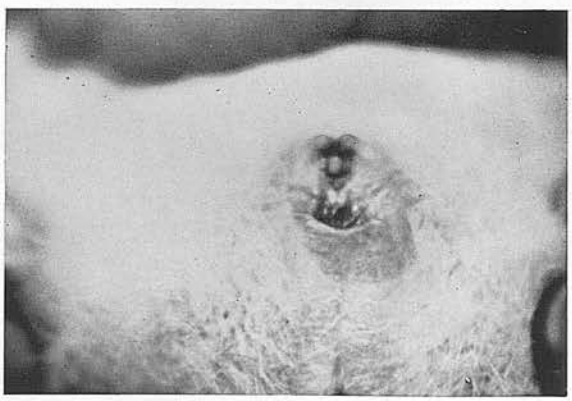


FIG. 1.—Normal female rat: urethral prominence (corpus urethræ) and vagina.





FIGS. 3 and 4.—Female rat, showing the effect of ante-natal administration of œstrin to the pregnant *mother*: same view as in fig. 1. Untouched photographs of the living animal.

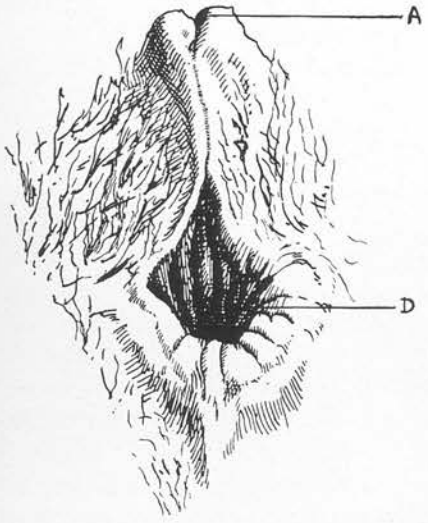


FIG. 2.—Diagrammatic sketch of fig. 1, showing (A) urethral prominence into which urethra opens. (D) vagina.

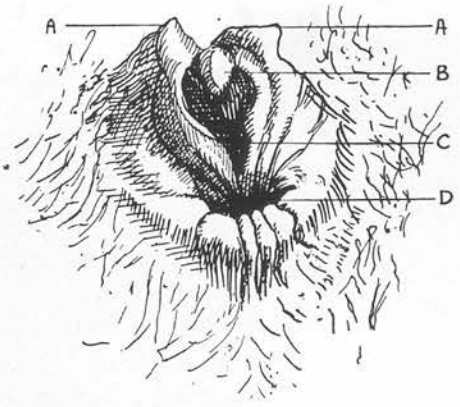


FIG. 5.—Diagrammatic sketch of fig. 3, showing (A) urethral prominence with deep fissure; (B) protuberance (? clitoris); (C) opening of the urethra; (D) vagina.

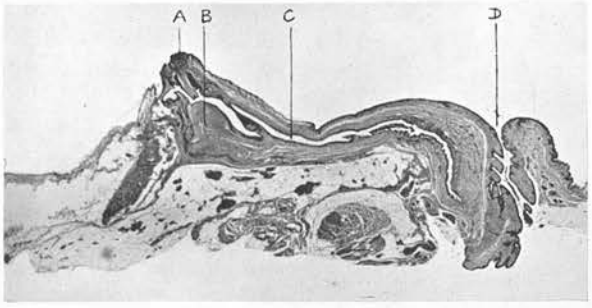


FIG. 6.—Normal female rat, photomicrograph. × 5. (A) urethral prominence; (B) protuberance; (C) urethra; (D) vagina.

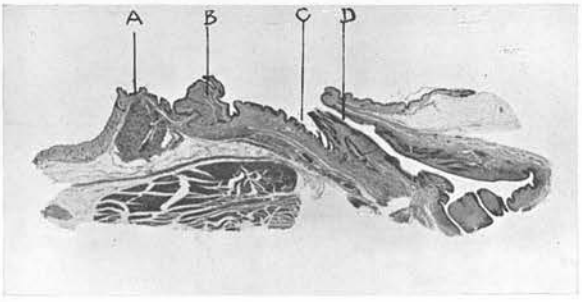


FIG. 7.—Abnormal female rat. (Litter-mate of rat illustrated in fig. 3.) Lettering as in fig. 6.

the two varying slightly in different animals. It will be noted in fig. 7 that the urethral opening is distal to a strong band of muscle which lies deep in the section, and this arrangement is associated with urinary incontinence which is a constant feature of these abnormal animals.

(ii) *Œstrin + Anterior Pituitary Extract or Extracts of Human Pregnancy Urine.*—Tests were confined to the larger quantities of œstrin, and were followed by amounts of anterior pituitary extract which, on the basis of previous experiments [Hain, 1932], were likely to interrupt pregnancy. Injections either immediately followed the last dose of œstrin or were given after an interval of 12–15 hours in three or five hourly doses; of the hypophyseal extract a total of 1·2 c.c. was administered, of the urine extract 0·5 c.c. (*i.e.* one mouse unit), and of Antuitrin "S," 0·3 c.c., 0·2 c.c., or 0·1 c.c. (*i.e.* 24, 16, and 8 rat units).

It is evident from a comparison of Tables I. and II. that:

(*a*) The addition of an extract of anterior pituitary or pregnancy urine to amounts of œstrin which, when administered alone, caused absorption or delayed abortion, did not cause immediate abortion.

(*b*) When œstrin was followed by such extracts, there was no significant increase in the percentage of delayed abortions.

(*c*) The number of instances in which pregnancy was prolonged was increased.

(*d*) The modification in the corpus urethræ of female fœtuses recorded in the previous group, in which œstrin alone was administered to pregnant rats, was observed in one litter in this group also, viz. rat Q2, which was injected with 0·45 mg. of œstrin in an oily solution on the 20th and 21st days of gestation, followed by 0·5 c.c. of an extract of pregnancy urine on the 22nd day. A litter of 16 was born on the following day, *i.e.* 23rd, of which 10 were females. Seven of the latter were retained, and all exhibited the cleft corpus and protuberance already described.

(iii) *Œstrin + Pituitrin or Pitocin.*—In order to ascertain the effect upon pregnancy of oxytocin in the absence of the administration of œstrin, 11 rats were injected between the 10th and 20th days of pregnancy with amounts ranging from 4 to 10 units. Administered at a single injection, these amounts failed to interrupt pregnancy, a result which is in keeping with the findings of Parkes [1930] in mice.

An investigation of the effects of œstrin in conjunction with oxytocin seemed especially desirable in view of the large amounts of œstrin which the pregnant rat can tolerate. Forty-two pregnant rats were injected with varying amounts of œstrin at hourly intervals; injections were spread over 13 hours only, unless otherwise stated; and pituitrin or pitocin followed the last injection of œstrin immediately, or at most, an hour later. Owing to the failure to secure immediate abortion with this procedure a longer period was allowed for the action of œstrin on

the uterus, so that in certain specified cases an interval of 12 hours to 3 days elapsed before the subsequent injection of the oxytocic extract. The only positive case of "immediate" abortion resulted in this way.

TABLE II.—THE EFFECT UPON THE PREGNANT RAT OF ŒSTRIN + ANTERIOR PITUITARY EXTRACT (AP) OR EXTRACT OF PREGNANCY URINE (PU, ANT. "S").

Œstrin.	Anterior pituitary extract.	No. of rats.	Days pregnant.	Results.
mg.				
0·1	AP or PU	2	10–13	2A
	PU	2	16–17	2DA*
	AP	1	18–19	1L
0·125	Ant. "S". (0·3 c.c.)	7	10–12	1DA, 6P (26–28)
0·2	AP	1	12–13	1P (27)
		1	16–17	1L
		1	18–19	1D
	PU	1	16–17	1DA
		1	20–21	1L
0·25	Ant. "S" (0·1–0·3 c.c.)	6	16–17	3DA, 1L, 2P (25) alive
0·45	PU	2	18–19	2L†
	AP	1	20–21	1L
	PU	1	20–21	1L‡
0·5	Ant. "S" (0·1 c.c.)	14	15–18	6DA, 3L, 5P (25–27)
1·0	PU	3	15	1DA, 2P (25–31)
	Ant. "S" (0·1 c.c.)	4	15–16	2DA, 2P (26)
	Totals .	48	..	16DA, 2A, 11L, 1D, 18P

* One aborted 40 hours after last injection.
† Litters not retained. ‡ Modified females.

The results of the immediate injection of oxytocin after large quantities of œstrin, as summarised in Table III., show that (*a*) immediate abortion does not occur in the rat even although 0·5 mg. or 1·0 mg. of œstrin is followed by 10 units of oxytocin. In a group of 6 rats receiving the larger amount, hourly injections were commenced at 4 a.m. and were followed by pitocin at 4 p.m., from which time the animals were watched continuously till 10 p.m. Abdominal contractions of moderate severity occurred from 5 to 6 p.m. only. An absence of the marked increase in the weight of the mother, that normally commences at this stage, indicated that considerable absorption occurred. Fœtuses born were generally either underweight and not viable, or decomposed owing to a prolongation of gestation. (*b*) The subsequent injection of oxytocin does not increase the incidence of delayed abortion.

(*c*) The prolongation of pregnancy was even more marked in this section than in that in which œstrin alone was administered. The birth of 5 or 6 fœtuses to some females and a drop of 30–50 g. at parturition in others indicates that the prolongation was not due to a mortal injury to the fœtus consequent on the contractions induced at the time the treatment was administered. (*d*) In two litters that survived, all the females again had the cleft corpus urethræ and clitoris-like structure previously described; the mothers were injected on the 16th and 17th days of pregnancy respectively.

TABLE III.—EFFECT ON PREGNANT RATS OF ŒSTRIN+PITUITRIN OR PITOCIN.

Œstrin.	Pituitrin or pitocin.	No. of rats.	Days pregnant.	Results.
mg.	units			
0·05	4*	1	10	1 (? immediate abortion)
0·1	2·5*	1	16	1L
	5·0*	1	16	1D
0·2†	2·5*	1	16	1L
	5·0*	1	16	1 (1 hour after injection of pituitrin)
0·125	5	4†	12	2A, 1D
		1	10	1A, 1P (26)
0·25	5	6	15–16	1DA, 3L, 2P (25, 30)
0·5	10	5‡	15	2DA, 3P (25–28)
		3	17–18	3DA
		6	16	3L, 1D, 2P (25—born alive)
1·0	10	10	15–16	2DA, 1§L, 2L (eaten), 2D, 3P (25, 26)
		2	17	1DA, 1§L
		42	..	11DA, 3A, 12L, 5D, 11P

* Pituitrin was given 20 hours after last injection of œstrin, except after 0·2 mg. œstrin when 3 days elapsed.

† Injections of œstrin spread over 28 hours.

‡ To 2 rats pitocin given after 12 hours.

§ Modified females.

II. ANTERIOR PITUITARY-LIKE HORMONE.

(i) alone
(ii*a*) with œstrin } 39 pregnant rats.
(ii*b*) with oxytocin

(i) Two Concentrated Extracts of Pregnancy Urine were employed:

(*a*) One prepared by Mr. P. G. Marshall and used in the experiments already described. Absorption occurred in 5 rats injected with 10 mouse units between the 5th and 10th days of pregnancy.

(*b*) Antuitrin "S." It is evident from Table IV. that, following its administration, pregnancy was more often interrupted than prolonged, and that interruption frequently occurred in the more advanced stages of gestation, *i.e.* at a point at which Mr. Marshall's preparation consistently caused a prolongation [Hain, 1932], yet all the rats (with two exceptions) received considerably in excess of the minimum amount calculated to produce this effect on the basis of the usual tests. Absorption was frequently accompanied by excessive hæmorrhage.

TABLE IV.—THE EFFECT OF ANTUITRIN "S" ON PREGNANT RATS.

Amount injected.	No. of rats.	Days pregnant.	Results.
0·08 c.c. (6 R.U.)	2	8–10	2A
0·3 c.c. (24 R.U.)	4	6–10	2A, 1D, 1P
	4	16–18	3A, 1D
0·6 c.c. (50 R.U.)	5	6–11	3A, 2P
	4	14–17	2A, 1L, 1P
Totals .	19	..	12A, 1L, 2D, 4P

(ii) Anterior Pituitary-like Hormone:

+(*a*) Œstrin.
+(*b*) Pitocin or pituitrin.

When either œstrin or oxytocin followed the injection of amounts of pregnancy urine extracts which had an interruptive effect upon pregnancy, a speedier termination of gestation did not ensue. The amount of œstrin injected was ·017 – ·025 mg.; of pitocin 10 units; of pituitrin 4 units (15 rats used).

III. SUBSTANCES FROM PARTURIENT RATS.

(*a*) *Placenta* (8 rats).—Implants of one, two, and three placentæ between the 11th and 17th days were without effect on pregnancy.

(*b*) *Uterus and Cervix* (9 rats).—Portions equal in size to a single placenta were used. Seven rats had normal litters; one prolonged and had still-born young; in the remaining rat absorption followed strong abdominal contractions which occurred at regular intervals 4 hours after the implantation.

(*c*) *Blood* (2 rats).—2·0 c.c. of parturient rats' blood [1] had no effect when injected on the 15th day of pregnancy.

[1] Approximately equal to one-sixth of the total blood-volume of a rat of 200 g. [Donaldson, 1924].

(*d*) *Hypophyses* (13 rats).—Single implants made on the 12th or 15th day caused no interruption of pregnancy. Rats which received 2 and 3 glands had still-born young at normal term; in 1 rat pregnancy was prolonged by 4 days.

(*e*) The rupturing of the bursa ovarica between the 13th and 21st days of pregnancy did not interfere with parturition or lactation (6 rats).

(*f*) *Prolactin* (26 rats).—Tests made with a preparation based on that described by Riddle, Bates, and Dykshorn [1932], and with a sample kindly supplied by Professor Riddle, were negative on pregnant, mature, and ovariectomised rats, and also on rats injected 4–7 days after their young were weaned. The rats were given the equivalent of 0·6 g. of tissue.

IV. Parturition in the Normal Rat.

The procedure adopted by Rudolph and Ivy [1930] in the dog formed the basis of that used in the observation of a rat in the act of labour. In a warm room and under ether anæsthesia, a ventral incision was made; iodised oil was poured over the uterine horns to keep these moist and warm, and the contractions were watched throughout the expulsion of a single fœtus.

It was observed that the right cornu contracted independently of the left. Circular contractions above the cervix were followed by a pause; this was succeeded by longitudinal contractions by which the blood above the fœtus was forced back (*i.e.* in a cephalic direction). Another pause was followed by relaxation longitudinally, causing a lengthening of the uterine cornu and a flow of blood in a cervical direction. Circular contractions were repeated and resulted in the blood being forced in both directions; during the relaxation which followed the fœtus advanced caudally. The blood within the uterus was again forced back to the accompaniment of circular contractions, to be succeeded by a forward movement as the muscle expanded longitudinally. At the same time the position of universal flexion of the fœtus was undone, so that there was a lengthening of its longitudinal axis. As a result of these movements, the fœtus was now partially engaged in the cervix, which measured 18 mm. in width. Marked circular contraction again occurred behind the fœtus, following which it was found protruding from the vagina, and the second fœtus was now in the position originally occupied by the first.

Discussion.

1. *Œstrin.*—A comparison of the results herein described with those reported by Parkes [1930] demonstrates a marked difference between the rat and the mouse in their response to œstrin, when either

administered alone or followed by the oxytocic principle of the pituitary. The data show that:

(*a*) Delayed abortion (*i.e.* 36–72 hours after the last injection) occurred with considerably less frequency in rats than in mice after multiple injections of œstrin, *cf.* 15 out of 74 as against 20 out of 30. There was no instance of immediate abortion (*i.e.* 1–6 hours after injection) such as occurred in mice.

(*b*) As was demonstrated by M. Smith [1926], the amount of œstrin required to interrupt pregnancy rapidly increases as pregnancy advances.

(*c*) The prolongation of pregnancy after the injection of œstrin, which was observed in 19 cases,[1] is difficult to explain, more especially as such prolongation was not attributable to the death of the fœtus, nor to any retardation in its development due to the injection of œstrin, since the weight of the mother usually remained stationary or fell only slightly during the last 2–3 days during which gestation was prolonged.

(*d*) The subsequent injection of gonadotropic extracts did not cause a significant increase in the incidence of delayed abortion which occurred when œstrin alone was injected.

In previous experiments [Hain, 1932 and 1934] in which gestation was prolonged after the injection of extracts of pregnancy urine and of growth hormone, it was pointed out that such prolongation of the normal gestation period could not be directly related to the ovarian effect which was common to both extracts, viz. "blood spots." The interruptive action of Antuitrin "S," observed late in pregnancy, in the present series of experiments followed the injection of amounts considerably in excess of those which ensured prolongation on the basis of a unit related to the production of "blood spots," and thus affords further evidence in support of our previous statement.

(*e*) *The Effect of Œstrin on the Female Fœtus in Utero.*—Twenty-seven females with the modification of the corpus urethræ and penis-like structure described and illustrated have been obtained up to date; they comprise eight litters. With only one exception, the administration of œstrin to the pregnant mother was confined to a single day and spread over 12–13 hourly injections; the earliest stage in pregnancy at which the modification was induced was the 16th day, the latest the 20th–21st. The abnormality, which is clearly discernible when the youngsters are 21 days old, affects *all* the female members of a litter, though occasionally some more markedly than others. The ovaries of 2 females which were examined macroscopically when the latter were 4 months old appeared entirely normal, though large; these females, and their sisters, mated, had normal pregnancies and suckled their young; their brothers are fertile and appear to be normal.

It is difficult to explain in a satisfactory manner how a female sex hormone could cause a modification in the structural development of

[1] Those in which œstrin was followed by anterior pituitary hormone are excluded.

the uro-genital system of the nature described, and why the male fœtus is not similarly affected, since the urethra is not completely developed in the male on the 20th day [Ruth, 1934]. The suggestion that the effect is not due to the direct action of œstrin on the fœtus, but to its action on other ductless glands, is being examined. In view of the brief period during which the hormone could have exerted its effect, consideration is also being directed to the possibility that the effective period of its action was extended by transmission through the mother's milk. The histological findings indicate that an inhibition of development and not a hypertrophy is involved. There is therefore no suggestion of a resemblance to the effect obtained in the immature and adult female guinea-pig by Papanicolaou and Falk [1934] and Steinach and Kun [1931] as the result of the injection of luteinising extracts of the anterior pituitary.

The literature provides few studies of the effect of hormones on the fœtus in utero. Proliferation of the vaginal epithelium of fœtal guinea-pigs was reported by Courrier [1924] following the injection of liquor folliculi into guinea-pigs approaching term, but no departure from the normal was encountered by Parkes and Bellerby [1927] in mice. In a study of the effect upon the chick embryo of the injection of female hormone (theelin and theelol) into the egg, Kozelka and Gallagher [1934] observed a variable degree of replacement of testicular by ovarian tissue in the periphery of the left testis of the Leghorn.

(*f*) The synergism between œstrin and oxytocin demonstrated by Parkes [1930] in the mouse does not, apparently, exist in the rat. In no animal did "immediate" abortion occur when œstrin was immediately followed by oxytocin, although as much as 1·0 mg. of œstrin +10 units of pitocin was injected. The fact that such amounts of œstrin had a modifying effect on the external genitalia of the female fœtuses, and that the subsequent injection of oxytocin failed to cause abortion, makes it highly improbable that the combined action of these two substances is responsible for parturition in the rat. The effect produced on the female fœtuses of rats injected with large quantities of œstrin late in pregnancy, and in which abortion did not take place, suggests that, where abortion occurred, this was due rather to an unfavourable effect on the fœtus than to œstrous conditions set up in the uterus, more especially as such have frequently not been demonstrated in association with abortion [D'Amour, 1934].

2. *Products from Parturient Animals.*—It has not been possible by the methods adopted to demonstrate the existence in the placentæ or blood of parturient rats of oxytocic substances of sufficient concentration to bring about parturition.

The experiments of Pencharz and Long [1931] suggested that the anterior pituitary played an important rôle in parturition since its removal on the eve of term did not cause that interference with the birth

mechanism with which its removal at an earlier date was associated. The failure of term hypophyses to cause an interruption of pregnancy does not preclude the possibility that such secretion is necessary for a prolonged period.

The part which the fœtus and fœtal movements may play in the act of parturition has not been fully investigated, but it appears likely from observations made in the living animal and herein described that normally this is an active one. As it is probable that the act of parturition is due to a *series* of events it is readily understood that the normal birth mechanism may be disorganised by interference with any one link in the chain, *e.g.* removal of the ovaries at term [Hain, 1934], and of the hypophysis after mid-pregnancy [Pencharz and Long, 1931], or by the injection of certain hypophyseal extracts [Teal, 1926; Hain, 1932; Snyder, 1934].

Conclusions.

1. "Delayed" abortion (*i.e.* 36–72 hours after the last injection) following multiple injections of œstrin was less frequent in rats than in mice; very large amounts of œstrin were required; there was no "immediate" abortion (*i.e.* within 1–6 hours of the last injection). The gestation period was sometimes prolonged by 3–5 days after the injection of large quantities of œstrin.

2. The subsequent injection of an extract of anterior pituitary or of an œstrin-free extract of human pregnancy urine did not cause a significant increase in the percentage of "delayed" abortions which occurred when œstrin alone was injected There was an increase in the number of prolonged pregnancies.

3. The synergism between œstrin and oxytocin demonstrated in the mouse does not, apparently, exist in the rat.

4. The administration to pregnant rats of placentæ, hypophyses, uterus, and blood of parturient rats did not cause abortion.

5. The process of parturition in the normal pregnant rat is described.

6. A modification of the corpus urethræ involving a displacement of the urethra occurred in all surviving female fœtuses born to rats injected with large quantities of œstrin late in pregnancy. The subsequent injection of oxytocin failed to cause abortion in animals in which this modification of the fœtal genitalia was induced.

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THE PHYSIOLOGY OF PREGNANCY IN THE RAT: FURTHER DATA ON THE PASSAGE OF HORMONES *VIA* THE PLACENTA AND THE MOTHER'S MILK.

By A. M. Hain (Carnegie Research Fellow). From the Institute of Animal Genetics, Edinburgh University.

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Previous experiments have demonstrated that when large quantities of crystalline œstrone are administered to the rat on a single day late in pregnancy, the female fœtuses that survive exhibit a morphological abnormality of the urogenital region, of the nature of partial hypospadias [Hain, 1935 *a* and *b*]. A similar effect was induced in the suckling female when the lactating mother was injected during the early puerperium [Hain, 1935 *c*]. In both instances females only were affected.

Further investigations sought to ascertain (*a*) the earliest and latest stages in gestation at which the effect could be induced and the minimum amount of œstrone required, and whether it was possible to affect the *male* fœtus by administering to the pregnant rat amounts of œstrone which were considerably in excess of the threshold amount producing abnormality in the female; (*b*) whether the action of œstrone on the fœtus might not be an indirect one and involve other ductless glands, or (*c*) be due to a change in the chemical structure of the compound. The existence of a number of synthetic compounds resembling œstrin and some of which possess œstrogenic activity has been demonstrated by Cook and Dodds [1933], and Dodds [1934]. (*d*) The possibility that the effective period of the action of œstrone on the fœtus was prolonged by transmission through the mother's milk was also examined. (*e*) All females exhibiting the abnormality were observed throughout the reproductive period for a possible effect on the attainment of puberty, their fertility, reproductivity, etc.

Material and Technique.

Experimental Animals.

Wistar (albino) and Hooded (black and white) rats were injected at known stages of pregnancy. In those rats that were injected prior to the appearance of placental sign the existence of pregnancy was

ascertained by means of laparotomy. All rats were weighed daily from the day of injection until parturition.

Extracts.

(i) *Œstrone.*—Two forms of œstrone were used:

(*a*) Œstrone crystals. These were dissolved in hot absolute alcohol and injected in a 10 per cent. aqueous solution; in the single-day experiments injections were given in one course lasting generally 10 hours and were made hourly, 0·2 c.c. being given at each injection. Rats which were used for testing the effect of œstrone over a period of several days were injected either once or twice daily. When new-born rats were injected, sesame oil was used instead of dilute alcohol.

(*b*) Œstrone benzoate, in oil solution, in the strength of 1·0 mg. per 1·0 c.c.

(ii) *Progestin*, having an activity of 2 rabbit units per c.c. olive-oil solution (McPhail, 1934).

(iii) *Eucortone* (Allen and Hanbury's).—According to the makers 1·0 c.c. was the equivalent of 30 g. of adrenal cortical tissue.

(iv) *Anterior Pituitary Hormone.*—Pregnyl and Antuitrin "S" were used, both being prepared from human pregnancy urine.

(v) *Male Hormone.*—The preparations used in the early stages of the experiment were found to have a strong œstrogenic action on ovariectomised rats, viz. hombreol, supplied by Organon, and a urine extract obtained from the British Drug Houses, Ltd. Through the intervention of Dr. A. S. Parkes it was possible to examine the effect of purified male hormones—androsterone and its diol derivative androstandiol; injection was made in a solution of nut oil.

(vi) 1 : 2 *Benzpyrene.*—This was supplied in two dilutions by Union Chimique Belge, Brussels; the highly concentrated oil suspension contained 300 mg. in 6·0 c.c., whereas the dilute preparation consisted of the same amount in 60 c.c. of oil. This compound belongs to the same form of condensed carbon ring compounds as cholesterol, Vitamin D, and the sex hormones [Cook, Hewett, and Hieger, 1932; Cook and Dodds, 1933], and possesses carcinogenic properties.

Results.

I. *Œstrone.*

In the previous experiment [Hain, 1935 *a*] it was found that the urogenital abnormality described occurred in all female fœtuses when 1·0 mg. of œstrone was injected on a single day late in pregnancy, and when this was spread over 10 or 12 hourly injections. Five live

litters containing 14 affected females were obtained in this way. Further experiments (grouped in Tables I. and II.) showed that:

(*a*) The smallest amount of œstrone capable of inducing the abnormality was 0·5 mg. In one litter only 5 out of 8 females were definitely affected, whereas 100 per cent. of females were affected when amounts in excess of 0·5 mg. were administered.

TABLE I.—INCIDENCE OF MORPHOLOGICAL MODIFICATION OF FEMALE FŒTUS AFTER INJECTION OF ŒSTRONE ON A SINGLE DAY LATE IN PREGNANCY (RAT).

(Gestation period 22–23 days. Average weight of new-born 5·25 g.)

Total amount of œstrone.	Stage of gestation at injection.	No. of rats injected.	Live litters.	No. of ♀♀ surviving showing "modification."	Remarks.
2·0 mg.	18th day	1	1	7	..
	20th ,,	4	3 *	10	..
	21st ,,	1	1	1	..
	22nd ,,	1	..	..	Still-births.
1·0 mg.	16th and 17th day	3	..	..	Absorption or abortion.
	18th day	1	..	..	Killed in act of parturition; histology.
	19th ,,	1	1	5	..
	20th ,,	5	3	8	2 litters still-born —1 averaged 2·5 g.
	21st ,,	3	3	12 †	..
	22nd ,,	3	..	..	Still-births.
0·75 mg.	15th ,,	1	..	..	Abortion 18th–19th day.
	16th, 17th, 18th day	7	..	..	Still-births 21st–22nd day; average weight of young 2–3 g.
	19th and 20th day	9	3 *	9	..
0·5 mg.	15th and 16th day	5	..	..	Still-births; average 2–3 g. on 21st–22nd day.
	19th, 20th, 21st day	7	3	11	..
0·25 mg.	12th day	2	..	..	Absorption.
0·125 mg.	10th ,,	3	..	..	,,
	Total	57	18	63	

* 1 litter contained ♂♂ only.

† A litter of 7 ♀♀ (all "modified") was born within 12 hours of the last injection of œstrone.

(*b*) The effect could be produced with regularity, and generally live litters could be obtained, when injections were made between

the 18th and 21st days. Three affected litters were secured in the previous experiment between the 16th and 17th days, but the administration of œstrone at or before that stage almost uniformly caused death of the fœtus. Injection on the 22nd day was equally injurious, and yet it is of interest that a litter containing 7 "modified" females was born within 12 hours of the last injection of œstrone.

TABLE II.—THE EFFECT OF DAILY INJECTION OF ŒSTRONE FOR SPECIFIED PERIODS DURING PREGNANCY.

Amount of œstrone at each injection. mg.	Period of gestation covered by injection.	No. of injections.	No. of rats injected.	Live litters.	Results.
·00250	1st to 15th day	twice daily	4	..	Pregnancy interrupted before implantation.
·00125	1st to 17th ,,	,,	7	..	Pregnancy interrupted before implantation.
·00250 and ·00125	4th to 15th ,,	,,	7	..	Pregnancy interrupted before implantation.
·00250	10th to 13th ,,	,,	4	..	Placental sign followed by absorption.
·00125	12th or 13th day to 19th day	once daily	6	..	5 dead litters; 1 absorption.
·00125	13th to 22nd day	,,	1	1	2 ♂♂, 11 ♀♀; all normal.
·00250	16th to 21st ,,	,,	2	..	Born alive but not viable or not suckled.
·00250	16th to 22nd ,,	,,	1	1	5 ♂♂, 6 ♀♀; all normal.
	Total		32	2	

(*c*) Increasing the amount of œstrone to 2·0 and 2·5 mg. did not cause additional anomalies in the female fœtus nor induce a similar effect on the male. It was, however, possible to detect the abnormality earlier, *e.g.* about the 14th day of life instead of the 21st day.

(*d*) Whereas a sudden excessive dose of œstrone administered on a single day late in pregnancy can affect the fœtus without interrupting gestation, the injection of minute amounts over a prolonged period fails to have this effect. Table II. shows that ·00250 mg. of œstrone per day prevents implantation of the ovum when administered at the beginning of gestation; ·005 mg. per day was equally injurious when given in mid-pregnancy, although gestation

was not immediately interrupted. When ·00125 mg. was injected daily from the 13th day to near term a single live litter was obtained from 7 pregnant rats; this exhibited no morphological abnormality. The injection of twice this amount of œstrone from the 16th day to term gave a similar result.

II. *The Effect of other Hormones and the Possibility of Indirect Action.*

(*a*) Since it was possible that the corpus luteum hormone might affect the fœtus in the same way as the follicular hormone, 2 rats were injected on the 19th and 20th days of pregnancy respectively with 6 rabbit units of Progestin (B.D.H.), administered in 3 injections. Live litters were born and showed no abnormalities.

In 2 affected females from which the ovaries were removed when they were 26 days old, the clitoris was less developed but the modification was fundamentally the same.

(*b*) *Eucortone.*—In view of the effect which disturbances in the pituitary-adrenal balance may have on the urogenital tract, a concentrated preparation of cortical tissue was injected into 7 rats on the 17th day of pregnancy; amounts equivalent to 60 and 120 g. of cortical tissue were administered in 7 injections, but had no effect on the offspring; one rat injected with the equivalent of 210 g. ate her litter.

(*c*) *Anterior Pituitary Hormone.*—Injections were confined to a single day late in pregnancy for purposes of comparison with the effect of œstrone.

(i) *Pregnyl.*—Thirteen rats were injected with amounts ranging from 20–100 rat units on the basis of the makers' reckoning of potency, the stage covered being the 15th to the 20th days of gestation. Six normal litters were born at term; prolongation of pregnancy, abortion, dead litters, and death of the mother owing to inability to litter, accounted for the remainder. In two of the latter the ovaries were found to contain a mass of follicles and no corpora were manifest. Both females died with 10 fœtuses in utero.

(ii) *Antuitrin "S."*—Of 6 rats injected with 100 or 400 rat units of this preparation on the 20th day of pregnancy, 4 had live litters which showed no structural abnormality.

The results do not indicate that either the adrenal or the anterior pituitary played a part in the process of the modification of the fœtus under the influence of œstrone.

III. *Other Compounds related to Œstrone in Chemical Structure.*

The possibility that the effect produced was not due to œstrone as such but to a breakdown product held in common with other related compounds was investigated.

(i) *Male Hormone.*—Owing to the similarity in the chemical formulæ of the male and female sex hormones, it appeared desirable to ascertain if male hormone also had an effect on the female fœtus, and whether the male fœtus might not be affected in its morphological development by male hormone. As in the case of œstrone, injections were of two types, viz. an excessive dose administered by frequent injections on a single day late in pregnancy, and injections once or twice daily of smaller amounts for a prolonged period throughout pregnancy. It was anticipated that the latter course might affect sex differentiation, and accordingly treatment was commenced on the 10th or 11th day. According to Buyse [1933] sex is not morphologically differentiated in the rat till the 14th day.

In all, 55 pregnant rats were used, but a proportion had to be discarded as the earlier preparations injected were contaminated with œstrin. The preparations used on these earlier attempts, and to which 35 rats were devoted, were: an extract prepared by the British Drug Houses, Ltd., and hombreol [1] (Organon). Briefly, the experimental data were as follows:—

Hombreol.—Fifteen capon units given on a single day in pregnancy between the 16th and 21st had no injurious effect, but 20 capon units given on the 12th day, and 10–30 c.u. spread over a period of 5 days at the same stage caused absorption, abortion, or still-births in 12 pregnant rats. Three rats injected on the 20th and 21st days of pregnancy with a total of 100–120 capon units of hombreol gave birth to litters containing "modified" females. These results caused the presence of œstrin to be suspected, of which confirmation was obtained by tests on ovariectomised rats and mice. Dr. Macbeth of Organon finally reported on œstrogenic activity exceeding 32 mouse units per capon unit, which was sufficient to account for the effect produced on the female fœtuses. It is of interest to note that the presence of male hormone did not prevent the action of œstrin described.

Tests were made with specially prepared "œstrin-free" hombreol administered to 8 rats on 2 days between the 19th and 21st day of pregnancy. Mortality was high, but 2 live litters were obtained from females injected with 115 capon units; these showed no abnormality.

Through the generosity of Dr. A. S. Parkes it was possible to test the effects of purified male hormones on 20 pregnant rats. The injection of 6·0 mg. of androsterone on the 20th and 21st days of pregnancy, and of 3·0 to 10·0 mg. on the 10th day, had no effect on the offspring; and normal litters were born to rats injected daily from the 11th to 15th days with totals varying between 2·5 and 4·0 mg. of male hormone. The amounts used were approximately equal to 30–100 capon units.

Androstandiol was similarly tested on a single day late in pregnancy and also over a prolonged period. When 3·75 mg. (*c.* 150 capon units) was injected on the 20th day in 10 injections at

[1] The makers had omitted to test this particular batch for œstrin before dispatch.

hourly intervals, 2 out of 3 litters born alive contained females fully "modified." In both instances some of the females in the litter were not affected, or hardly perceptibly, and 1 litter was quite normal. In 1 of 2 other litters born after the mothers were injected with 5·0 mg. all the females were "modified."

When these amounts (3·75 mg. and 5·0 mg.) were injected over a period of 10–12 days, *i.e.* from the 11th to the 20th or 22nd day of pregnancy in 5 rats, no external abnormality was induced in either the female or the male offspring. Two litters were still-born.

(ii) *Benzpyrene* (15 rats).—Investigation of the effects of benzpyrene on the fœtus was prompted not only by the chemical relation of benzpyrene to œstrone and its known œstrogenic activity [Cook and Dodds, 1933], but primarily to ascertain if the fœtus could be affected by means of its carcinogenic action [Cook, Hewett, and Heiger, 1932] at the same stage of pregnancy at which fœtal development had proved susceptible of modification under the influence of œstrone and of androstandiol. The effects of benzpyrene alone and in conjunction with œstrone were investigated; the amounts administered of the former alone were 20, 25, 40, 50, 70, and > 100 mg., *i.e.* the range extended from the carcinogenic unit [*c.* 16 mg., Pourbaix, 1935] to the level at which œstrogenic activity was observed by Cook and Dodds [1933], viz. 100 mg. The latter administered on the 20th–21st days of pregnancy had no effect on the morphological development of the female fœtuses. When given in conjunction with 0·5 to 1·0 mg. œstrone benzoate it neither prevented nor augmented the typical œstrone effect on the fœtus; thus 2 litters born after injection of the larger amount of œstrone contained females exhibiting the partial hypospadias described.

(iii) *Œstrone Benzoate.*—Since it has been shown [Zondek, 1934, in the rat, and Cohen *et al.*, 1935, in the human subject] that the animal body is capable of converting large quantities of œstrin into a physiologically inactive form, it seemed desirable to ascertain whether the effect observed in female rat fœtuses could be induced by a form of œstrone which was very slowly hydrolised in the body. As the benzoate has a more prolonged action than œstrone [Zondek, 1934], its effect was examined (12 pregnant rats). Amounts varying from 0·5 mg. to 2·5 mg. were administered, and the effect of a single injection was compared with that of the same amount in divided doses.

The obvious inequality in the results obtained (Table III.) as compared with the remarkable consistency observed when œstrone was employed (Table I.) may have been related to the slower rate at which the benzoate is hydrolised in the body and the lower peak of concentration reached. This may account for the fact that 0·5 mg. did not cause fœtal abnormality when injected on the 20th day, whereas it produced this effect when administered on the 18th day. The same argument covers the non-effective-

ness of 0·8 mg. on the 20th day. However, such individual variations may be due to other causes, as a litter of " modified " females was born in the benzpyrene group when 1·0 mg. of the benzoate was injected at the same stage (20th day). Including the 2 affected litters in the latter group, 5 affected litters have been obtained after the injection of œstrone benzoate. The instances of delayed abortion (*i.e.* 3 days after injection) following a single

TABLE III.—EFFECT OF ŒSTRONE BENZOATE INJECTED ON A SINGLE DAY LATE IN PREGNANCY (RAT).

Total amount of œstrone benzoate.	Doses.	Stage of gestation at injection.	No. of rats injected.	Litter results.
0·5 mg.	5	18th–19th day	1	Normal litter.
	1	20th day	1	,, ,,
	1	18th ,,	1	4 ♀♀ survived, all "modified," 1 slightly.
0·8 ,,	8	17th ,,	1	Normal litter.
	1	20th ,,	1	,, ,,
1·0 ,,	5	17th ,,	1	2 ♀♀ survived, 1 slightly "modified."
	1	18th ,,	1	5 ♀♀ survived, all "modified," 1 slightly.
1·5 ,,	6	17th–18th day	1	Delayed abortion.
	1	18th day	1	,, ,,
2·5 ,,	6	19th–20th day	1	Born alive, but died next day.
	1	18th day	1	Delayed abortion.

injection late in pregnancy are of interest; fœtal growth was retarded as shown by the average weights of the fœtuses aborted on the 21st day, viz. 0·6 g., 2·1 g., 2·4 g. The difference in potency as between single and divided doses is not marked as determined by the effect on fœtal development, since a slight effect was observed following 1·0 mg. administered in 5 injections. The results also indicate that the unit of œstrone and of the benzoate affecting fœtal morphology is approximately the same in both cases.

IV. *Was the Effective Period of Œstrone confined to Pregnancy or prolonged by Transmission via the Mother's Milk?*

The latter possibility was suggested by the birth of a litter of affected females 12 hours after the last injection of œstrone. In order to investigate this point, females from litters known to be affected [1] were, at birth, and before they had had milk, transferred to uninjected mothers who had just littered, and *vice versa*. This exchange of mothers

[1] The regularity with which 1·0 mg. of œstrone affected fœtal development made such an early assumption possible.

did not prevent the "modification" from taking place; no normal female placed with an injected rat developed the abnormality. It was clear that the influence was exerted and completed while the fœtus was in utero.

Two experiments were performed which showed, however, that the development of the female rat is capable of structural modification even after birth.

(*a*) In the course of an investigation into the effects of œstrone on the *lactating* rat [Hain, 1935 *c*] 3 litters were obtained containing females with the same developmental abnormality as occurred after the administration of œstrone late in pregnancy. The amount of hormone required to produce the modification was considerably in excess of the unit in the pregnant rat: 2·0 to 3·0 mg. *cf.* 0·5 mg., and the effective period was limited to the earliest stage of the puerperium. Since the publication of these results the effect of the benzoate of œstrone has also been ascertained on the 1st day of suckling. Two of the three rats used were injected before the sucklings had had any milk; 1·0, 1·2, and 2·0 mg. were given in a single dose to each rat. Only 1 litter contained slightly "modified" females, viz. that of the rat which received 1·0 mg. This inequality of response in the lactating animal was not peculiar to the benzoate, as it was observed also when œstrone was used. It is of interest that as little as 1·0 mg. of the benzoate affected the sucklings, whereas this amount was ineffective when œstrone itself was administered. This may indicate a greater potency in the benzoate, yet 2·0 mg. did not arrest lactation, cause the death of any of the sucklings, or markedly affect their rate of growth.

(*b*) *Direct injection of œstrone* (in a highly concentrated oily solution) into new-born females gave further proof of the plastic nature of the urogenital region even after birth; 9 affected females were obtained following the injection of 1·0, 2·0, and 3·5 mg. of œstrone. An effect somewhat similar was reported by Wiesner [1935] to have occurred after direct injection of a preparation of male hormone at a later stage of development.

V. *The Subsequent History of Litters which came under Hormonic Influence in Early Development.*

(*a*) *Females.*—By means of vaginal smears taken daily for a period of 3 weeks in 21 females "modified" through the injection of œstrone into the pregnant mothers, it was ascertained that the sex cycles of such females showed no abnormality. In rats that came under the influence of œstrone either by direct injection on the day of birth or *via* the mother's milk in the early days of suckling there was a tendency for the vagina to open at an unusually early age, *e.g.* on the 18th and 24th days in the first group, and on the 20th–26th days in the suckling

group (*cf.* 40th day, normal). Vaginal smears were taken till the 38th day, during which time no animal came in œstrus. This precocious opening of the vagina was not confined to affected females, but occurred in litters in which no abnormality was present; it was not observed in the offspring of rats injected during pregnancy whether with œstrone, benzpyrene, or androstandiol.

Reproductivity in affected females is normal, but fertility appears to be below the average. This is especially true of those affected by injection in pregnancy. Parturition is apparently difficult in "modified" animals, and there seems to be difficulty in expressing the fœtuses through the vagina. Other factors may play a part, however, in view of the frequency with which lactation proved to be either inadequate or entirely lacking, resulting in the death of the litter 2 or 3 days after birth as shown by the following group particulars:—

TABLE IV.—REPRODUCTIVE HISTORY OF "MODIFIED" FEMALES.

Affected ♀♀.	How "modification" was caused.	No. pregnant.	No. littered.	No. reared litters.	Remarks.
48	Œstrone single day late in pregnancy.	14	12	6	6 litters not reared because mother had no milk.
7	Hombreol late in pregnancy.	6	4	0	..
8	Œstrone during lactation.	5	5	2	1 still-birth, 1 whole litter died for want of milk and another only partly reared.
11	Œstrone direct on day of birth.	5	5	3	Remaining females mated several times.

Of 48 "modified" females of reproductive age housed with fertile males (which were changed from time to time) only 6 had litters that lived, although 14 became pregnant; the young born were normal. Mating occurred with moderate frequency.

In the case of the 7 females affected as the result of injection of hombreol late in pregnancy, 6 became pregnant and 4 had live litters. These, which consisted of 6, 6, 6, and 14 young respectively, were dead by the end of the 3rd day, when the mother's nipples were found to be under-developed and tipped with blood. Females affected by the injection of purified male hormone into the pregnant rat have not yet reached maturity.

The 3 litters containing "modified" females following the injection

of œstrone during lactation comprised 10 females, of which 4 were fully affected, 4 slightly, and 2 not at all. The 4 affected had live litters, but 1 failed to suckle at all and another reared 2 out of 10 youngsters. Only 1 still-born litter was born to the remaining 6, although all mated.

Of 11 females "modified" as the result of the direct injection of œstrone on the day of birth, 5 have had live litters, of which 3 have been reared successfully. The remaining females have mated several times, but no pregnancy has resulted as yet.

One of the 2 litters obtained after injection of œstrone over a period of several days during pregnancy was retained for observation of reproductivity, etc. This consisted of 2 males and 11 females, all of which were outwardly normal. Six of the females were kept and all had live litters, but only 2 were reared.

TABLE V.—REPRODUCTIVE HISTORY OF LITTERS BORN TO FEMALES INJECTED DURING LACTATION.

Œstrone.	No. of ♀♀ injected.	No. of ♀♀ born and observed.	No. of these that littered.	No. of offspring.	Remarks.
0·25–0·5 mg.	23	67	61	451	3 ate litters; 6 mated but had no pregnancy.
1·0–2·0 ,,	20	43	32	250	9 did not rear litters; 11 had no pregnancy.

The reproductivity and fertility of females which came under the influence of œstrone in the early stages of development, but without being affected morphologically, could be observed only in the case of the litters of those injected during lactation (with the single exception of those mentioned in the paragraph immediately preceding), as females injected during pregnancy either aborted or gave birth to affected females. As a general rule no marked impairment of fertility was observed, but the data in Table V. suggest that the injection of 1·0 and 2·0 mg. between the 1st and 4th days of lactation (whether on a single day or spread over 4 days) may have been responsible for the lower fertility observed in these groups. They show that 25 per cent. of the offspring of rats injected with 1·0 to 2·0 mg. œstrone between the 1st and 4th days of lactation proved infertile, and over 25 per cent. of those that littered were unable to rear their young as compared with 9 per cent. and 5 per cent. respectively in the offspring of the group injected with 0·25 to 0·5 mg. The significance of the figures is diminished, however, by the fact that *all* the members of a

litter could not be retained owing to the exigencies of space and the requirements of other experiments.

Mortality among affected females has been rather high; there have been 16 deaths, of which 12 were the result of abscesses (11 ovarian and 1 Bartholin's); severe œdema of the hind legs occurred in 4 rats accompanying the inflammatory condition, and was present in a fifth female without obvious associated symptoms. In 5 cases death occurred at the age of 3–4 months.

(*b*) *Males.*—Fertility was not seriously impaired. Thirty-one males that were litter-mates of "modified" females were killed when 3–6 months old; such occasional abnormalities as were found at necropsy could not be ascribed to the treatment.

Discussion.

In view of the large quantities of œstrin that are excreted in the urine of pregnant women, the possibility of inducing by the injection of œstrin even a passing abnormality in the development of the offspring would be of interest. The production of a morphological defect that is not merely clearly demonstrable but remains a permanent feature justifies a deeper concern,[1] and an effort has been made to determine, if possible, the nature of the substance or substances involved in the effect induced; the *modus operandi* remains obscure.

The experiments which have been described have failed to demonstrate that any ductless gland was an intermediary between œstrone and its action on the fœtus.

That male hormone was capable of exerting a similar effect to that induced by œstrone suggested either that some chemical change had taken place in the latter, or that neither hormone was responsible for the effect but a breakdown product common to both. The first alternative receives some support from the consideration that both male and female hormones are to be found in the same individual—in man [the literature is reviewed by Fee, Marrian, and Parkes, 1929], in woman [Womack and Koch, 1932], and in the stallion [Zondek, 1934]. The separation of the two hormones, as determined by the accepted tests on the capon and ovariectomised rodent, was effected by Womack and Koch [1932], and recently completely negative results have been obtained by Warren [1935] on ovariectomised mice with as much as 10 mg. of androsterone. However, the results herein described, together with the fact that Butenandt [1935] has demonstrated that two unsaturated substances related to androsterone—transdehydro androsterone and androstendione—possess an œstrogenic action on the

[1] The placentæ of man and rodents are hæmochorial [Kuttner and Ratner, 1923; Ratner *et al.*, 1927].

immature rat, suggest a similar possibility in the case of the saturated compound, androstandiol.

Although it is possible that under certain conditions the male and female hormones are capable of producing similar effects, numerous instances exist to show that their action is not always identical. In the experiments here recorded the highly interruptive effect of minute quantities of œstrone in early and mid-pregnancy was in marked contrast with the innocuous effect of androstandiol administered in large quantities at the same stages. This fact, together with the absence of any œstrogenic activity in androsterone demonstrated by Warren [1935], makes it unlikely that the morphological effect on fœtal development was due to a conversion of the excess of male hormone into female hormone, a change which Zondek [1934] suggests takes place in the normal male organism, unless, indeed, such a change is dependent on the presence of a gonad, and could not, therefore, be detected in ovariectomised animals. Moreover, Skowron [1935] reports that when an abortifacient amount of œstrin was followed by or injected simultaneously with male hormone into the pregnant rat, gestation was not interrupted. Such facts make one speculate on the possible function of the excessive amount of male hormone present in the pregnant woman [Womack and Koch, 1932].

The results obtained with the benzoate form of œstrone do not point to any radical change in the composition or nature of the hormone in process of transmission across the placental barrier or *via* the mother's milk. Judged by weight, the effective amounts in the two forms were approximately the same. If the œstrogenic unit of the benzoate had been considerably less than that of œstrone it might have been argued that fœtal development could have been affected by the inactivated form; however, in tests made on ovariectomised rats the benzoate was actually found to be less potent.[1] Further, the same length of time elapsed between the administration of both forms of œstrone, and an effect on the fœtus, *e.g.* delayed abortion, occurred 3 days after injection, and "modified" females were born when the hormone was administered as late as the 20th day of gestation. It would seem, therefore, that it is the concentration of the hormone that matters and not the duration of its activity.

The negative results obtained with benzpyrene, although demonstrating that this compound is not responsible for the effects obtained with œstrone and androstandiol, do not exhaust the possibility that these may be due to another derivative common to both, and investigations are proceeding.

The absence of any permanent modification of the male fœtus is remarkable not only on account of the imperfect development of the

[1] On the basis of a single injection; both oily and aqueous solutions were examined.

urogenital region in the male at the time of injection [Ruth, 1934], but also in view of the effects observed in the prostate of intact and castrated rodents as the result of repeated injections of œstrin [literature reviewed by Parkes and Zuckerman, 1935].

The ante-natal administration of excessive amounts of œstrone seems to cause a lowering of reproductivity such as has been observed by Wade and Doisy [1935] following prolonged injection of œstrin from the time of weaning.

Summary.

1. The effect of the ante-natal administration of œstrone on the morphological development of the female fœtus (rat) was further examined; the minimum amount of œstrone capable of modifying fœtal morphology was ascertained, and the earliest day at which the effect could be induced. Large amounts of œstrone did not cause further abnormalities or affect the male in a similar manner.

2. Experiments with preparations of various ductless glands failed to demonstrate that the action of œstrone was an indirect one.

3. Other compounds related to œstrone in chemical structure were examined: the di-hydroxy derivative of androsterone (male hormone) was found to affect the morphology of the female fœtus in the same way as œstrone.

4. The benzoate of œstrone has a similar action on the fœtus; the effective amounts of the two forms are compared. The suckling female also is capable of modification when the benzoate is injected into the lactating mother.

5. The urogenital region of the female rat is capable of modification by the direct injection of œstrone on the day of birth.

6. The subsequent reproductive history of litters which came under the influence of œstrone in the early stages of development is examined.

7. The significance of a similar action on the fœtus by both male and female hormones is discussed.

8. The results obtained with the benzoate of œstrone suggest that the concentration reached by the hormone is of more importance in determining the effect on the fœtus than the duration of its activity; they indicate that the effect is brought about by the hormone in its physiologically active form.

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hombreol, by Dr. Macbeth of Organon, and Antuitrin "S," by Parke, Davis & Co., both free of charge; also 1:2 benzpyrene by Union Chimique Belge, Brussels, at cost price.

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CONTENTS

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THE EFFECT OF SUCKLING ON THE DURATION OF PREGNANCY IN THE RAT (WISTAR ALBINO)

By A. M. HAIN, Ph.D.
(Carnegie Research Scholar.)
(Institute of Animal Genetics, University of Edinburgh.)

(*Received 20th December*, 1933.)

It is an established fact that in the mouse and rat ovulation normally takes place between 12 and 32 hours after parturition (Long and Evans, 1922). Only rarely, however, does a pregnancy result from a mating at this time if the mother is suckling a litter (Kirkham, 1916), and in those instances where a second pregnancy does ensue it is of longer duration than is the case in the normal non-suckling animal. Daniel (1910) was of the opinion that an exact relationship existed between the number of young suckled and the extent by which gestation was prolonged. The absence of any such correlation was demonstrated by Kirkham (1916), who also investigated the cause of the prolongation of pregnancy in such cases. This was found to be due to a delay in implantation of the ova released at the post-partum ovulation, a delay which he ascribed to the inhibitory action of the mammary glands upon the uterus; he recognised that this activity was modified by the metabolic idiosyncrasies of the individual.

In further support of the arbitrary nature of the prolongation of pregnancy in the suckling mouse, data have been presented by Mirskaia and Crew (1931), in whose opinion the delay in implantation and consequent prolonged gestation is to be attributed to the inability of the corpus luteum to respond to the double demand made upon its hormonic activity: the maintenance of pregnancy plus lactation. Since lactation can proceed in the absence of the ovaries (Corner, 1930; Hain, 1934), it is questionable whether the last-named explanation can be regarded as adequate. Kirkham's suggestion of a direct relationship between the mammary glands and the uterus, though supported by Bradbury (1932), is difficult to uphold in face of the experimental data adduced by Corner (1930), Nelson (1932) and others. Moreover, since deciduomata can form in the uterus of the lactating animal (Long and Evans, 1922), one must presuppose some extra-uterine source for the inhibition exercised upon the implantation of the ova. The indications tend to favour the existence of interaction between the mammary glands and the anterior lobe of the pituitary, and it is possible that, when in full play, this is of a nature inimical to the inception and maintenance of a normal pregnancy.

The particulars which follow are given for the following reasons:

(*a*) They demonstrate that the lack of correlation between the number of foetuses carried and suckled and the extent to which gestation is prolonged is common to both the rat and the mouse.

(*b*) They illustrate, also, that, contrary to the experience in mice, namely that any in excess of two young suckled suffice to delay implantation and so to prolong pregnancy (Kirkham, 1916), the suckling of as many as four young does not necessarily have this effect (rat RR 16).

(*c*) The delay in the development of the embryo in its initial stages has no adverse effect on its subsequent development and the foetuses born are of normal size. In view of the fact that Jones (1925) attributed the inheritance of an optical defect in rats to experimentally delayed implantation of the ovum, this point seemed of sufficient importance to merit investigation in a stock in which similar eye abnormalities have occurred.

The non-suckling rat has a gestation period of 22 days. In Table II, therefore, the extent by which pregnancy is prolonged in the suckling animal is estimated by reference to this figure, but allowance must be made for a variation within 24 hours, since mating (and ovulation) may take place at any time within this interval after parturition. It follows that in a "suckling" pregnancy of 23 days, gestation cannot be regarded as having been prolonged. After the post-partum ovulation both oestrus and ovulation are suspended until after the termination of the normal period of lactation unless only one or two young are suckled, in which case oestrus is not inhibited and normal cycles occur (Long and Evans, 1922; Crew and Mirskaia, 1930). The inhibition of ovulation during lactation in the rat was proved experimentally by Long and Evans by means of vital staining of the ovary and by the vaginal smear method.

Table I. *Pregnancy in suckling rats dating from other than post-partum ovulation.*

Rat	Date of 1st parturition	Litter size: 1st	Date of 2nd parturition	Litter size: 2nd	Interval days	Prolongation of pregnancy days
QQ25	8. viii. 33	2 +3 from 22. viii. to 2. ix	14. ix. 33	11	37	Normal oestrous cycles: mated 22. viii. 33. No prolongation
QQ12	30. vii. 33	8	12. ix. 33	5	43	Young with mother till 2. ix. 33. Mating occurred 21. viii. 33: 2nd gestation not prolonged

Two instances have been given of pregnancies dating from other than the post-partum ovulation (Table I). In rat QQ25 normal oestrous cycles occurred as only two were suckled. It is interesting to note that the addition of three suckling rats on the 15th day of lactation did not cause ovulation to be inhibited and had no effect on a pregnancy dating from this time. The other member of this group—QQ12—illustrates the fact demonstrated by Parkes (1926) and Crew and Mirskaia (1930) in the mouse, that the dioestrum which persists during the three weeks of suckling following parturition is not extended by a continuance of suckling. According to Long and Evans (1922) the typical oestrous smear in the rat does not return until at least 4 days after the suckling young are removed, from which Long and Evans conclude that the fundamental cause of oestrus cannot operate so quickly after a

period of lactation as it can under normal conditions. However, definite changes were observed in the corpora lutea of lactation within 24 hours of the cessation of lactation, and it is clear from the case of rat QQ 12 that pregnancy can arise from an ovulation that takes place on the 21st–22nd day of lactation or the last day of the lactation dioestrum.

The first three rats in Table II demonstrate that where three or four young are suckled (irrespective of the number born) the second gestation period is not prolonged. According to Donaldson (1924) the period of gestation is always prolonged when a female is suckling six or more young. The arbitrary nature of the effect of the number of young suckled upon the extent to which pregnancy is prolonged is well illustrated in five rats which suckled nine, ten or eleven young: QQ25, QQ45, QQ7, RR 5 and QQ37. In the first four pregnancy was extended by 12, 13 and 14 days; in the last-named by 5 days only, although this rat was carrying ten young, as against five in two of the others.

Table II. *Effect of lactation on pregnancy dating from post-partum ovulation.*

Rat	Date of 1st parturition	Litter size: 1st	Date of 2nd parturition	Litter size: 2nd	Interval days	Prolongation of pregnancy days
V20	2. x. 33	10 (3 suckled)	25. x. 33	8	23	—
QQ14	1. viii. 33	13 (3 suckled)	24. viii. 33	6	23	—
RR16	9. ix. 33	5 (4 suckled)	1. x. 33	8	22	—
QQ25	14. ix. 33	11	19. x. 33	9	35	13
QQ45	10. viii. 33	11 (9 suckled)	14. ix. 33	5	35	13
QQ7	1. ix. 33	10	7. x. 33	12	36	14
RR5	25. viii. 33	11	28. ix. 33	5	34	12
QQ37	21. ix. 33	12 (10 suckled)	18. x. 33	10	27	5
QQ12	12. ix. 33	5	6. x. 33	14	24	2
QQ54	11. ix. 33	5	6. x. 33	14	25	3
QQ33	9. viii. 33	10 (8 suckled)	6. ix. 33	11	28	6
QQ35	8. viii. 33	8	6. ix. 33	9	29	7
QQ48	29. ix. 33	12 (3 suckled)	23. x. 33	11	24	More than 3 were suckled at first but died
QQ27	10. ix. 33	9 (8 suckled)	7. x. 33	12	27	5
NN9/2	2. ix. 33	7	1. x. 33	9	29	7

The size of the litter *carried* plays hardly any part in prolonging pregnancy in the suckling mother. Remarkable instances of large litters carried by suckling mothers are afforded by rats QQ 12 and QQ 54, both of which gave birth to litters of 14, all of which were alive. The extension of the gestation period by 2 and 3 days respectively was such as one might anticipate where five young are suckled, though King (1913) found that the gestation period in the lactating rat is of normal length if the female is suckling five or fewer young and is carrying five or fewer young, and that it may be prolonged where this number is suckled, provided that six or more young are carried.

The material used afforded an excellent opportunity for ascertaining if any connection existed between delayed implantation of the ovum and microphthalmia, as in several instances matings were made with microphthalmic males (QQ33, QQ35, QQ7, QQ48). In three of these delayed implantation occurred (as determined by the prolongation of pregnancy by 6, 7 and 14 days), yet in not a single instance were the offspring other than normal. Rats QQ12 and QQ14 were sisters of microphthalmic males: the first had normal young; the second gave birth to one blind female in the first litter but had normal young in the second. Pregnancy was not prolonged. Rats QQ27, QQ37 and V20 had parents or grandparents heterozygous for the defect and were mated with their brothers. In two, gestation was prolonged by 5 days without adverse effect on the offspring. In the case of V20, in which parturition occurred at the normal time, defectives were born in both litters. Similarly abnormal young were born in both litters of rat RR16, yet there had been no delay in implantation of the ova. This rat belonged to the strain of microphthalmics and her sister gave birth to four defectives.

It is evident that the incidence of microphthalmia cannot be explained on the basis of delayed implantation, as not only did the defect occur where no such delay had taken place, but where the delay existed none of the offspring exhibited the defect even though the parents belonged to the defective strain.

SUMMARY.

1. As is the case in mice, pregnancy is prolonged in the rat when the pregnant mother is suckling young. When only three or four are suckled the ensuing pregnancy may be of normal duration.

2. No correlation exists between the number suckled and the extent by which gestation is prolonged, and there are individual variations in the effect upon gestation of the same number of young.

3. In a stock in which microphthalmia had been encountered, the delayed implantation of the ova due to suckling was found to have no effect upon the distribution of the abnormality.

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EFFECT UPON LACTATION OF OÖPHORECTOMY DURING PREGNANCY. (ALBINO RAT.) By ANNIE M. HAIN (Carnegie Research Scholar). Institute of Animal Genetics, University of Edinburgh.

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EFFECT UPON LACTATION OF OÖPHORECTOMY DURING PREGNANCY. (ALBINO RAT.)

By Annie M. Hain (Carnegie Research Scholar). Institute of Animal Genetics, University of Edinburgh.

(*Received for publication* 1*st March* 1933.)

The intimate relation which exists between the anterior pituitary and lactation has been clearly demonstrated by Corner [1930], who induced lactation in spayed virgin rabbits by injecting anterior pituitary extract. More recently, Riddle, Bates, and Dykshorn [1932] claim to have isolated the anterior pituitary hormone which is responsible for lactation ("Prolactin"), but from their experimental data it would seem that the secretion of œstrin is a necessary adjunct, as the effect was only obtained in the mature rabbit, or in the male guinea-pig after the injection of œstrin.

Whereas it is possible in the rabbit to elicit milk secretion in the absence of the ovaries, Evans and Simpson [1931 *a* and *b*] have been unable to evoke a similar response in the rat under the same conditions. Although mammary development was induced by means of injection of an alkaline extract of anterior pituitary in the virgin and immature rat, no effect was obtained in the oöphorectomised animal. Accordingly, Evans and Simpson insist on the necessary concurrence of the ovary in the mammary response to the anterior pituitary in the rat.

The experiment just described [Hain, 1934], in which the ovaries of a number of rats were removed at various stages of pregnancy either after previous injection of anterior pituitary extract or without such treatment, afforded an opportunity for further study of the mechanism of lactation in this animal.

Results.—Twelve rats were under observation, and with the exception of the members of one group (rats injected with alkaline extract of anterior pituitary), each rat was submitted to several tests by the substitution of fresh young when a definite drop in their weight indicated that no nourishment had been obtained by those previously foster-mothered.

A perusal of the table shows that oöphorectomy at normal term, whether preceded by the injection of anterior pituitary extract or not, interfered with lactation. In such rats parturition was delayed for 2 to 3 days, but, although the percentage of still-births was high, living fœtuses were born in all litters. In the uninjected group two rats gave

a little milk for a brief period, but this was not maintained. The interference with lactation in a rat in which the ovaries were merely raised, but not removed, indicates that the withdrawal of ovarian secretion may not have been solely responsible for the effect obtained in oöphorectomised rats. The results of oöphorectomy at stages earlier in pregnancy confirm this fact, as partial milk secretion occurred in one rat after oöphorectomy performed on the 17th day, and complete (though

Effect upon Lactation of Oöphorectomy during Pregnancy (Rat).

	Rat No.	Result.	No. of Tests.
Oöphorectomy at normal term .	X2	No milk.	1
	S8	Little milk for 4 days.	3
	V5	,, ,, ,, 8–9 days.	5
Oöphorectomy at normal term after injection of alkaline extract of anterior pituitary (equivalent to 2–2½ grams tissue).	A9	No milk.	1
	E1	,, ,,	1
	E2	,, ,,	1
	T9	,, ,,	1
Laparotomy at normal term .	X4	No milk.	3
Oöphorectomy on 17th day .	V2	Little milk for 1 day (*i.e.* at normal term).	3
	V3	Milk from 2 days after normal term (*i.e.* also 2 days after completion of parturition) and continuously for 5 weeks.	2
	V6	No milk.	1
Oöphorectomy on 18th day .	X7	No milk.	4

delayed) lactation was established in a second rat—V3—which, over a period of 5 weeks, foster-mothered five young, all of which, when weaned, exceeded in weight controls of the same age. In both of the rats described above, parturition occurred at normal term but was protracted, extending from the 20th to 22nd days, and the fœtuses were undersized and still-born. In other rats from which the ovaries were removed at the same stage of pregnancy (17th and 18th days) either no milk was secreted or lactation was not successfully established. It was observed that lactation sometimes commences before parturition is complete and while the rat is still pregnant.

With the exception of rat V3 (in which lactation persisted for 5 weeks), all the rats used in the experiment described were autopsied on completion of the test, but no trace of ovarian tissue was found. Rat V3 was not immediately killed but received injections of a concentrated,

protein-free extract of human pregnancy urine. Vaginal smears were taken thereafter and demonstrated that the ovaries had been completely removed. This finding was also confirmed later by laparotomy.

Discussion.

The experimental data, although collected from a small number of animals, are sufficient to show, when allowance is made for individual variation, that the removal of the ovaries at any stage of pregnancy in the rat may prevent lactation or diminish it to almost negligible proportions. At the same time it has been demonstrated that lactation equal in every respect to that in the intact rat can occur after oöphorectomy performed as early as the 17th day of pregnancy, and without the assistance of any endocrine extract. It appears that, although the ovaries may play an important part in the development of the mammæ, especially in the early stages of their development, their secretion for a period equivalent to the full gestation period is not necessary in all cases for lactation in the rat. Also, since lactation commenced 6 to 7 days after removal of the ovaries (rat V3), it would seem that ovarian secretion does not play an essential part in initiating or maintaining the process of lactation. The experiments demonstrate, furthermore, that the stimulus of suckling is not sufficient to induce a continuance of milk secretion.

Emphasis has been laid by Evans and Simpson [1931 *a* and *b*] on the essential part played by the ovaries in the early development of the mammary glands in the rat, and there are indications that in the rabbit ovarian secretion may play a rôle of almost equal importance. In Corner's experiment [1930] the rabbits used for injection of anterior pituitary extract were spayed at the beginning of the experiment, generally on the day of the first injection. The extract would, accordingly, be injected in the presence of œstrin. Corner found that a rabbit spayed ten days before injection showed only a delayed and partial result. He concludes: "Very likely, the mammary glands must first be subjected to the influence of œstrin and thus be brought into the full pubertal condition at the time of spaying and the beginning of injections." This observation has since received confirmation by Nelson and Pfiffner [1931] in the guinea-pig and by Bradbury [1932] in the mouse.

Since lactation was induced by the injection of an alkaline extract of anterior pituitary in the virgin rat [Evans and Simpson, 1931 *a* and *b*] it is all the more remarkable that this effect was not elicited by the same treatment in the pregnant rat,[1] and that lactation was in any way interfered with by oöphorectomy at normal term even in the uninjected

[1] Only one test was made in each rat in this group; it is possible that a response would have been obtained with further tests.

animal. Apparently there is no question of the birth of living as against dead fœtuses playing any part in the distribution of lactation within the group, as in many cases when live fœtuses were born no lactation occurred. In the opinion of Bradbury [1932] lactation is due to the release of the inhibition placed on the mammæ "by some uterine substance which is lost at parturition," but it is clear that the mere act of parturition itself is not sufficient to induce lactation.

There appear to have been few instances in the literature of the study of lactation after oöphorectomy in the pregnant animal. Hammond in 1917 removed the fœtuses from the rabbit on the 15th day of pregnancy and noted an arrest of mammary development with immediate appearance of milk in the duct. These experiments were only partially confirmed. Evans and Simpson [1931 *a*] did not carry their experiments into advanced pregnancy. Johnson and Challans [1930] removed the ovaries from three rats on the 19th day of pregnancy and state that in none were the mammæ functional; in only one out of four did they function after removal at 21 days, but details are not given of the duration of lactation. However, when an extract of corpus luteum was given following bilateral oöphorectomy on the 19th day, the mammæ were functional in all cases, also after removal of the 13th day when this was followed by the same treatment. Similarly, Corner [1930] obtained lactation in rabbits deprived of their ovaries 18 hours after conception when injected with corpus luteum extract. It was pointed out that this action was not due to the corpus luteum, as it was not obtained in spayed, non-pregnant rabbits when given the same treatment. Also it was demonstrated in Corner's Laboratory by Jares [1930] that although the corpora lutea formed in non-pregnant rabbits by the injection of extracts of pregnancy urine caused a prolonged pseudopregnancy, they did not cause any changes in the mammæ. Therefore, Corner [1930] concluded that "the corpus luteum acting alone in the absence of pregnancy (even when prepared by œstrin) does not induce proliferation of the mammary glands or lactation."

The experiment which has herein been described affords evidence that ovarian secretion is not the regulator of lactation nor the activator of that secretion of the anterior pituitary which, according to recent research, probably controls this function.

Conclusions.

1. Removal of the ovaries at any stage of pregnancy in the rat may either prevent lactation or diminish it to almost negligible proportions. In one animal, however, normal lactation occurred after oöphorectomy on the 17th day.

2. Injections of alkaline extract of anterior pituitary failed to promote lactation in rats oöphorectomised during pregnancy.

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THE EFFECT (*a*) OF LITTER-SIZE ON GROWTH AND (*b*) OF ŒSTRONE ADMINISTERED DURING LACTATION (RAT).

By ANNIE M. HAIN (Carnegie Research Fellow). From the Institute of Animal Genetics, University of Edinburgh.

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By Annie M. Hain (Carnegie Research Fellow.) From the Institute of Animal Genetics, University of Edinburgh.

(*Received for publication* 1*st August* 1935.)

Data have been published under both of the above headings for the mouse [Parkes, 1926 *b*; Robson, 1935], but similar information is lacking for the rat. For this reason alone the investigation appeared justified, but considerations arising out of experiments with œstrone on pregnant rats rendered imperative an examination of the lactating rat under similar treatment.

The experiments to be described were designed to ascertain:

(*a*) The effect of litter-size on the growth of the individual, and the maximum milk-yield of which the rat is capable.

(*b*) The effect of ketohydroxyœstrin (œstrone) on (*a*).

(*c*) The possible effect of a sudden excessive dosage of œstrone on the morphological development of the suckling female, such as had been previously demonstrated on the fœtus in utero [Hain, 1935 *a* and *b*].

The expenses of this research were defrayed by grants from the Carnegie Trust and the Medical Research Council. The author is indebted to the British Drug Houses, Ltd., for supplies of œstrone at a greatly reduced price.

I. Methods.

Wistar albino rats kept under standard conditions [Hain, 1934] were employed; these were generally primiparæ and 3–4 months old, but some second and a few third litters have been included. For the purposes of ascertaining the normal growth in relation to litter-size litters were selected containing every number between 2 and 13 youngsters. Both control and experimental litters were weighed daily until the 26th day of life; the average weight per rat on each day was ascertained, and the averages for all rats of a group (*e.g.* 2–7 in a litter, or 8–13) were totalled and divided by the number of litters. In this way the growth curves give reliable information as to the daily rate of growth, with and without treatment. The frequency distribution of litters in both cases is shown in Table I.

TABLE I.—FREQUENCY DISTRIBUTION OF LITTERS.

Controls.		Injected with œstrone.				
Size of litter.	No. of litters.	Size of litter.	No. of litters.			
			2–2·5 mg.	1·0 mg.	0·5 mg.	0·25 mg.
2	2	2	..	1	1	..
3	5	3	6	2	..	1
4	7	4	2	4	2	2
5	2	5	..	4	2	..
6	..	6	4	2	1	4
7	2	7	3	5	3	3
8	3	8	3	2	2	3
9	3	9	3	5	2	3
10	3	10	3	2	3	1
11	3	11	1	6	2	..
12	2	12	..	2	..	..
13	3	13	1	..	1	..
Total	35	Totals	26	35	19	17

2–7 in litter: 18 litters, 71 rats.
8–13 in litter: 17 litters, 177 rats.

2–7 in litter: 52 litters, 263 rats.
8–13 in litter: 45 litters, 436 rats.

Crystalline ketohydroxyœstrin (B.D.H.) was used throughout in a 10 per cent. aqueous alcohol solution (with the single exception of the rat injected with 3·0 mg. of œstrone, when the medium employed was sesame oil). Since the main purpose of the experiment was to determine the effect of a sudden excess of œstrone rather than its prolonged administration, injections were made mainly during the first few days of lactation, and were given hourly for 10 hours either on one day only or on four consecutive days. The time of injection was between the 1st and 6th days, but in a limited number of animals the effect of a given amount of œstrone was spread over a longer period, *e.g.* for 9 or 10 days when injections were made twice daily; in such rats the stage at which treatment commenced varied from the 4th to the 11th day.

Both in control and experimental animals vaginal smears were taken daily till the 26th day; in some rats these were continued until the termination of the lactation diœstrus and the litter was meantime left with the mother.

II. NORMAL GROWTH IN RELATION TO LITTER-SIZE.

It is well known that growth is much more rapid when only a small litter is being suckled [Parkes, 1926 *b*; Parkes and Bellerby, 1927].

Since a rat can suckle as many as eighteen youngsters, this animal seemed a profitable subject on which to ascertain the effect of numbers on individual growth and on milk-yield capacity.

Thirty-five control litters were used, or a total of 248 rats. When the average weights per rat of all litter-sizes were compared it was found that in litters containing 2–7 young the averages were comparable, and again in those containing from 8–13 young; accordingly two groups were made.

From fig. 1 it will be seen that, while throughout growth is more rapid in the 2–7 group than in the 8–13 group, the difference is specially

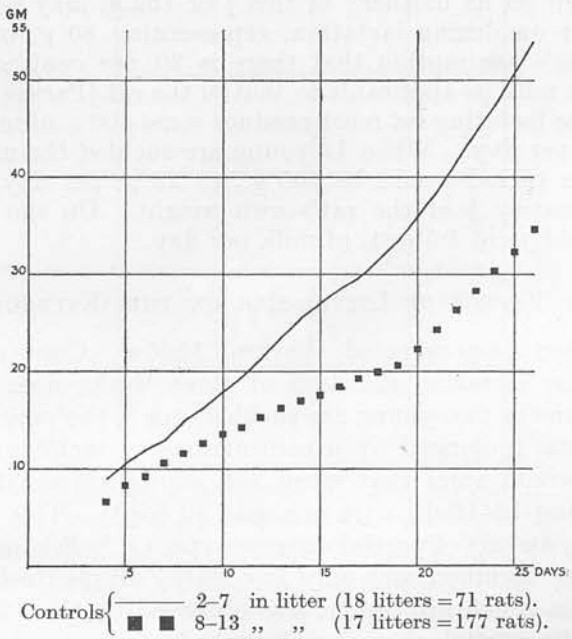


FIG. 1.—Growth curves of young of normal mothers during first 26 days of life.

marked between the 10th and 20th days. The stage of rapid growth upon which the larger group enters at the 19th-20th day is less abrupt in the 2–7 group, although it clearly exists. It commences at the same time in both groups, and is independent of the size of the youngster, *e.g.* the rat of the 2–7 group weighs 35 g., while that of the 8–13 group weighs only 22 g. One would have expected that the advantage in weight held by the 2–7 group would hasten the stage at which the rapid increase commenced. From this time the rate of increment is almost the same in both groups, a fact which indicates that the retardation of growth from the 10th to 20th days when a large litter was suckled was due to lack of adequate nourishment.

The growth curves run parallel, not only in the period of rapid growth dating from the time at which the youngsters commence to

feed for themselves, but also during the first 9 days of life. At this period growth is at a very high rate, and actually in the larger group the daily increment is greater than at any other time during the first 19 days, not only relatively, but absolutely; thus the young rat adds 10–20 per cent. of its own weight daily between the 4th and 10th days, and almost doubles its weight in that time. In the 2–7 group the rate of growth is almost constant throughout the 19 days.

The amount of milk secreted by a rat is probably slightly less relatively than that produced by a mouse. A litter of six rats weighs about 216 g. at 20 days old, when it ceases to be wholly dependent on its mother; of this $\frac{3}{4}$ or 162 g. may be held to have been put on during lactation, representing 80 g. of dry matter. If Parkes's assumption that there is 20 per cent. of dry matter in mouse milk be applicable to that of the rat [Parkes and Bellerby, 1927], the lactating rat must produce some 400 g. of milk in 20 days, or 20 g. per day. When 12 young are suckled the milk-yield over the same period would be 500 g., or 25 g. per day, representing approximately $\frac{1}{8}$ of the rat's own weight. On the same basis a cow would yield 1·5 cwt. of milk per day.

III. The Effect of Litter-size on the Œstrous Cycle.

It has been demonstrated [Parkes, 1926 *a*; Crew and Mirskaia, 1930] that the lactation dicestrus of three weeks does not occur in mice if only one or two young are suckled, nor is the period of dicestrus (when it exists) prolonged by a continuance of suckling. In the rat, however, it would seem that when 2–6 young are suckled there is a dicestrus lasting 12–16 days (in one case 19 days). This was generally terminated by smears of partial œstrous type, *i.e.* consisting of epithelial cells becoming cornified and only few fully cornified cells; in one or two animals in which full œstrus was observed on the 12th–16th days a smear of the partial character described was also observed on the 4th day and/or 8th day, but this was exceptional. The œstrous period, terminating the fortnight's dicestrus, was not followed by a resumption of the normal 5-day cycles, but by another period of dicestrus of 10–13 days' duration.

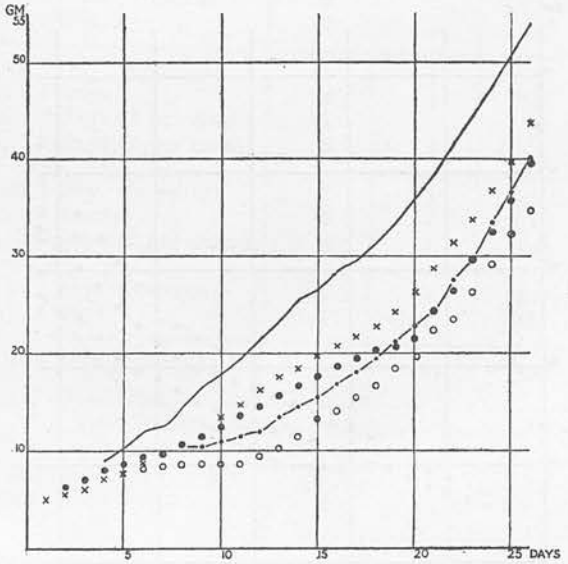
When 7–11 young were suckled the lactation dicestrus persisted for 22–28 days, and in the case of litters containing 12–13 this period was extended to 40 days and longer if the youngsters were not withdrawn. This is in agreement with Long and Evans's data [1922].

IV. The Effect of Œstrone on:

(a) *The Growth Rate.*

[When the same total amount of œstrone, variously administered, gave similar results within a group the average weights were combined.]

From a comparison of figs. 2 and 3 it is evident that a given amount of œstrone has a more marked effect on the growth of small litters than on those in which a large number is suckled; indeed, the effect on the former is similar to that produced by raising the litter-size to that of the 8–13 group. It is observed, also, that although the deleterious effect on growth increases with the amount of œstrone administered, this increase is not in direct ratio to the amount given, so that there



2–7 rats in litter.
——— Controls: growth of normal rats 5–26 days old.
× × 0·25 mg. œstrone in 1 day and in 4 days.
● ● 0·5 ,, ,, 9 days and in 4 days.
— · — 2·0 ,, ,, 1 day and in 4 days.
○ ○ 2·5 ,, ,, 4 days.

(*N.B.*—1·0 mg. œstrone same effect as 0·5 mg. so not shown.)

FIG. 2.—Effect on young rats of œstrone administered to the lactating mother: 2–7 rats in litter.

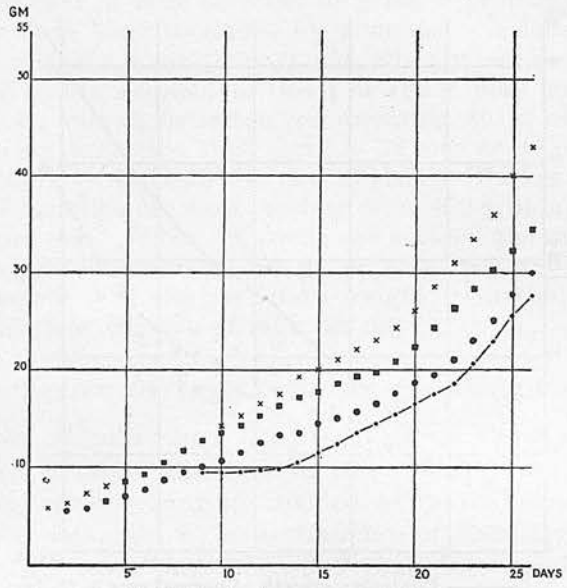
is little difference between the curves for 0·25 mg. and 2·0 mg. of œstrone. The details given in Tables II. and III. correct this somewhat erroneous impression. In both groups there is a considerable death-rate when 1·0 and 2·0 mg. of œstrone are injected, and this is especially marked when the latter total is given in hourly injections in one day. The effect of the minimum dose used (0·25 mg.) in the two groups is in marked contrast; the injurious effect observed in the "smaller" group is entirely absent from the "larger."

It is of interest to note that, when as much as 2·0–2·5 mg. of œstrone was injected on the first day of the puerperium only, 3 out of 13 litters died; of 10 other rats in which injections of 0·5–2·0 mg. were spread over the first four days 2 litters died. The ability to withstand such large quantities of œstrone at this early stage of development may be

related to the rapid growth which takes place normally during the first 8 or 9 days of life [Parkes, 1926 *b*].

The effect on milk-yield for the 20 days (if the figures for 0·5 mg. be taken) is to reduce the total of 400 g. to 250 in the "smaller" group, and 500 g. to 425 in the "larger" group. The yield per day is $12\frac{1}{2}$ g. cf. 20 and 21 g. cf. 25.

With the possible exception of the 2·0 mg. total in the "larger"



8–13 rats in litter.
■ ■ Controls.
× × 0·25 mg. œstrone in 1 day and in 4 days.
● ● 0·5 ,, ,, 1 day and in 9 days.
— · — 2·0 ,, ,, 1 day.

(*N.B.*—1·0 mg. œstrone in 1 day and in 4 days almost identical with curve for 0·5 mg. so not shown.)

FIG. 3.—Effect on young rats of œstrone administered to the lactating mother: 8–13 rats in litter.

group œstrone did not modify the time at which the sucklings entered upon their stage of rapid growth.

The cause of death deserves further investigation. It was at first thought that this was due to an inhibition of milk-secretion in the mother, as the young died of starvation. The peculiar behaviour of the mother threw some doubt on this assumption. The first indication of abnormality was that at about the 5th or 6th day after injection the mother deserted her nest and showed no interest in her young, which were found scattered in various directions and quite cold. Up to this time the rate of growth had been normal, and it should be noted that the fall in weight which subsequently took place occurred after and not before the mother's change in attitude toward her young.

TABLE II.—2–7 YOUNG IN LITTER.

Treatment.	No. of litters.	Whole litter died.	No. of rats suckled.	No. survived.	Wt. at 26 days. g.
Controls	18	..	71	71	54
Œstrin injected:					
0·25 mg. in 1 day (hourlies)	3	..	18	18	45
,, ,, 4 days ,,	7	..	36	32	42
0·5 mg. in 1 day (hourlies)	1	1	2	..	..
,, ,, 4 days ,,	4	1	23	15	43
,, ,, 2 days (3 per diem)	1	..	4	4	53
,, ,, 9 days (2 per diem)	3	1	18	14	36
1·0 mg. in 1 day (hourlies)	7	1	47	36	36
,, ,, 4 days ,,	5	1	19	14	44
,, ,, 10 days (2 per diem)	6	1	25	20	41
2·0 mg. in 1 day (hourlies)	8	4	37	20	41 *
,, ,, 4 days ,,	4	1	19	16	39 *
2·5 mg. in 4 days (hourlies)	3	..	15	15	34

* 1 litter in each section contained "modified" females.

TABLE III.—8–13 YOUNG IN LITTER.

Treatment.	No. of litters.	Whole litter died.	No. of rats suckled.	No. survived.	Wt. at 26 days. g.
Controls	17	..	177	177	35
Œstrin injected:					
0·25 mg. in 1 day (hourlies)	2	..	16	16	46
,, ,, 4 days ,,	5	..	45	45	39
0·5 mg. in 1 day (hourlies)	5	..	51	50	29
,, ,, 4 days ,,	..	..	..	..	..
,, ,, 2 days (3 per diem)	2	..	22	22	31
,, ,, 9 days (2 per diem)	3	..	26	26	31
1·0 mg. in 1 day (hourlies)	8	2	79	50	30
,, ,, 4 days ,,	5	1	51	40	31
,, ,, 10 days (2 per diem)	4	3	41	8	27
2·0 mg. in 1 day (hourlies)	7	3	69	33	28
,, ,, 4 days ,,	2	..	20	10	35
2·5 mg. in 4 days (hourlies)	1	1	8	..	..
* 3·0 ,, 2 days ,,	1	..	8	8	38 †

* [Oil].
† Litter contained "modified" females.

A practice was made of squeezing the nipples in order to ascertain if milk were available, and almost without exception considerable quantities of milk could be expressed. The youngsters were warmed by hand and a nest made and the mother was coaxed to nurse them, but refused to do so; usually this occurred on three successive days, and on the fourth day the litter was dead, when little or no milk could be expressed from the nipples. Occasionally, when the litter was a large one, the mother might recover her "maternal instinct" in time to rear a few of the hardier survivors.

(b) *Effect of Œstrone on Recurrence of Œstrus.*

Although as much as 2 and 3 mg. of œstrone was injected into the lactating rat either immediately after littering or during the first four days of lactation in no case was a full œstrous smear obtained, the typical smear being EE–C (*i.e.* epithelial cells in their ordinary state and in process of losing their nucleus). Moreover, the existence of such cells in the smears over a period of 6–7 days, such as frequently (but not always) occurred following the injection of 1 and 2 mg. of œstrone, was in no way related to the death of the litter, and was as often associated with normal development of the offspring.

The effect of œstrone on the lactation dicestrus was generally to lengthen the interval in both groups. When litters containing 3 or 4 were suckled, dicestrus lasted between 20 and 33 days, and in only 2 out of 12 animals was it terminated by a fully cornified smear. One rat was in partial œstrus on the 10th day, but a dicestrus of more than 23 days followed. When 5–13 youngsters were suckled the lactation dicestrus lasted from 26–46 days.

(c) *Effect on Urogenital Development of the Suckling Female.*

The permanent modification of the external genital region of the female fœtus which was observed after the injection of the pregnant rat with 0·5–1·0 mg. of œstrone on a single day late in pregnancy [Hain, 1935 *a* and *b*] occurred also after the injection of the lactating rat. Larger amounts of œstrone were required, viz. 2 and 3 mg., and the effect was obtained with much less regularity than when the pregnant rat was injected. Such "modified" females occurred in three litters: when 2·0 mg. was injected over the first 4 days after parturition; when the same amount was confined to a single day, viz. the third after parturition;[1] and when 3·0 mg. was given in oil during the first 2 days of the puerperium; in the last two groups the effect was less marked and later in being manifest: total number of affected females, 8. In these abnormal females the urethral prominence is deeply cleft, exposing the clitoris, and the terminal course of the

[1] Young weighed < 5 g. on day of injection.

urethra is lacking, in consequence of which the urine escapes by a fistulous opening immediately in front of the vagina and there is incontinence. Usually the vagina of such youngsters opens precociously, *e.g.* when the rats are 19–21 days old, but œstrous cycles are not established before the 33rd day.

Since the results did not preclude the possibility that a breakdown product from œstrone and not œstrone itself was responsible for effecting the modification *via* the mammary gland or placenta, two experiments were performed. (*a*) The milk of treated mothers was injected into ovariectomised rats, when it was found to contain rather less than 5 R.U. per c.c. (*b*) Ten new-born female rats were injected with 1·0, 2·0, and 3·5 mg. of œstrone, the total amounts injected being 0·2 and 0·35 c.c. in 2 and 4 injections (sesame oil); in one injected litter of five females two were kept as controls. Nine youngsters exhibited in full the morphological abnormality described, *including the two controls*. It was thus evident that the effect of injection into the new-born rats was not solely attributable to direct treatment but in considerable measure traceable to the transmission of œstrone *via* the mother's milk. This seems less likely to have occurred in those rats that received 1·0 mg., as this amount injected into the lactating rat produced no instance of abnormality in the offspring, and as much as 3·0 mg. of œstrone in *oil* solution produced only the slightest effect when similarly injected.

Discussion.

The particulars given regarding the rate of growth of the rat during the first 26 days of life complete the data previously collected regarding the Wistar rat in Great Britain [Hain, 1934], besides providing a control for the effects of œstrone injection.

The tendency of œstrone to cause an extension of the lactation dicestrus seems to indicate an effect of the hormone upon the anterior pituitary whereby luteinisation was encouraged—an hypothetical action suggested by Hohlweg [1934] and Hisaw *et al* [1934], but one which requires further experimental support. It is to be noted that although the vagina opened precociously in œstrone-treated youngsters, œstrous cycles were not prematurely established, showing that the anterior pituitary had not been activated.

It would seem that the starvation of litters which sometimes occurred after the injection of the lactating rat with large quantities of œstrone was generally due to a psychological rather than a physiological effect, and that œstrone, at least in excessive amounts, is inimical to "maternal behaviour" [Wiesner and Sheard, 1934]. Starvation was not due, as in mice [Robson, 1935], to a failure of mammary secretion, except indirectly and ultimately.

It is manifestly impossible to compare the effects of œstrone admin-

istered in lactation and in pregnancy in relation to the development of the offspring. At any time during the first two-thirds of pregnancy even minute amounts of œstrone are fatal to the ovum or embryo. A comparison is only possible between the latter third of pregnancy, *i.e.* from the 16th to the 21st days, and the first 4–5 days of lactation. Throughout both periods the morphological development of the young rat is capable of similar modification under the influence of œstrone. Since a greater degree of plasticity exists in the fœtus in utero than in the youngster at and after birth, it is only reasonable that development should be affected more readily, *i.e.* with a smaller amount of œstrone than was required in the early puerperium. Owing, perhaps, to variations in the degree of development already attained by the offspring at birth, as well as to individual variations in the transmission of the effective agent from the lactating mother, the modification of the external genitalia occurred with much less regularity in the suckling rat than in the fœtus in utero.

Since the presence of considerable quantities of "folliculin" has been demonstrated in human colostrum [Lacassagne et Nyka, 1934], the possibility was suggested that the injection of œstrone had caused a colostrum-like fluid to be secreted instead of milk. It is exceedingly unlikely, however, that litters of 6 and 8 rats (in which "modified" females occurred) could add 20 and 24 g. to their respective weights between the second and fifth day of life if colostrum provided their sole nutriment. It was intended, however, to examine the effect of human colostrum in this connection. Colostrum, withdrawn on the first day of the puerperium, was kindly supplied for this purpose by the Royal Simpson Memorial Hospital, Edinburgh. This was fed to 10 newly born female rats at 2-hourly intervals by means of a glass pipette, to the fine end of which a small piece of No. 1 rubber tubing was attached for sucking. Fresh colostrum was obtained every twelve hours; the pipettes were boiled between each "feed" and fresh tubing attached; and the youngsters were bathed with warm olive oil several times. Only one rat survived for 43 hours. This was given to a foster-mother, but was crushed later in the day. The experiment does not appear to be practicable.

Summary.

1. The effect of litter-size on the growth-rate of the individual rat is given, and groups containing 2–7 and 8–13 in a litter are compared. The effect of numbers on milk-yield and on the length of the dicestrous interval is also examined.

2. The injection of œstrone into the lactating rat did not induce œstrus; it had an injurious effect on the growth of the young, but not in direct ratio to the amount administered. When litters were starved as the result of the injection of the mother, this was not due to

an inhibition of mammary secretion, but to disinterestedness on the part of the mother.

3. There was a tendency to a prolongation of the dicestrous interval in lactating rats injected with œstrone, and this was more marked in rats suckling small litters.

4. It was found possible to cause a morphological abnormality in the development of the urogenital region of the suckling female by injecting large quantities of œstrone into the lactating rat. The effect is thus induced through the channel of the mother's milk.

5. A similar effect was produced by the direct injection of œstrone into new-born females.

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An Effect on the Rat of Antenatal and Postnatal Administration of Œstrin

BY

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AN EFFECT ON THE RAT OF ANTENATAL AND POSTNATAL ADMINISTRATION OF ŒSTRIN.*

By A. M. HAIN, Ph.D.

Carnegie Research Fellow, Institute of Animal Genetics, Edinburgh.

IN the course of a somewhat comprehensive investigation into the cause or causes of the onset of labour, and in testing the effect of œstrin in this connection, I observed in 1933 that the females of a litter born to a rat injected during pregnancy with œstrin had a genital deformity the nature of which will be described later. It was not until 1934 that a similar occurrence suggested that a connection existed between the administration of œstrin to the mother and the abnormality of the offspring.

A prolonged series of experiments has demonstrated that the *female fœtus* is indeed affected in its morphological development by the antenatal administration of œstrin to the mother late in pregnancy. There appears to be only one instance in the literature of an effect on the fœtus due to such treatment: Courrier [1] has reported a proliferation of the vaginal epithelium of the female fœtus of the guinea-pig.

In ascertaining the amount of œstrin that is required to interrupt pregnancy, I found that the rat could tolerate large amounts in the latter half of pregnancy without abortion ensuing, and that every female fœtus born to an œstrin-treated mother showed a marked modification of the external genital region. In the normal female rat the urethra opens on the apex of the prominence which encloses the clitoris, and behind this prominence, separating it from the vaginal opening, there is a considerable area of skin which may be compared to that of the vestibule in the human female, and which covers the latter portion of the urethra which lies fairly superficially. In the females modified by œstrin injections into the mother, the urethral prominence is deeply cleft, and within the fissure so formed there is exposed the clitoris. The cleft splitting the prominence also runs backwards as far as the vaginal opening and, in doing so, splits open the floor of the urethral canal in its terminal course from the front of the vagina to the normal point of exit on the prominence. In other words, a state of partial hypospadias has been produced in as far as the floor of the terminal urethra is lacking. In consequence of this modification, the urine escapes by a fistulous opening immediately in front of the vagina. This

* Read at a Meeting of the Edinburgh Obstetrical Society, 8th May 1935.

FIG. 1 A.

Photograph of External Genitalia of normal new-born female rat.

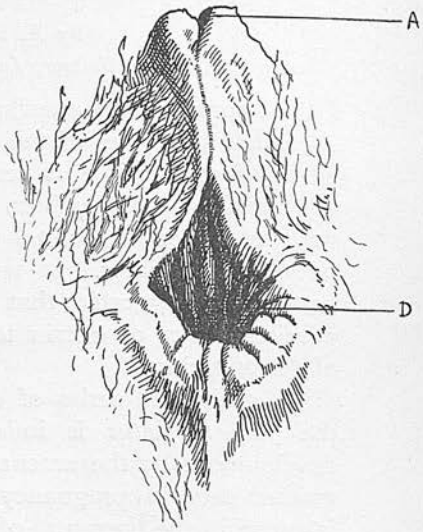

FIG. 1 B.

Detailed drawing of External Genitalia of normal female rat.

(A) Prominence on summit of which urethra opens.
(D) Vaginal opening.

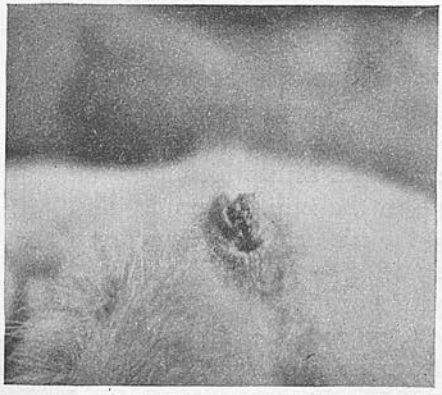

FIG. 2 A.

Photograph of modified External Genitalia of female rat after injection of mother with œstrin.

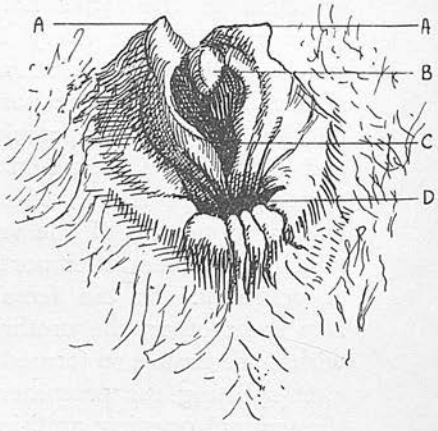

FIG. 2 B.

Detailed drawing of modified External Genitalia of female rat.

(A) Cleft prominence.
(B) Clitoris exposed.
(C) Exposed dorsal surface of urethral canal.
(D) Vaginal opening.

arrangement is associated with incontinence—a constant feature in these abnormal animals. It must be emphasised that this urogenital deformity was present in 100 per cent. of female babies in affected litters.

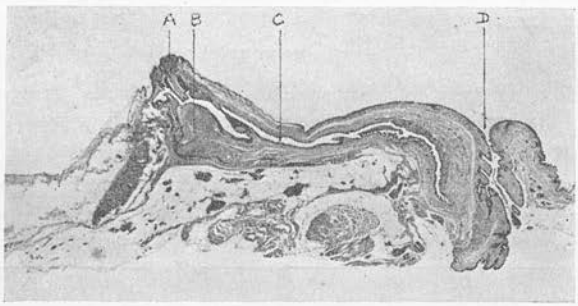

FIG. 1 C.
Microphotograph of mesial section of External Genitalia of normal female rat.
(A) Prominence on summit of which urethra opens. (B) Clitoris.
(C) Urethral canal. (D) Vaginal opening.

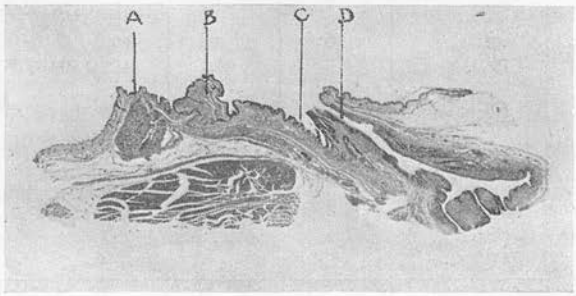

FIG. 2 C.
Microphotograph of mesial section of modified External Genitalia of female rat.
(A) Prominence. (B) Clitoris exposed.
(C) Deficient urethral canal. (D) Vaginal opening.

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Technique.—In all, 70 pregnant rats were used in this experiment and injected with œstrin. The date of conception of each rat was known, and injections were given in *one course lasting over not more than 12 hours*, and were made hourly. Pure crystalline ketohydroxyœstrin, obtained from the British Drug Houses Ltd., was employed, in aqueous solution. The total amounts injected varied from 0·5 to 1·0 mg.—0·5 to 1·0 mg. œstrin are equivalent roughly to 150 and 300 rat units respectively.

That the injection of large quantities of œstrin in late pregnancy in the rat is liable to cause death of the fœtuses is shown by the death-rate, which was high—only 21 live litters were born to 70 pregnant females injected; however, in these 21 live litters there were 79 females and *every one of these showed deformity*.

TABLE I.

Incidence of morphological modification of female fœtus after injection of œstrin during pregnancy (Rat).

(Gestation period 22-23 days.)

Total Amount of Œstrin.	Stage of Gestation at Injection.	No. of Rats Injected.	Live Litters.	No. of Females Alive or Surviving, all Showing Modification.
1·0 mg.	22nd day	4	...	...
	21st ,,	4*	4	22
	20th ,,	7	6	19
	19th ,,	2	1	5
	18th ,,	4	1	2
	17th ,,	11	2	6
	16th ,,	9	1	1
0·75 mg.	20th day	6	2	10
	19th ,,	3	1♂	... (♂ males only)
	18th ,,	1	...	...
	17th ,,	2	...	...
	16th ,,	4	...	...
	15th ,,	1	...	...
0·5 mg.	21st day	7	3	14 { 11 modified; 3 hardly affected
	16th ,,	2	...	...
	15th ,,	3	...	...
	Total . .	70	21	79

* A litter of 7 ♀♀ (all "modified") was born within 12 hours of the last injection.

Stage at which Deformity is caused.—The normal gestation period in the rat is 22 days. Large amounts of œstrin given before the 16th day invariably caused death of the fœtuses. This was probably due to the very immature state of fœtal development up to this time; actually only one live litter was obtained from 19 pregnant females injected on the 15th and 16th days. The fœtus enters a stage of very rapid growth at this point and consequently on the 16th day and thereafter, it was possible to get live litters. Observations, therefore, on live offspring were possible only in the case of rats treated from the 16th day onwards. Actually, injections given on any day from the 16th to term induced the

abnormality; and, what is more, œstrin given to a rat *within 22 hours of parturition* was effective.

Regarding the *dose* of œstrin used, it was found that any amount ranging between 0·5 mg. and 1·0 mg. was sufficient to affect the fœtuses, but less than 0·5 mg. caused no abnormality. Amounts in excess of 1·0 mg. are being tested to see if additional abnormalities occur.

The date of parturition was unaffected by œstrin, and lactation was quite normal.

The fact that injections, started even within the 22 hours preceding littering, produced modified females, suggested that the action of the hormone might not cease at parturition but be carried on and be transmitted to the sucklings through the mother's milk. In order to investigate this point, females from abnormal litters were, at birth, transferred to uninjected mothers who had just littered, and *vice versa*. This exchange of mothers, so to speak, did not prevent the modification already described from taking place. Thus it is clear that the modifying influence of œstrin injected during pregnancy takes effect while the fœtus is still *in utero*.

I am indebted to Dr Edwin Robertson for suggesting the possibility that a similar effect could be induced by injecting the *lactating mother* with œstrin. The investigation has met with extraordinary success. It has been found that 2·0 mg. of œstrin, when spread over the four consecutive days following parturition, produces an effect on the sucklings in every way similar to that induced by œstrin given before parturition. In this case the œstrin was injected hourly during a period of 10 hours on each of the four days. When, however, the same amount was injected on one day only—the day of littering, instead of for four days—no modification was induced. This, of course, is probably due to the fact that lactation is not established at once, and in these cases the fœtuses did not receive an effective dose of œstrin.

That these effects were due to the excretion of œstrin in the mother's milk is shown by the fact that when the milk of treated mothers was injected into test rats, mild œstrous changes occurred in these rats. The amounts of œstrin present were, however, very small, there being less than 1 rat unit in 0·2 c.c. of milk.

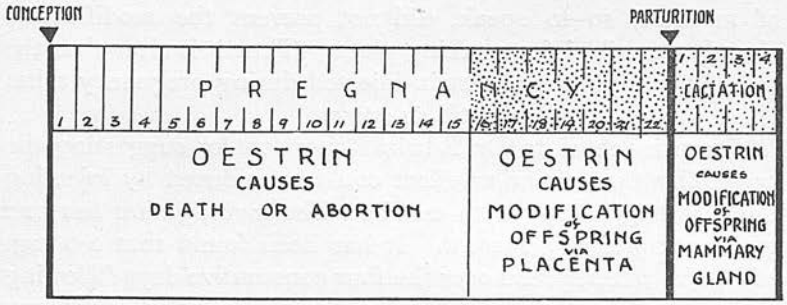
Further experiments were designed to ascertain if other hormones besides œstrin could produce a similar effect during pregnancy.

Progestin.—Two rats each injected with 6 rabbit units of progestin on the 20th day of gestation had normal litters.

Anterior Pituitary Hormone.—Pregnyl, antuitrin "S," and prolan (B.D.H.) were injected in large amounts at the same stages of pregnancy and over the same number of hours as œstrin had been administered. Of 24 rats so injected only 12 had live litters, the members of which have no macroscopic abnormality. The effects of implants and crude alkaline extracts of anterior pituitary tissue and preparations of growth hormone were also examined. When given during the first half of gestation, absorption of the embryos was encountered, and when administered later, the only peculiarity noted was a

TABLE II.

Modification of Offspring by Œstrin injected into the Pregnant and Lactating Mother.



prolongation of pregnancy for 4-10 days, and, unfortunately, dead litters were born in each case. Similar results were also obtained with a concentrated extract of human pregnancy urine. In these experiments only 21 live litters were obtained from 102 injected animals, but in no instance was any abnormality observed in the young surviving.

In view of the effect which disturbances in the pituitary-adrenal balance may have on the urogenital tract, a concentrated preparation of cortical tissue was investigated; injections of eucortone were made into 7 rats on the 17th day of pregnancy; and amounts equal to 60 and 120 grams of cortical tissue were used; there was, however, no effect on the offspring.

Since the chemical formulæ of the male and female sex hormones are somewhat similar, there was a possibility that the breakdown products of both hormones might have an effect on the female fœtus. In order to examine this, and

also to ascertain if a genital abnormality could be produced in the *male* fœtus as well, two preparations of *male hormone* of known potency were tested: Androsterone and hombreol were administered to 6 pregnant rats under the same conditions as œstrin (*i.e.* at hourly intervals for 10 hours on a single day late in pregnancy). The total amounts ranged from 15 to 100 capon units. The rat which was injected with 100 capon units of hombreol has given birth to females that are fully "modified." Rats receiving smaller quantities of male hormone had normal offspring. It is understood from the proprietors of this preparation that there can be only inappreciable amounts of œstrin present in the material used, as judged by the usual tests on ovariectomised animals. Similar amounts of androsterone given prior to sex-differentiation affected neither morphological development nor the sex-ratio. [According to Buyse,[2] sex is not morphologically differentiated in the rat till the 14th day. Injections were made therefore on the 11th and 12th days.]

The *subsequent history* of females exhibiting the abnormality is being watched. In the animals treated in the prenatal stage, the vagina opens about the 40th day, which is the average of the stock; whereas in those treated through the lactating mother there is a precocious opening of the vagina on the 20th day.

By means of vaginal smears taken daily for a period of three weeks in 21 "modified" females, it was ascertained that their *sex cycles* showed no abnormality, in that they came into œstrus every 5th day, as is usual.

Reproductivity is normal, although fertility appears to be below the average. Of 29 females of reproductive age and housed with males, only 6 had litters that lived, although 13 became pregnant. These litters consisted of 47 youngsters, all of whom were normal.

Parturition, apparently, is difficult in the "modified" animals. It seems that there is difficulty in expressing the fœtuses through the vagina. This may be due to inflammatory changes at the introitus, which is continually soaked with urine.

Discussion.—The effect upon the female rat of the antenatal and postnatal administration of large quantities of œstrin to the mother is of special interest for several reasons.

It was established in 1927 by Aschheim and Zondek[3] and M. Smith,[4] working independently, that large quantities of

œstrin are excreted in human urine during pregnancy, so that at the end of the gestation period the urine may contain as much as 20,000 M.U. of œstrin per litre or even more ; 100,000 units per day have been reported.[5] At this time the blood contains from 800-1000 M.U. per litre (Zondek [6]) and the full-time placenta 5000 M.U. It is possible that these amounts do not represent the total amount of œstrin formed in the body, since experiments on the ovariectomised and menopausal human subject have shown that only a small fraction of the œstrin injected is recoverable from the urine [7, 8, 9, 10]. However, as little is known of the renal factors controlling the excretion of œstrin, conditions of excretion persisting in the non-pregnant subject may not necessarily be applicable to the gravid state, and thus the amount of œstrin recovered may be fairly representative of that produced. Provided that a parallel can be found between conditions in the pregnant rat and the gravid human subject, the existence normally of a barrier between œstrin and the fœtus is a logical conclusion, and where morphological abnormalities resembling those herein described are encountered, the possibility that they may be due to a breakdown in this hormone barrier must not be overlooked.

Again, the experimental findings described are of interest as providing the *first recorded instance of a demonstrable effect* of œstrin upon the fœtus.

They show, further, that morphological development is plastic even up to the 4th day of life and can be modified through the channel of the food ingested.

In conclusion, three further observations arising out of these experiments may be mentioned. I have already referred to the original object for which these experiments were designed, namely, an investigation into the causes of the onset of labour. It is therefore of importance to note that, in rats, large doses of œstrin followed by oxytocin *did not* produce abortion even when the amounts of œstrin were sufficient to pass to the fœtus and to cause the modification which has been described. It was found that oxytocin did not accelerate parturition in the œstrin-sensitised animals, nor did it affect the modification of the babies subsequently born at term. That an effect produced by the female sex hormone can be produced equally by the male hormone would seem to throw a little more light on the puzzling enigma of the existence of large quantities of both hormones in the same individual—in man, in woman,

and in the stallion [11]—a side-issue which does not, however, come within the scope of this paper.

Lastly, since the urogenital region of the *female* fœtus can be modified, it is of interest to note that the *male* fœtus does not exhibit any abnormality, and this in spite of the fact that development of the urogenital region was probably incomplete at the time œstrin was injected.[12] The males within affected litters are apparently quite normal in structure, and also in their degree of reproductivity; a histological examination of their gonads and accessories will form a separate study.

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INCREASE IN WEIGHT OF THE MOTHER AND OF THE FŒTUS DURING PREGNANCY (RAT). By Annie M. Hain. From the Macaulay Laboratory, Institute of Animal Genetics, Edinburgh University.

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By Annie M. Hain.
From the Macaulay Laboratory, Institute of Animal Genetics, Edinburgh University.

(*Received for publication* 22*nd March* 1932.)

Introduction.

Teel (1926) observed that the growth of the fœtus of the rat was unaffected by alkaline extracts of the anterior pituitary when injection ceased before the twelfth day of pregnancy; at this stage the fœtus of the uninjected animal enters upon a period of rapid growth which becomes marked from the fifteenth day. In the rat pregnancy lasts approximately for 22 days, but this period is prolonged in animals injected with gonadotropic hormones (Teel, 1926; Hain, 1932).

It is well known that, whereas in the rabbit intra-uterine growth is most rapid between the ninth and fifteenth days (Minot, 1907), in the majority of animals the greatest growth takes place in the last third of pregnancy. In the absence of statistics of the actual increase in the weight of the fœtus and of the mother during pregnancy, the following figures may prove of interest. In the course of a recent experiment a study was made of :

(*a*) The growth of the fœtus and placenta from the first to the fifteenth day of pregnancy.

(*b*) The growth of the fœtus and placenta from the fifteenth day to term.

(*c*) The increase in the weight of the mother during pregnancy—

- (i) Normally.
- (ii) Under injection of an alkaline extract of anterior pituitary.
- (iii) Under injection of an extract of human pregnancy urine.
- (iv) Under implantation of anterior pituitary tissue.

(*d*) The increase in weight of virgin rats under the same conditions. These served as controls.

Experimental Results.

In all, 125 rats were employed. The figures for the weight of the fœtus and placenta at term are based on general observation of the laboratory stock of Wistar rats (155 fœtuses were weighed).

(*a*) Twenty-seven pregnant albino rats, weighing 160 to 200 grams when mated, were killed on the fifteenth day of pregnancy and the fœtuses and placentæ were weighed to the nearest mgm. The average weights of 134 placentæ and fœtuses were:

	mgm.
Placenta at fifteenth day . .	187 ± 6·5.
Fœtus at fifteenth day . .	277 ± 12·5.

(*b*) These weights were compared with the averages of the placenta and fœtus at term:

	mgm.
Placenta at term . . .	417 ± 12·0.
Fœtus at term	5260 ± 0·0.

The average weight of the fœtus is a little lower than the average given by Long and Evans (1922)—5·8 grams. On the basis given above, the growth between the fifteenth day and term is:

Placenta (approx.) 55·15 per cent. of its total growth.
Fœtus ,, 92·3 ,, ,, ,,

(*c*) *The Increase in Weight of the Mother during Pregnancy.*—Sixty-eight rats were weighed daily throughout pregnancy. Of the total, only 16 comprised normal pregnancies; the remaining 52 were treated with alkaline extract of anterior pituitary (as prepared by Teel, 1926), or an extract of human pregnancy urine (Wiesner and Marshall, 1931), or implantation of anterior lobe tissue. The increase in weight at various stages of pregnancy, for each of these groups, is set forth in Table I.

All the animals were nulliparous and, with fourteen exceptions, weighed between 150 and 200 grams on the day of mating. Of the 14 which weighed between 140 and 150 grams on the first day of pregnancy, only *one* animal was injected with alkaline extract. The increase in weight in this rat during the first seven days (which covered the period of injection) was 22 grams, as against 20·8 grams, which is the average for the group; the "gross" increase during pregnancy was 75 grams as against 74·4 grams; and the "permanent" increase was 36 grams as compared with 43·4 grams for the group. The slight difference in the initial weight had, therefore, no appreciable effect on the result obtained.

The remaining 13 rats, of which the initial weights came

between 140 and 150 grams, are included in the total of 41 normal pregnant rats, as none was injected during the first seven days of pregnancy (Table I., column 1). The average increase in weight of the thirteen during the period was 12·3 grams as against 10·5 grams, the average for the 41 animals: the difference is hardly significant. It was thought advisable to calculate their weights separately, as EVANS and SIMPSON

TABLE I.—AVERAGE INCREASE IN WEIGHT AT DIFFERENT STAGES OF PREGNANCY (ALBINO RATS).

	Days 1–7 (grams).	Days 7–15 (grams).	Day 15-max. (grams).	Gross increase (grams).	Permanent increase during pregnancy (retained after parturition) (grams).
Normal 16 rats	10·5 ± 0·9 [1]	22·0 ± 1·8	40·0 ± 2·0	72·5	24·0 ± 1·5
Injected with alkaline extract of pituitary . 28 rats	20·8 ± 1·2	19·0 ± 1·3	34·6 ± 2·0	74·4	43·4 ± 2·8 [2]
Injected with extract of human pregnancy urine . 20 rats	10·5 ± 0·9 [1]	21·4 ± 1·4	32·8 ± 1·6	64·7	22·4 ± 2·6
Implantation of anterior lobe tissue . 4 rats	10	23	40	73	20

[1] This figure is based on 41 normal pregnant rats.
[2] This figure is based on 15 rats.

(1931), in comparing the weights of their animals under injection of growth hormone, used only "plateau" animals, *i.e.* rats which had reached a stage at which the increase in weight was constant—approximately 10 grams in 20 days. Such rats weighed 150 grams and upwards. It is impossible to state if the "plateau" stage is reached at the same point in the Wistar stock employed by us, as complete statistics are not available. It will be seen in Table II. that the average increase in 14 days for 9 normal virgin rats weighing between 150 and 200 grams was 4·5 grams. However, apart from the small number of animals used, there was considerable variation within the 9 rats. In the absence of figures for our own stock, the average given by EVANS and SIMPSON (1931) has been assumed to be applicable, viz. that the animals are "plateau" animals at 150 grams and that the increase thereafter is not more than 10 grams in 20 days.

Of the 20 rats described in Table I. as being injected

with an extract of human pregnancy urine, only 3 were so injected with urine of the first 7 days of pregnancy, and, as the increased weight in these did not differ from that in normal rats, the average for the latter has been given in this column. This figure, and that in the same column for normal pregnant rats, is based on 41 animals.

The average increase in weight occurring during pregnancy is that increase which is retained at parturition and may be called the "permanent" increase. It is arrived at by subtracting the weight on the first day of pregnancy from that taken a few hours after parturition. The total increase due to the pregnant condition + the permanent increase is shown in the column immediately preceding as the "gross" increase. The average weight of fœtuses + placentæ + membranes, etc. is obtained by subtracting these two columns.

A comparison of the average increase at the various stages set forth in Table I. shows that:

	Grams.
From the first to seventh day the pregnant rat gains (approx.)	10·5
From the seventh to fifteenth day ,, ,, ,, ,,	21
From the fifteenth to its maximum ,, ,, ,, ,,	38
During pregnancy the rat gains and retains approximately .	22
The gross increase in weight due to the pregnant condition is approximately	71
The average weight of fœtuses, placentæ, membranes, etc., is therefore 71 less 22 grams	49

Relation of Gross Increase to Body-weight of the Pregnant Rat.

The gross increase in weight due to the pregnant condition in the 68 animals, viz. roughly 70 grams, is equivalent to 41 per cent. of the body-weight of the rat, if the average initial weight be taken as 170 grams, which was the actual average weight of all the animals used. Of this amount 32 grams are added during the first 15 days of pregnancy, and 38 grams between the fifteenth day and the attainment of the maximum (which does not always coincide with term in injected animals). It follows that approximately 54 per cent. of the increase in weight which occurs during pregnancy takes place after the fifteenth day. It is interesting to note that SLONAKER'S figures (1931) are 68 and 66 grams; the average number per litter in his case and in ours was 5·5.

Increase in Weight during Pregnancy (*i.e.* weight after parturition compared with initial weight): average 22 grams.

Whether the duration of pregnancy be taken as 22 days or 24 days, the permanent increase in weight was considerably in excess of that which occurs in the normal virgin rat of the same age (10 grams in 20 days). SLONAKER (1928) found that the maximum weight reached by animals which were permitted a normal number of litters and to nurse these, was 40 grams (or 15 per cent.) in excess of the maximum reached by hysterectomised rats which were permitted to mate, and 46 grams or 18 per cent. in excess of that reached by virgin rats. This increase in weight cannot be attributed to the deposition of fat attendant on lactation, as animals which were bred intensively but not allowed to nurse their litters reached the same maximum. In a more recent experiment SLONAKER (1931) used exclusively females weighing over 200 grams on the day of mating. These showed an increase of 16, 18, 18, 21, 16 grams respectively for the five groups into which his stock was divided. It is possible that the non-specific metabolic stimulant in the gonad, which is at the height of its activity during pregnancy, together with the general increase in the size and weight of the whole reproductive system resulting from the pregnant condition, accounts in some measure for the increase in weight of the pregnant animal over the virgin during the same period. It will be shown that the excessive increase in the pregnant rats which were injected with alkaline extract (43·4 grams) is comparable with that of virgin rats under the same treatment (Table III.).

Effect of Alkaline Extract of Pituitary on Weight of Pregnant Rats.

In contrast with the pregnancy urine extract, the alkaline extract of pituitary has a marked effect on weight in the early stages (days 1 to 7); animals so injected show an excess weight of 100 per cent. over the average for normal pregnant rats. As injection of the alkaline extract during the first 7 days of pregnancy delays the implantation of the ovum (TEEL, 1926), this increase could not be due to embryonic development. TEEL injected larger quantities than were employed in these experiments (1·0 c.c. daily throughout pregnancy, as against a total of 3·2 c.c. to 6·0 c.c. during the first 6 days),[1] and he obtained a growth of 40 grams in the first 10 days. It is interesting to note that the excess of growth over the average at this stage of pregnancy is apparently retained, as the "permanent" increase of such rats after parturition is almost 100 per cent. greater than that of the controls. It is possible that this effect is due to deposition of fat; body-length was not measured before and after pregnancy. The effect is not likely to be due

[1] 1·0 c.c. of extract was equivalent to 1 gram of anterior lobe tissue.

to mammary development, as the greatest growth occurred at a stage when mammary development does not normally take place.

(*d*) *Virgin Rats.*—Thirty virgin rats were employed as controls; with a single exception, these weighed over 150 grams at the commencement of the experiment; 4 weighed between 200 and 220 grams. The rat, of which the weight was below 150 grams, showed an increase in weight during 7 and 14 days of 17 and 19 grams respectively, and did not, therefore, exceed the average for its group.

Tables II. and III. set forth the average increase in weight of the normal and injected rats; the period of injection is covered in the figures assigned to the treated animals.

TABLE II.—NORMAL VIRGINS—INCREASE IN WEIGHT IN 14 DAYS.

Rat No.	First day (grams).	Fourteenth day (grams).	Increase (grams).
J1	165	168	3
J2	197	201	4
J7	206	213	7
M7	165	164	−1
R3	209	220	11
Q1	202	203	1
P2	151	159	8
B9	172	176	4
1804/XI.	170	174	4
A2	158	160 (7th day)	?
		Total 9 . .	41
		Mean . .	4·5 grams.

TABLE III.—AVERAGE INCREASE IN WEIGHT OF VIRGIN RATS (INJECTED AND NORMAL).

		During 7 days (grams).	During 14 days (grams).
Normal	9 rats	. .	4·5 ±1·3
Injected with alkaline extract of anterior pituitary	11 ,,	17·5	19 ±1·91
Injected with pregnancy urine extract .	11 ,,	1	1 ±1·80

As has been indicated, owing to the small number of normal virgin rats used as controls, the figures of both pregnant and non-pregnant

rats have been compared with the average of EVANS and SIMPSON (1931) based on several hundred virgin rats, viz. an average increase of 10 grams in 20 days. If the normal increase in the unmated rat, weighing 170 grams, is roughly 3 grams in 7 days, and if the increase in the weight of the pregnant animal for the same period is approximately 10·5 grams,[1] a comparison of the weight of a rat on the date of mating with that of the same animal on the seventh day *should* provide a fair indication as to the existence of pregnancy. Unfortunately it cannot be regarded as an infallible sign, as there are considerable individual variations. Of the 41 normal pregnant rats, 12 gained less than 10·5 grams in the first 7 days of pregnancy, *i.e.* approximately 30 per cent. SLONAKER (1929) reports a similar increase in pseudopregnant rats.

The alkaline extract causes an increase in the weight of injected rats proportionate to the amount of extract administered, *e.g.* those receiving a total of only 2·0 c.c. showed an increase of 13 grams as against 19 grams for rats injected with 6·0 to 8·0 c.c. It will be seen that, after the very rapid increase which occurs during the period of injection, the weight of injected animals remains practically stationary. When allowance is made for the normal excess over virgin rats, the increase in the weight of pregnant rats receiving this extract is comparable with that of virgin rats over a similar period given the same treatment.

The figure for rats injected with the extract of human pregnancy urine suggests the possibility of toxic substances being present. This is not manifest in the figures given for the pregnant rats so injected, as all animals in which absorption occurred (*i.e.* those injected in early pregnancy) have been excluded from the count.

As the effect of the implantation of a whole ox-pituitary was observed in only one animal for the entire 14 days the figure is not given, being of no statistical value.

SUMMARY.

1. Approximately 92 per cent. of the growth of the fœtus of the rat takes place between the fifteenth day and term, *i.e.* during the last third of pregnancy.

2. Approximately 54 per cent. of the increase in the weight of the mother during pregnancy occurs after the fifteenth day.

3. The gross increase in weight due to the pregnant condition is 71 grams, which is roughly equivalent to 41 per cent. of the body-weight of the rat.

4. The "permanent" increase in weight during a single pregnancy (no lactation) is considerably in excess of the increase which takes place over the same period in virgin rats.

[1] It would appear from the graphs given by SLONAKER (1931) that his average increase during this period was 14, 12, 15, 12, and 10 grams respectively for his five groups.

5. Rats injected with alkaline extract of anterior pituitary gain an excess of 100 per cent. in weight over controls during the first seven days of pregnancy; this increase is retained, and is comparable with that of virgin rats under the same treatment.

The author desires to acknowledge the assistance and advice of Drs WIESNER and ROBSON in the work recorded in this and the preceding paper.

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"The Assay of Oestrone in the Rat and the Mouse."

by A.M. Hain and J.M. Robson.

N.B. The Assay on the Rat was done entirely by A.M. Hain; that on the Mouse by J.M. Robson

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COMPARATIVE ASSAY OF OESTRONE IN THE RAT AND THE MOUSE

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Recent investigations into the effects of oestrin in rats and mice in pregnancy have revealed marked discrepancies between the reactions of these two species to the hormone. This suggested the need for a careful comparative assay of oestrone and its benzoate in the rat and mouse and led to the present investigation.

The experiments of a number of authors have yielded results which differ widely according to the method used (1–6).

METHOD

In order to reduce to a minimum the various factors which might affect the standardisation, the following precautions were taken:

a. The same animals were used throughout the experiment (the rats were Wistar albinos of a highly inbred stock). All animals were ovariectomised when 4 to 5 weeks old, and first injected at about 3 months old. The average weight of the rats at the first injection was 180 grams and at the conclusion of the whole investigation they averaged about 220 grams.

b. At least 2 to 3 weeks elapsed before any group of animals underwent a further course of injection.

c. Injections were made in one of the following ways:

(i) A single injection given in the morning;

(ii) Twelve injections at hourly intervals;

(iii) Four injections administered in the morning and evening of two consecutive days.

[1] Carnegie Research Fellow.

[2] Beit Memorial Fellow.

d. A sample of the international standard of oestrone was used; for the benzoate, crystalline material specially prepared for assay by the British Drug Houses Ltd. and having a M.P. of 219°C. was employed. The material was dissolved in absolute alcohol, the necessary amount added to oil of Sesame and the alcohol blown off to make the oil solutions. The aqueous solutions were made by diluting with distilled water and thus contained up to 10 per cent of alcohol. In five instances indicated in table 1 material in absolute alcohol was injected.

e. In order to eliminate as far as possible individual errors, all vaginal smears (stained with Giemsa or methylene blue) were jointly examined under the microscope by the investigators.

f. The amount giving a positive result in 50 per cent of a group of 20 or 40 animals was taken as the unit. A smear was considered positive when it contained only fully cornified cells, and negative when more than an occasional cell not fully cornified was present.

g. Smears were taken at least three times daily; owing to the known short duration of oestrus in the rat four smears were made during the 12 hours, i.e., at 4-hourly intervals. None were taken between 10.30 p.m. and 8.30 a.m. Smearing was continued until the presence of leucocytes and nucleated cells clearly showed that the maximum result was passed.

RESULTS

The data are collected in tables 1 and 2 and illustrated in figures 1 and 2. They demonstrate that the rat unit of oestrone is approximately 0.0033 mgm. when it is given in 4 doses in oil, and 0.0025 mgm. when similarly administered in 10 per cent alcohol. On this basis the rat unit in oil is equivalent to about 33 international units, and in an aqueous medium to about 25 international units. The equivalent figures for the mouse receiving oestrone in 4 injections over 2 days are 0.00009 mgm. (i.e., 0.9 international unit) when the material is given in oil, and 0.00025 mgm. when administered in an aqueous medium.

A considerable reduction in potency as compared with the above data was observed both in the rat and in the mouse when

oestrone was administered either in a single injection or in a course of hourly injections over a period of 12 hours. Thus in the rat 0.01 mgm. given in a single dose in oil (i.e., more than 3 times the unit based on 4 injections) failed to induce full cornification in any of the animals in a group of 20, and in the mouse a similar result was obtained when 0.0005 mgm. (i.e., more than 5 times

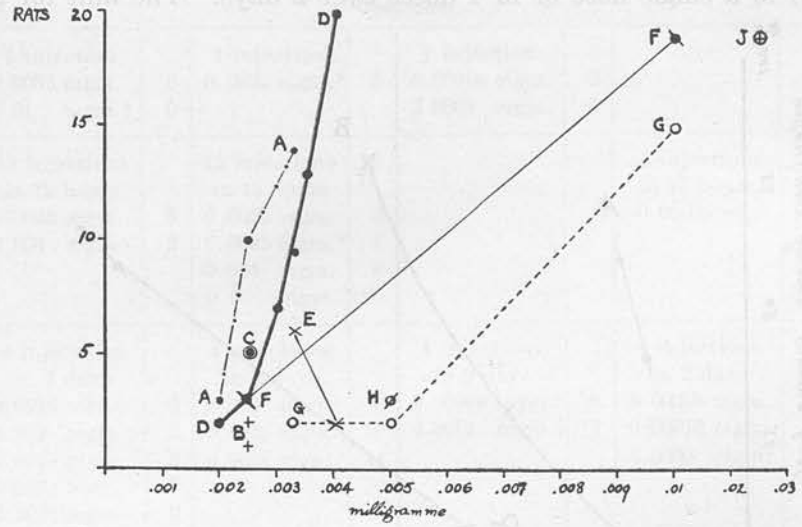

FIG. 1. SHOWING THE REACTIONS OF GROUPS OF 20 OR 40 RATS TO OESTRONE AND ITS BENZOATE

Oestrone in aqueous alcohol: Unit = 0.0025 mgm. —·— A = 4 injections in 0.4 cc. + B = 12 injections in 12 hours: total 1.2 cc. ◉ C = 1 injection in 0.1 cc.

Oestrone in oil: Unit = 0.0033 mgm. •——• D = 4 injections in 0.4 cc. x——x E = 12 injections in 12 hours: total 1.2 cc.

Oestrone benzoate in alcohol: ● F = 1 injection in 0.4 cc. ⊕ J = 12 injections in 12 hours: total 1.2 cc.

Oestrone benzoate in oil: ○——○ G = 1 injection in 0.4 cc. ϕ H = 4 injections in 0.4 cc.

the unit) was given. The results when an aqueous solution was employed were of the same type (see table 1).

The results obtained when oestrone was administered in repeated injections over a period of 12 hours (either in aqueous or oil solution) show very marked variations, thus, e.g., 0.0025 mgm. given over 12 hours in aqueous solution produced cornification in 2 out of 20 rats, whereas 0.004 mgm. administered in

the same way failed to cause cornification in any animal. It is noteworthy that when oestrone was given over a period of 12 hours in absolute alcohol instead of in 10 per cent alcohol, no increase in potency resulted.

The preliminary experiments with the benzoate showed that the result was very similar whether this was administered (in oil) in a single dose or in 4 doses over 2 days. The unit for a

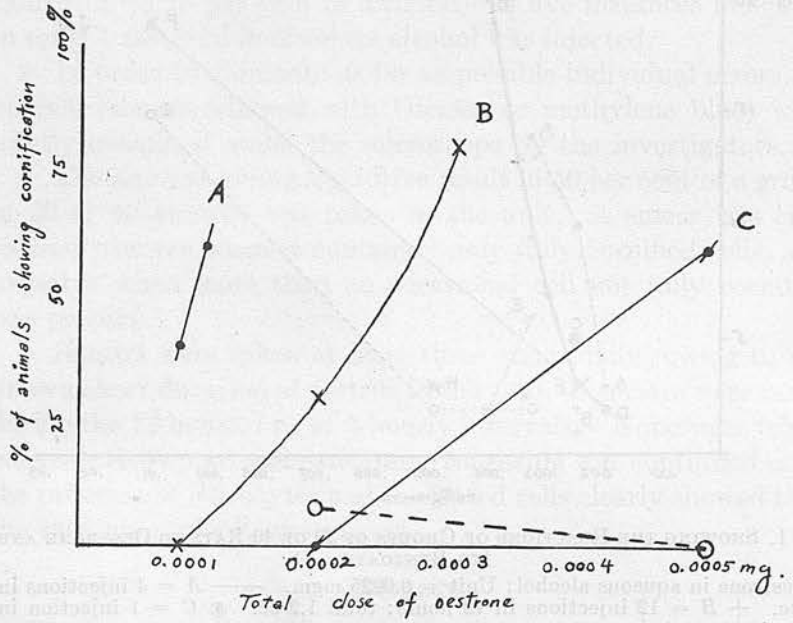

FIG. 2. SHOWING THE REACTIONS OF GROUPS OF 20 OR 40 MICE TO OESTRONE AND ITS BENZOATE

○, oestrone in oil: 1 injection in 0.1 cc.
●, oestrone in oil: 4 injections in 0.4 cc. = *A*. Unit = 0.00009 mgm.
×, oestrone in aqueous alcohol: 4 injections in 0.4 cc. = *B*. Unit = 0.00025 mgm.
⊗, oestrone benzoate in oil: 1 injection in 0.1 cc. = *C*. Unit = 0.00045 mgm.

single injection of benzoate was then approximately determined. In the rat it lies between 0.005 and 0.01 mgm. and in the mouse it is slightly under 0.0005 mgm.; thus in both species a single injection of oestrone benzoate in oil is more potent than a single injection of oestrone in oil; on the other hand, the administration of the benzoate in one injection produces less effect than the same weight of oestrone given in 4 injections.

TABLE 1

Comparative assay of oestrone in rats and mice

RATS				MICE			
In oil		In aqueous alcohol		In oil		In aqueous alcohol	
	Positive in 20 rats		Positive in 20 rats		Positive in 20 mice		Positive in 20 mice
Oestrone							
1 injection		1 injection		1 injection			
0.0033 mgm.	0	0.0025 mgm.*	5	0.00018 mgm.	2		
0.01 mgm.†	0			0.0005 mgm.	0		
12 injections in 12 hours		12 injections in 12 hours				12 injections in 12 hours	
0.0033 mgm.	6	0.0025 mgm.	2			0.00008 mgm.	1
0.004 mgm.	2	0.0025 mgm.*	1				
		0.004 mgm.	0				
		0.1 mgm.	20				
4 injections in 2 days		4 injections in 2 days		4 injections in 2 days		4 injections in 2 days	
0.0018 mgm.	0	0.002 mgm.	3	0.00008 mgm.	8	0.00008 mgm.	0
0.002 mgm.	2	0.0025 mgm.†	10	0.0001 mgm.†	12	0.00018 mgm.	6
0.0025 mgm.	3	0.0033 mgm.	14			0.0003 mgm.	17
0.003 mgm.	7						
0.0033 mgm.	9						
0.0035 mgm.	13						
0.004 mgm.	20						
Oestrone benzoate							
1 injection		1 injection		1 injection			
0.0033 mgm.	2	0.0025 mgm.*	3	0.00018 mgm.	0		
0.005 mgm.	2	0.01 mgm.*	19	0.0005 mgm.	12		
0.01 mgm.	15						
4 injections in 2 days							
0.005 mgm.	3						
12 injections in 12 hours		12 injections in 12 hours					
		0.025 mgm.*	19				

* In absolute alcohol.

† Group of 40.

DURATION OF REACTIONS

At the level of both units of oestrone (i.e., in 10 per cent alcohol and in oil given in 4 injections) full cornification was reached in the rat at 72 hours after the first injection. When amounts in excess of the respective units were used, the maximum reaction occurred earlier, e.g., 56 to 60 hours; in several instances the maximum was definitely passed at 80 hours.

In the mouse the maximum was reached slightly later (76 to 80 hours) and was maintained for a rather longer period, so that at 96 hours several animals still showed the maximum effect.

TABLE 2

Oestrone assay—rat and mouse units

	RAT		MOUSE	
	Unit in milligram	Equivalent in international units	Unit in milligram	Equivalent in international units
Oestrone: unit in oil (4 injections in 36 hours)	0.0033	33	0.00009	0.9
Oestrone: unit in 10 per cent alcohol (4 injections in 36 hours)	0.0025	25	0.00025	2.5
Oestrone benzoate in oil (single injection)	Between 0.005 and 0.01		0.00045	

Although the oestrone unit in oil in the rat (0.0033 mgm.) was less effective when given in 12 hourly injections than in 4 injections spread over 36 hours, those animals that responded in the group injected hourly did so at an earlier stage, e.g., full cornification occurred 36 to 52 hours after the first injection. A similar occurrence was observed when the aqueous unit (0.0025 mgm.) was administered in 12 hourly injections. When 40 times the aqueous unit (0.1 mgm.) was similarly injected, every rat responded: 1 with full cornification at 32 hours after the first injection, and 19 at 48 hours; of these, 15 remained fully cornified for 24 hours, 4 for 32 hours, and 1 for 44 hours. When the maximum was attained, a potent male was placed in each cage containing 5 rats but no mating occurred.

In both species full cornification was sometimes observed at a single smearing period, and 4 hours later the maximum was already passed.

The reaction to the benzoate was generally more protracted, and frequently the maximum was attained very late, e.g., when 0.01 mgm. was given in a single injection in alcohol full cornification occurred in one rat at 120 hours after injection; in this group the maximum was maintained for periods varying between 33 and 60 hours. When an amount of the benzoate equal to the oestrone unit in oil was given, the two responding rats attained their maxima at 84 and 97 hours after injection. When 0.01 mgm. of the benzoate was similarly administered, the maxima were reached at the following points: 75 hours (7 rats), 80 to 84 hours (6 rats), 105 hours (2 rats) and were maintained for periods varying between 21 and 48 hours.

In the mouse, the reaction to the benzoate in oil was observed at a maximum at about the same time as in the case of oestrone, i.e. 76 to 80 hours after injection, but this maximum was maintained for a much longer period, e.g., in the group injected with 0.0005 mgm., 11 animals out of 12 still showed full cornification at 100 hours after injection, 5 at 144 hours, and 2 at 172 hours; at 240 hours one mouse was still almost fully cornified.

DISCUSSION

The essential data on the units of oestrone and its benzoate in the rat and mouse are collected in table 2.

These assays demonstrate that, under the experimental conditions described, the relationship between the rat and the mouse units of oestrone is much higher than that adopted by the London Conference of 1932. Moreover, the comparative potency of oestrin in the rat and the mouse varies both with the particular compound used and also with the method of administration (viz., the solvent used and the number of injections given). Thus, when the units of oestrone are based on 4 injections in oil spread over 36 hours, the rat unit (0.0033 mgm.) is some 36 times greater than the mouse unit (0.00009 mgm.). When, however, the injections are given at similar intervals in an aqueous-alcohol medium, the rat unit is equal only to 10 M.U., the potency being

increased in the rat by using an aqueous instead of an oily solvent, but decreased in the mouse.

When oestrone was administered either in a single dose or in 12 injections in oil at hourly intervals, its potency was very considerably reduced in both species, and the results obtained showed a great degree of variability. The data obtained for a single injection in oil in the rat are very different from those reported by Bülbring and Burn (6). We have had the opportunity of discussing this question with Professor Burn and arrived at the conclusion that the discrepancy between our results and those of Bülbring and Burn is due to differences in the end-point adopted in reading the vaginal smears (Professor Burn and one of us (J. M. R.) have actually read smears together in order to make sure of this difference). Bülbring and Burn consider a smear to be positive when it contains a considerable proportion (about 30 per cent or more) of cornified cells; when repeated smears from one animal showed a great preponderance of epithelial cells the result was also taken as positive. The authors, on the other hand, considered smears to be positive only when they were composed exclusively or almost exclusively of cornified cells.

In the case of the benzoate, the conditions appear to be rather different, since a test in the rat showed that the potency was of a similar order when the hormone was given in a single injection or in 4 injections over 36 hours (see table 1). In both the rat and the mouse the benzoate is more potent than oestrone when both are administered in a single dose in oil. When oestrone is given in 4 doses, however, its potency is considerably greater than that of the benzoate given in one injection. It is to be noted that the relation between the rat and the mouse units of the benzoate administered in a single injection is about 18, thus giving a different figure from those obtained for oestrone. That the benzoate exerts its action for a more prolonged period than oestrone was also demonstrated by the results of Butenandt (7) though the duration of full oestrus which we observed in mice was appreciably shorter than that reported by Butenandt.

The conclusions as to the importance of the method of administration are further emphasised by an examination of the litera-

ture, which shows very great discrepancies on the question both of the biological unit (as compared with the international unit) and of the relation between the rat and mouse units for a given method of standardisation. Thus various authors have concluded that the rat unit equals anything between 10 and over 30 international units of oestrone (1–6). The results of Hinglais and Hinglais (3) are, indeed, in close agreement with ours, as they find that the rat unit averages 32.2 international units but the agreement is only apparent as their unit is based on cornification in 12 out of a group of 15 animals. Furthermore, the data obtained for the biological mouse unit vary with different authors, and the figure has actually been put as high as 0.0005 mgm. for oestrone given in 3 injections in oil (5).

The question arises as to what light the above results throw on the known action of oestrin during pregnancy in these two species. In the mouse, the administration of the hormone causes interruption of pregnancy, and when the injections of oestrone are followed by oxytocin live foetuses may be born within a comparatively short time (8–10). In the rat, pregnancy is less easily interrupted and the abortion of live foetuses following treatment with oestrin and oxytocin has not been observed (11). Now in the mouse, Marrian and Newton (9) have shown that 0.03 mgm. of oestrone spread over 12 hours is sufficient to sensitize the uterus to the action of oxytocin. If this amount is multiplied by 36 (the greatest divergence between the rat and the mouse units obtained in the present experiments) about 1.0 mgm. is obtained, and this amount was found by Hain (11) to be insufficient to sensitize the uterus to oxytocin in the rat. These results support the view that abortion is not dependent on morphological changes related to the known oestrus-producing action of oestrin.

SUMMARY

1. The units of oestrone have been ascertained in the rat and in the mouse in two media: oil and dilute alcohol.

2. The unit differs according to the method of administration. A comparison is made between the effect of oestrone and its benzoate.

3. The duration of the oestrous response has been examined and the relation between the rat and mouse units is briefly discussed.

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SPONTANEOUS "DECIDUOMATOUS TUMOURS" IN THE PSEUDOPREGNANT RAT. By Annie M. Hain. From the Macaulay Laboratory, Institute of Animal Genetics, Edinburgh University.

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SPONTANEOUS "DECIDUOMATOUS TUMOURS" IN THE PSEUDOPREGNANT RAT.

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Introduction.

It was shown by Loeb in 1909 that mechanical stimulation of the endometrium causes deciduomatous formation in the absence of pregnancy; this effect was obtained only when functional corpora lutea were present in the ovary. In 1928 Evans reported that similar structures can form spontaneously in the pseudopregnant rat, but are apparently of rare occurrence; he observed a frequency in normal pseudopregnant rats of only 3 to 4 per cent., but this was increased to 60 per cent. by a dietary regimen lacking in vitamin E. Innes and Bellerby (1929) observed similar growths in rats in which pseudopregnancy had been lengthened by injection of an alkaline extract of pituitary. The outward manifestation of the presence of the tumours is hæmorrhage from the vagina, which occurs in the uninjected animal about the eleventh day, but was apparently deferred in those rats which Innes and Bellerby injected. A study has been made of two cases noted recently and of the effect of the injection of pituitary extract upon their incidence.

Experiments.

Twenty-two Wistar albino rats, three to five months old and weighing 150 to 200 grams, were employed on the experiment, and by attention to diet, all possibility of a deficiency of vitamins playing a part in the result obtained was excluded. The rats were mated to vasectomised males, and from the day of mating vaginal smears were taken daily till the ninth day; thereafter they were recorded twice daily until either hæmorrhage or cornification occurred. The effect of pituitary hormone on pseudopregnancy-hæmorrhage was also examined, the following groups of animals being used:—

7—Injected with extract of human pregnancy urine, prepared as described by Wiesner and Marshall (1931). Injections were made from the day of mating for eight to ten days.

5—Injected with alkaline pituitary extract (TEEL's preparation, 1926).
2—Each received a single implant of anterior lobe tissue equivalent to a whole gland.
3—Portions of one uterine horn were removed on the sixth day after the sterile mating.
5—Given no treatment.

Hæmorrhage was observed in two animals on the eleventh day: in one rat injected with the alkaline extract for four days prior to bleeding —Rat 1812 (0·3 c.c. daily), and in one which received no treatment—Rat 1816; thereupon both were killed. Macroscopically, the outward appearance of the uterus denoted an early pregnancy, but the swellings were irregular and resembled the condition seen where atrophic fœtuses are being resorbed. Blood could be seen in both uterine horns, especially above the swollen portions. The swellings in the injected rat (1812) were only 1 cm. below the ovary in both uterine horns; those in the untreated rat (1816) were much larger and situated near to the cervix.

HISTOLOGY.

The genital tracts of both animals were preserved in 5 per cent. formol saline. Paraffin sections were stained with hæmatoxylin and eosin.

The Ovaries were strongly luteinised; the corpora lutea were in process of degeneration. Ovulation was approaching in several follicles, as indicated both by the size of these and by the presence of polar bodies. In the ovary of the injected rat the theca showed rather more luteal development than in the untreated animal and the whole ovary was exceedingly hyperæmic and hæmorrhagic.

The Vagina of both animals was mucified but the mucous stratum was not so high as in pregnancy and portions of the epithelium were being shed. In addition to mucus, blood, which had escaped from the uterus, was found in the vaginal orifice.

The Uterus.—Immediately above the tumour, *i.e.* in the most distended portion of the uterus, the lumen, in cross-section, was distended by a large, loose mass of mucus, red blood-corpuscles, and polymorph leucocytes. Proximal to this portion (*i.e.* nearer the tube) was an area in which distention was less marked and along a part of its length the endometrium exhibited the structure typical of the twelfth day of pseudopregnancy. It was apparent here that the blood which was collecting in the lumen had escaped through the stroma and epithelial layers and was certainly not wholly derived from the tumour; mucus had exuded from the stroma glands.

The tumours from the two animals differed from each other in their shape and structure; apparently both were in the process of degenera-

tion, but degeneration had proceeded farther in the untreated animal (Rat 1816).

Opposite the apex of the tumour in Rat 1812, the epithelial layer was entirely absent along the greater part of the uterine surface, leaving an uneven and ragged edge. The endometrium was not thrown into crypts and folds as in normal pseudopregnancy, but three-fourths of the lumen were filled by a wedge-shaped papilla, which, at its base, was embedded in, and adherent to, the muscle layer of the uterus. This layer was intermingled with stroma and decidual cells, many of which had a cystic appearance. The character of the tumour was decidual as a whole. Decidual cells predominated nearest the lumen; below this were ordinary stroma cells, and still lower fibroblastic cells. At the outer margin were masses of leucocytes and, along a portion of the apex, indications of the formation of a membrane. At one point the apex was broken and blood and leucocytes were being released into the lumen of the uterus. Blood-cells were detected also throughout the mass, but at no other point were there indications of a cavity formation.

In the oval-shaped tumour found in the untreated rat (1816) degeneration had also set in. Leucocytes, lymphocytes, and ordinary stroma-cells preponderated over decidual cells. The tumour was of comparatively loose texture and the cell structure was embedded in a muco-fibrinous exudate. It was essentially hæmorrhagic; but, owing to disintegration, the cavity seen in Rat 1812 was less clearly defined. Almost surrounding the tumour and separating it from the uterine mucosa was a fibro-muscular wall. The remainder of the lumen was filled with the same material, but more hæmorrhagic than the structure already described. The uterine cavity, which was oval in section, gave the impression that the muscle layer had been hollowed out by the pressure of the apices of the two egg-shaped bodies resting upon it.

Sections cut transversely and longitudinally below the tumour in Rat 1812 showed that the uterine distention described in the vicinity of the tumour was merely local, and that elsewhere the mucosa was thrown into crypts and folds as in a normal pseudopregnancy of about the twelfth day (ALLEN, 1931). In the lumen were blood-corpuscles and leucocytes, but far fewer than above the tumour. A small amount of blood and mucus was found also in the horn in which no tumour was present.

DISCUSSION.

The points of difference which have been noted in the structures described in the two animals are probably due to difference in the age of the tumours and do not indicate any dissimilarity in their character. In their capacity to erode the muscle, their shape, the existence of a lacuna giving rise to a hæmorrhage, and of a surrounding fibro-muscular

wall, they bear a marked resemblance to the endometriomata described by BAILEY, 1924. The term "deciduomatous tumour," chosen by EVANS (1928) and adopted by INNES and BELLERBY (1929), rightly emphasises their decidual character.

The structures are characteristically hæmorrhagic. INNES and BELLERBY (1929) report that blood was found in the lumen of the uterus only below the level of the growths, but this was not our experience. In Rat 1812 sections were made immediately following on those of the ovary and up to the point where the tumour began, and, as has been stated, the portion of greatest distention was directly above the tumour and was filled with blood. As INNES and BELLERBY state, the bleeding and general hyperplasia of the uterus was due to the presence of the growth: such degeneration of the uterus as was observed was also attributable to this cause and has its counterpart in an ordinary pregnancy where the placenta involves destruction of tissue and leucocytic infiltration (ALLEN, 1931). At the end of the normal pseudopregnancy in the rat there is, apparently, no denudation of the surface epithelium (ALLEN). It would seem, therefore, that the hæmorrhage which is associated with the growths and represents their outward sign, is similar to the "placental sign" of pregnancy which is found in some rodents and in the macaque.

Certain circumstances suggest that the hæmorrhage which occurs in the presence of placentæ and deciduomatous tumours is not to be explained entirely by local irritation. It may be emphasised that blood was found in greatest abundance *above* the tumours; the excessively hæmorrhagic condition of the ovary in one animal has been pointed out. It is also interesting to note that blood was found in the horn in which no tumour existed. The uterus of the rat, though bi-cornuate, may be considered a unit, and what affects the blood-supply of one horn may affect that of the other; it is doubtful, however, if one can explain the bleeding in the empty horn by any simple mechanism regulating blood-supply. One must not lose sight of the possibility that the tumour, by some specific or non-specific mechanism, exerts a general and not merely a local effect.

> A somewhat similar occurrence was observed in a mouse from which one ovary had been removed. Subsequent to the operation, the mouse became pregnant; when placental sign occurred on the eleventh day, blood was found not only in the horn which contained fœtuses but also in the horn from which the ovary had been removed a month previously.

In the experiments described, the injection of extracts containing anterior pituitary hormone did not increase the frequency of occurrence of the tumours; it is possible that their growth was delayed or their degeneration postponed in the only injected animal in which tumours were found (Rat 1812), since in the rats injected with alkaline extract

of pituitary by INNES and BELLERBY (1929) the first sign of bleeding was only observed two to three weeks after the sterile mating, owing to a lengthening of the period of pseudopregnancy. These authors suggest that "the luteal tissue produced in the ovaries as a result of the injection of anterior lobe extract is abnormally active in producing a dysgenic hyperplasia of the uterus during pseudopregnancy." The formation of the tumours is, however, not clearly linked with or a necessary accompaniment of prolonged pseudopregnancy. This may be concluded in view of the experiments under discussion, for, where the urine extract was given, though pseudopregnancy lasted between 19 and 25 days, and periods of 16 to 19 days followed injection of the alkaline extract of pituitary, yet in none of these animals did tumours form. From the quotation given above, it would be anticipated that injection of the alkaline extract into the pregnant animal would tend to increase the amount of, or duration of, "placental sign," but this is not the case (TEEL, 1926; HAIN, 1932). TEEL found that prolonged injection in the early stages of pregnancy delayed its onset, as apparently also in the pseudopregnant animal.

SUMMARY.

1. A brief histological description is given of the ovaries, uteri, and "deciduomatous tumours" of two rats in which hæmorrhage was observed towards the end of pseudopregnancy.

2. Although preparations of gonadotropic hormone prolonged the period of pseudopregnancy, they did not increase the frequency of the occurrence of the "tumours."

3. The phenomena attendant upon "placental sign," and those associated with the hæmorrhage which accompanies the tumours, have many points of resemblance in common.

4. The possibility that the tumours exert a general and not merely a local effect, is touched upon.

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1934. Some facts regarding the growth of the Wistar rat under standard conditions in Britain. (Derivative Edinburgh stock)

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Body growth of Wistar strain at Edinburgh

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SOME FACTS REGARDING GROWTH OF THE WISTAR RAT UNDER STANDARD CONDITIONS IN BRITAIN (DERIVATIVE EDINBURGH STOCK) [1]

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The classical work of Donaldson ('24), in providing exhaustive data regarding the development of the Wistar albino rat has rendered invaluable service to all those using this animal for experimental purposes. The data given by Donaldson naturally relate to certain conditions of food, temperature and habitat, and might be modified by altered circumstances. This consideration, to some extent, prompted the present investigation in which are set forth certain particulars regarding the growth, litter size, sex ratio, etc., of a branch of the Wistar stock bred in Edinburgh. In spite of the smallness of the numbers involved, the material is sufficient to form a basis of comparison—a comparison eminently desirable as recently certain structural abnormalities have been observed which might be interpreted as due to physical depreciation of the stock.

Housing conditions and food

The rats were kept in a well-ventilated hut which was heated electrically during winter. Wooden boxes 30 × 30 × 20 cm. were employed; these were provided with a central partition separating the food compartment from the sleeping quarter, and were fitted with removable lids having a strong wire mesh 1 cm. square. Two rats or one pregnant rat occupied a box, and the distance between boxes was 20 to 25 cm.

[1] These observations were made in connection with experiments performed during the tenure of a Carnegie Research Scholarship.

The daily ration consisted of: flaked maize, dried liver and yeast, cod liver oil and salt. This mixture was made up with a modicum of hot water, and, during winter, whole wheat which had heated slowly in water overnight, was mixed with the maize, etc. Skimmed milk was given three times a week and water on the other days. Lettuce and cauliflower leaves were given two or three times weekly from May to November. On Sundays brown bread was given, and sheep-lights twice weekly during the cold weather. The supply of lettuce, cod liver oil and liver and yeast was on a liberal scale; the daily allowance for 450 rats (ranging from 3 weeks old to 9 months) was approximately 175 cc. cod liver oil, i.e., roughly 0.4 cc. per rat, and 500 gm. of dried liver and yeast or roughly 1.0 gm. per rat. The important part played by the essential vitamins contained in the three substances last named, in promoting growth and fertility and in stimulating lactation has been repeatedly instanced in the literature (Evans, '31; Nelson et al., '28; Dann, '32; Mapson, '32; Bahrs, '33; and Evans and Burr, '28).

The data which follow cover a period of 12 months from October 1932 to October 1933, but no attempt has been made to distinguish between growth and fertility at the different times of the year.

Litter size and sex ratio, etc.

Of 307 litters (2843 individuals) for which full particulars are available, the average size was 9.26 ± 0.182 (compare King, '16; average 6.7 for 3955 individuals). The following table shows the distribution of the largest litters:

TABLE 1

64 litters consisted of 10 or 11 young
31 litters consisted of 12 young
23 litters consisted of 13 young
17 litters consisted of 14 young
4 litters consisted of 15 young
3 litters consisted of 16 young
2 litters consisted of 17 young (all alive)
1 litter consisted of 18 young (1 dead)

It appears that the record of eighteen has been exceeded by a rat which had a litter of nineteen. In each of the three last-named litters on the list, seventeen rats reached maturity.

The sex ratio of 2744 rats born was 104.68. This figure includes stillborn animals, but comprises no litters in which young were eaten if the sex was indistinguishable. King ('24) records a sex ratio of 105.2 ± 2.0 for 4992 rats. This figure embraces the entire breeding period, as many as thirteen litters being recorded for some animals.

Records were available in 150 cases of the increase in weight of the mother during pregnancy (i.e., computed by subtracting the weight on the day of mating from that taken a few hours before littering). The average increase previously reported on the basis of sixty-eight rats (Hain, '32) was 71 gm., but the following table indicates the extent to which this figure may be exceeded.

TABLE 2

The gross increase in weight of rats during pregnancy

35	rats gained	80– 89 gm.
22	rats gained	90– 99 gm.
21	rats gained	100–109 gm.
19	rats gained	110–119 gm.
10	rats gained	120–129 gm.
5	rats gained	130–139 gm.
2	rats gained	140–149 gm.

Age and weight at maturity

If the establishment of the vaginal orifice be accepted as the criterion of maturity, the female rat in this branch of the Wistar stock matures, on an average, at 40 to 45 days of age, when weighing 75 to 80 gm. (318 rats). According to Long and Evans ('22), who made a study of 200 rats, the vagina opens at about 72 days (range 34 to 109 days). Both Donaldson and later experimenters have observed that, with increasing time, the Wistar rat tends to mature at an earlier date than formerly.

An analysis was made of those rats (seventy-nine) falling within the age/weight group in which maturity was anticipated but in which the vaginal orifice was not yet established.

TABLE 3

Vaginal orifice was not established in the following

WEIGHT OF RAT	AVERAGE AGE	NUMBER OF RATS
Grams	*Days*	
70– 74	48.5	16
75– 79	44	21
80– 84	48.5	19
85– 89	46	4
90– 94	45	12
95– 99	50	4
100–104	66	1
105–109	71	1
110–114	40	1

The last rat in table 3 is of interest in that, in spite of its abnormally rapid skeletal development, it was somatically immature.

Since it has been shown (Mirskaia and Crew, '30) that a truer standard of somatic maturity is provided by the ability to produce and rear young, the records have been examined for those rats that had normal pregnancies to matings which took place when the female was 8 weeks old or under.

TABLE 4

Age of female rats at fertile mating

14 were exactly 8 weeks old.
6 were 2, 3 or 4 days less than 8 weeks old.
1 was 7 weeks old.
7 were 2, 3 or 4 days older than 8 weeks old.
4 aged 8 weeks gave birth to dead litters.

In thirteen of the above the female mated with her brother, and in one instance the male was only 53 days old. From both aspects, therefore, it would seem that the stock under survey matures at an earlier age than the original from which it was derived. It is of interest to record that five instances of mating during pregnancy were observed; Long and Evans ('22) reported two such.

Weight in relation to age

The figures given in table 5 are based on over 2500 weighings. Rats were weighed (fasting) fortnightly from the date of weaning, and, in the case of females, on the date of mating and again after littering. To facilitate comparison, Donaldson's weights are placed alongside our own, the weight which he gives for the mode being taken in each group. In some of the older groups the paucity of animals rendered the calculation of standard error unnecessary.

TABLE 5

Body weight in relation to age

MALES			FEMALES		
Age in days	Body weight, grams	Donaldson's weights, grams	Age in days	Body weight, grams	Donaldson's weights, grams
17– 19	29.7 ± 0.805[1]	18	17	29.0 ± 1.056	17.3
20– 29	35.4 ± 0.773	23.9	20– 29	35.7 ± 0.700	25.4
30– 39	57.8 ± 1.594	35.4	30– 39	55.6 ± 1.044	37.0
40– 49	83.6 ± 2.105	50.6	40– 49	82.0 ± 1.477	51.1
50– 59	110.7 ± 3.86	69.5	50– 59	108.4 ± 2.105	67.9
60– 69	141.8 ± 0.099	92.0	60– 69	130.6 ± 2.608	87.3
70– 79	179.0 ± 5.136	118.3	70– 79	150.87 ± 2.105	109.3
80– 89	239.4 ± 2.234	142.0	80– 89	165.85 ± 2.746	128.1
90– 99	241.6 ± 6.966	158.4	90– 99	179.97 ± 2.726	140.6
100–109	264.0 ± 4.022	172.7	100–109	191.1 ± 4.577	151.4
110–129	290.8 ± 3.470	190.9	110–129	205.0 ± 2.470	165.2
130–149	300.0 ± 4.701	210.5	130–149	208.0 ± 2.820	179.9
150–169	298.2 ± 8.883	226.0	150–169	219.0 ± 4.097	191.6
170–189	336.5 ± 7.730	238.6	170–189	226.6 ± 3.925	201.0
190–199	376.36 ± 8.259	246.2	190–199	231.2 ± 5.00	206.4
200–215	326.5 ± 4.15	251.9	200–215	246.1 ± 7.790	210.6
216–229	381.3 ± 14.83	257.8	216–229	267.7 ± 13.202	215.4
230–249	364.4 ± 16.165	263.3	230–249	242.3 ± 4.870	219.2
250–269	389.0 ± 9.826	268.5	250–259	228	223.0
270–289	384	272.5	260–279	280.5	224.5
340–350	400	279.3	280–289	255	226
360–375	398	280.0	290–299	243	227
380–395	385		300–319	242	228.7
400–415	385		340–350	312	230.3

[1] Standard error.

In his table 157 A Donaldson quotes the weights recorded by Greenman and Duhring ('23) for Wistar rats receiving special care and feeding. These show an increase on the figures given by Donaldson himself but the American averages in both cases are far exceeded by those here reviewed. The difference is especially noticeable in the males: at 365 days old, Donaldson's male weighs 280 gm., and his female 230 gm. These figures correspond to a male aged 110 days and to a female of 170 days in table 5. Donaldson's standards indicate that the male rat grows from 60 gm. to 200 gm. in approximately 80 days. When special diet was used, Bryan and Gaiser ('32) reduced this period to 38 days, and accelerated growth still further by administering growth hormone to rats fed on this diet. According to table 5 the male rat grows from 60 to 200 gm. in, roughly, 40 days—a period approximating very closely that of Bryan and Gaiser.

An interesting fact which is common to both sets of figures is made manifest in table 5, namely, that there is little difference between the weight of a male and female during the first eight weeks of life. Actually Donaldson's female from 20 to 50 days old is heavier than his male of the same age. From the eighth week of life the divergence in weight is very marked.

In tables 6 and 7 the relation between the age and size of male and female is compared in the two stocks. As Donaldson relates size to body weight and not to age, the figures here given (columns 3 and 5) correspond to the age-weight table, thus, the body weight of a particular age being known, the size of an animal corresponding to that weight has been recorded.

By body length is meant the measurement from the nose to anus. This was taken immediately after killing, with the animal extended on its back.

For a given body length the female has a greater body weight than the male, e.g., 215 mm. in the male corresponds to 179 gm.; 215 mm. in the female corresponds to 209 gm. It is observed, also, that in the average female of this stock growth is much more rapid at an early stage (i.e., before

70 days) than in the female of the American stock, but in both cases the weights reach a maximum at about 150 days and show little further increase thereafter.

TABLE 6

Size related to age and weight of testes. Male

AGE IN DAYS	SIZE, MILLIMETERS	DONALDSON'S, MILLIMETERS	TESTES, GRAMS	DONALDSON'S, GRAMS
70– 79	215	172	2.35	1.52
80– 89	217	182	2.35	1.74
90– 99	...	188	...	1.87
100–109	240	193	2.8	1.98
110–129	243	199	2.73	2.12
130–149	244	204	2.7	2.22
150–169	240	209	2.65	2.33
170–189	248	212	2.66	2.40
190–199	255	214	3.04	2.44
200–215	...	215	...	2.46
216–229	265	217	3.1	2.50

TABLE 7

Size related to age. Female

AGE IN DAYS	SIZE IN MILLIMETERS	DONALDSON'S, MILLIMETERS
70– 79	205	167
80– 89	208	176
90– 99	209	181
100–109	206	185
110–129	210	191
130–149	215	195
150–169	221	199
170–189	220	202
190–199	220	204
200–215	221	205
216–229	222	206
230–249	235	207

According to Slonaker ('28) rats which are allowed sexual indulgence show a tendency to grow more rapidly in early life than abstainers. The application was made both to males and females on the basis of weight. The opportunity was afforded to compare the rate of growth of bachelors and mated males in the same litters. The figures relating to eleven mated males and ten bachelors in table 8 are, on the whole,

in favor of the bachelors. However, these appear at a disadvantage in two groups: X 3's and V 10's in that the advantage which their initial weight gave them over their mated brothers was not maintained.

TABLE 8[1]

The growth of mated males and bachelors (litter mates) compared

MALES	DAYS OLD	GROWTH IN GRAM AVERAGE WEIGHT		TOTAL GROWTH IN GRAMS		PERCENTAGE GAIN IN WEIGHT	
		Mated	Bachelors	Mated	Bachelors	Mated	Bachelors
		②	②				
Q2's	48	104	101				
	77	214.5	220.5	110.5	119.5	106.25	118.31
		①	①				
Born	56	159	152				
28/12/32	77	215	216				
	90	253	255	94	103	59.12	67.76
		①	②				
X3's	47	163	169				
	64	239	238				
	83	321	301				
	103	355	326				
	127	406	368	243	199	149.08	117.75
		①	①				
V10's	51	115	162				
	58	144	191				
	68	188	225				
	87	241	287				
	107	272	294				
	121	285	303				
	131	305	319	190	157	165.22	96.91
		④	②				
Q1's	46	110	92				
	61	173.5	152				
	81	212	204				
	95	237	233				
	105	245.5	263				
	117	254.5	272				
	131	268	289	158	197	143.63	214.13
		②	②				
P4's	36	109	81				
	53	187	159				
	72	258	240				
	92	292	284				
	106	315	308				
	119	332	323	223	242	112.84	298.76

[1] The figure within the circle indicates the number of rats in that group.

Sufficient data have been given to demonstrate that the Wistar rat has suffered no deterioration as the result of changed conditions and that this branch of the Edinburgh stock can bear comparison with the parent stock even when the latter is kept under the most favorable conditions.

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CONGENITAL UROGENITAL ANOMALIES IN RATS INCLUDING UNILATERAL RENAL AGENESIA

BY

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"Congenital Urogenital Anomalies in Rats including Unilateral Renal Agenesia."

by A.M. Hain and Edwin M. Robertson.

N.B. The abnormalities were detected by A.M. Hain who made the macroscopic observations. These were confirmed and amplified under the microscope by Dr. Edwin M. Robertson who also supplied the detailed anatomical descriptions and drawings.

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CONGENITAL UROGENITAL ANOMALIES IN RATS INCLUDING UNILATERAL RENAL AGENESIA[1]

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Structural abnormalities which have been observed in certain rats of a stock bred primarily for experimental purposes appear to be worth recording, not only on account of their rarity, but also because of the existence of a close relationship between the affected individuals, and of the fact that similar anomalies have been encountered in man (Collins, 1932; Campbell, 1928; Eisendrath, 1924; and others).

The stock consists of pure albino rats (Wistar) and a cross between these and a "hooded" variety in both of which abnormalities are rarely found, although microphthalmia has occurred in the pure-bred stock and in the crosses. There is no known record of the existence of the abnormalities to be described either in mice or in rats. The stock has been injected with endocrine preparations over a period of years and more especially pregnant animals have been used; it has been impossible, however, to find any direct association between such treatment and the anomalies observed. The genealogical table reproduced suggests that the character is present as a recessive with various modifications of its expression, and its association with a microphthalmic ancestry diluted by outcrosses points to a possible connection with the factor for microphthalmia, such as has been cited by Collins (1932) in the list of anomalies associated with renal agenesia collated by him from the literature bearing on the human subject. From the descriptions which follow it would seem that, whereas unilateral renal agenesia in the rats observed was always accompanied by genital maldevelopment, the latter can occur in related individuals without renal defects.

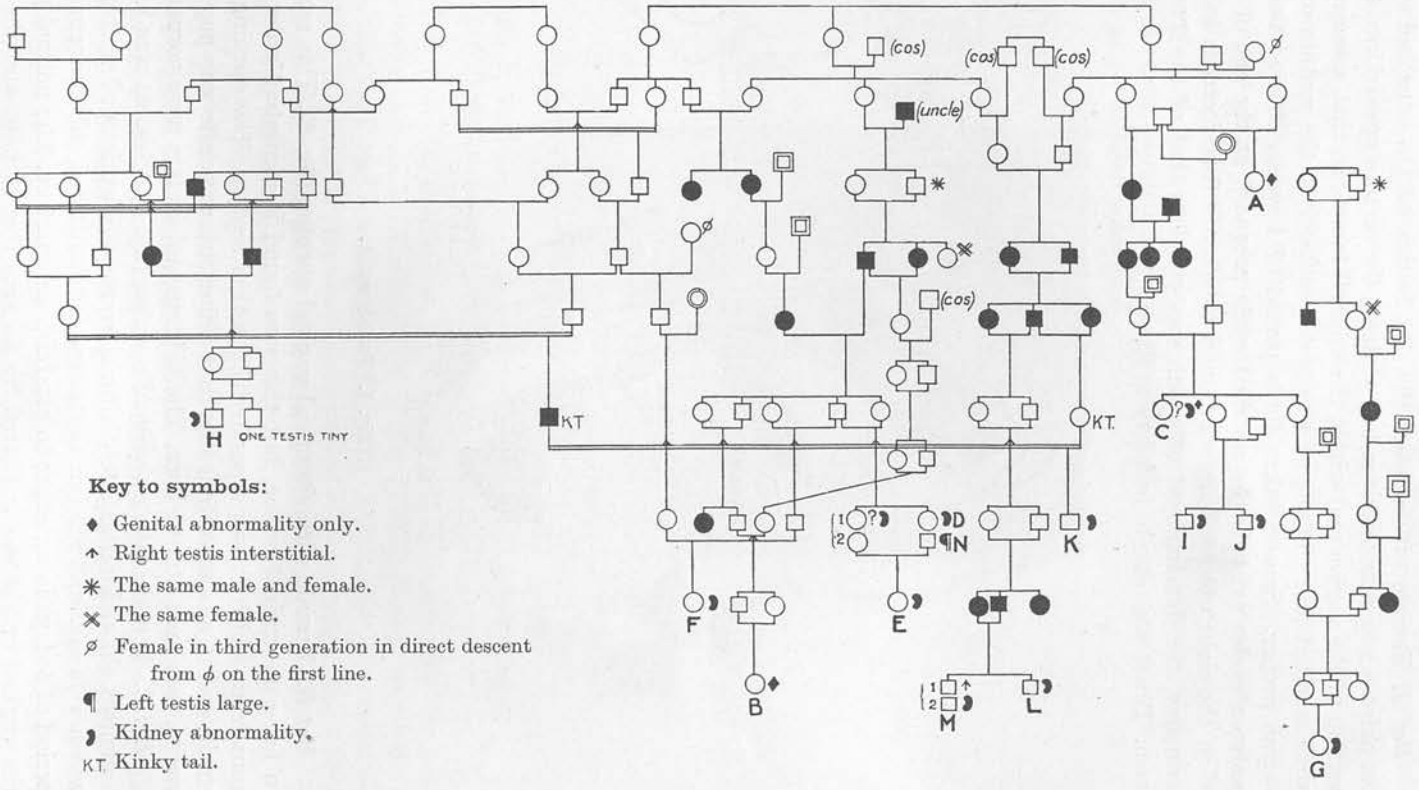
DESCRIPTION OF THE ABNORMALITIES

It will be found that towards the end of this investigation the abnormalities are more fully recorded, as it was seen that the earlier observations were not sufficiently comprehensive. In the description which follows, the rats bear the initials given to them in the genealogical table.

[1] The expenses of this investigation were largely defrayed by grants from the Medical Research Council and the Carnegie Trust for the Universities of Scotland.

Pedigree of rats having renal and genital anomalies

A circle denotes a female; a square denotes a male. Outcrosses (i.e. a different strain of rats) are represented by a circle within a circle and a square within a square. Solid squares and circles denote microphthalmics.



Key to symbols:

- ♦ Genital abnormality only.
- ↑ Right testis interstitial.
- ✳ The same male and female.
- ✕ The same female.
- ⌀ Female in third generation in direct descent from ϕ on the first line.
- ¶ Left testis large.
- ❜ Kidney abnormality.
- KT Kinky tail.

Rat A. Female with a cloaca (fig. 1). In this rat the anus and vagina did not exist as separate openings but, instead, the rectum opened into the dorsal portion of the vagina and within the vaginal lumen, so that a smearing wire, when inserted into the vagina penetrated either into the rectum or into the vagina proper. Smears taken for a period of 4 weeks showed that normal oestrous cycles existed; the rat died while pregnant at the age of 4 months when the right uterine horn was found to contain four foetuses and the left horn five, the development of which was equal to that of the 22nd day or term. Death was due to inability to litter.

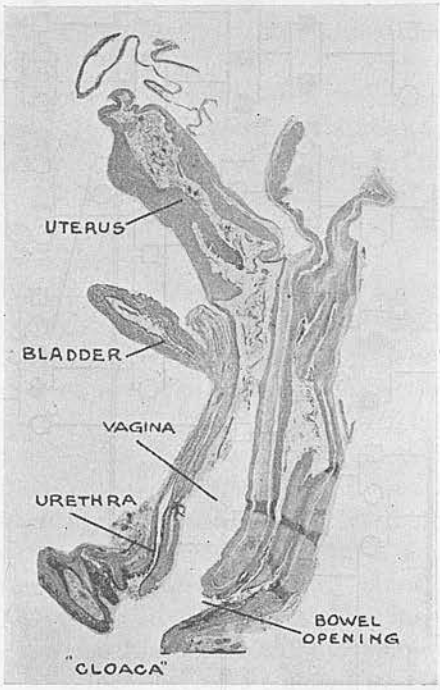


Fig. 1. Female rat A.

Rat B. Female with abnormal vaginal opening (fig. 2). This rat appeared to have no vaginal orifice, but this was found immediately anterior to the anus from which it was separated by a thin septum. The opening was very small, and it was with difficulty that a fine suture needle was passed up the vagina into each uterine horn. The kidneys and ureters were normal.

Rat C. Female with no vaginal opening (fig. 3). This rat was killed when 5 months old and immediately after a course of injections of oestrone, during which 1·44 mg. or 7500 int. units were administered subcutaneously over a period of 4 days in an effort to establish an aperture. The normal position of the vaginal orifice was indicated by an area free of hair, and the occluding

membrane appeared to be of a uniform thickness. The distance from the anus to the centre of this area measured 11·75 mm. At necropsy the right uterine horn was found to be greatly distended with fluid and was 8 mm. wide × 45 mm. long. A sharp constriction at the cervix marked its entrance into a distended and closed canal 14 mm. in diameter and 42 mm. long, which proved to be the vagina. This did not connect with the natural site of the vaginal orifice but followed the course of the rectum along its entire extent although it did not open at the anus.[1] The left uterine cornu was represented by a small spherical sac situated immediately below the ovary; it measured only 11 × 8 mm. and was filled with fluid.

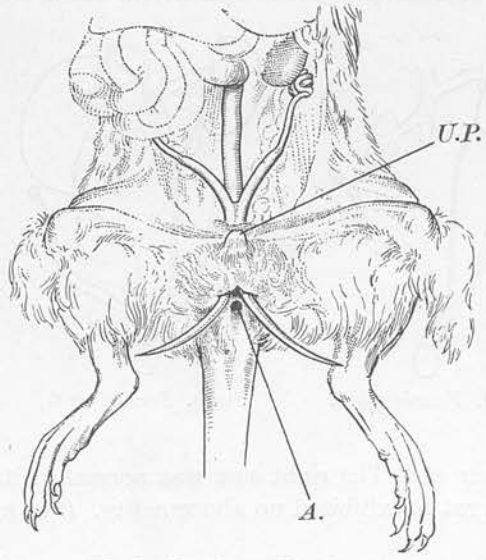


Fig. 2. Female rat B. *A*. anus, *U.P*. urethral prominence.

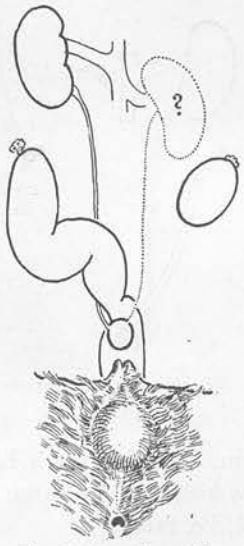


Fig. 3. Female rat C.

It is likely from the data about to be presented demonstrating the association between defects in development of the genital system and kidney anomalies, that rat C had a hypoplastic kidney on the defective side; however, this was the first animal in this series encountered with such an abnormality, and as no such association had at that time been observed, the existence of both kidneys was not ascertained.

Absence or hypoplasia of the left kidney

In the rats about to be described the absence or hypoplastic development of a kidney has always been found in association with genital abnormalities on the same side of the body, and it has always been the left side which has been affected.

[1] A similar defect has been observed in a mouse, but both uterine cornua were normally developed.

Rats D and F. Female rats in which neither kidney, ureter nor renal blood vessels were to be found on the left side (fig. 4). The adrenal gland and the ovary were present and the latter was normally situated, but no left uterine cornu existed. The right side of both rats was, in all respects, normal.

A female in the same litter as rat D was found to have no left cornu; this rat was unfortunately destroyed without complete examination.

Rat E. Female rat with hypoplastic left kidney (fig. 5). On the left side the place of the kidney was taken by a hard, yellowish body the size of a pea, which, on histological examination, proved to be a cystic, hypoplastic kidney. The renal artery and vein on this side were normal, but no ureter was found. Below the defective kidney were a normal ovary and a portion of uterus about

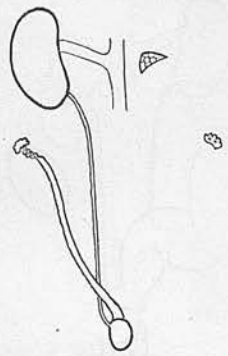


Fig. 4. Female rats D and F.

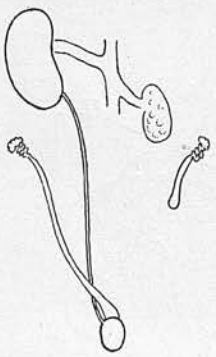


Fig. 5. Female rat E.

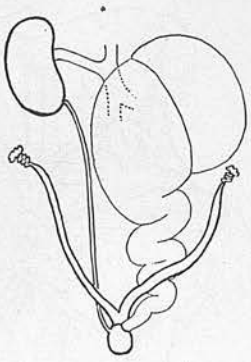


Fig. 6. Female rat G.

10 mm. long, having a blind lower end. The right side was normal. Littermates killed at the same time as rat E exhibited no abnormality. (See male rat N, i.e. father.)

Rat G. Female rat with hydronephrotic kidney and hydroureter on the left side (fig. 6). This female was killed when 42 days old; at that time she weighed only 15 gm. as compared with females in the same litter which weighed 90 and 92 gm. A litter-mate of her mother had a similar tiny female in her litter, but this was eaten on the day that rat G was killed. It is possible that a similar condition existed.

On the left side in place of the kidney there was a large translucent, hydronephrotic sac; the renal pelvis was greatly enlarged, also the ureter which was much kinked on itself. Under the dissecting microscope it was found that the left ureter was obstructed at its junction with the bladder and terminated at the site of its normal opening in a cystic dilatation projecting into the base of the bladder. The condition was apparently one of stenosis of the lower end of the ureter at its entry into the bladder, with subsequent distension of the occluding portion and also of the renal tract proximal to the barrier. Microscopic examination did not reveal any clue as to whether the cause lay in the ureter, the bladder wall or the bladder mucosa. On the embryological data,

however, it is evident that a secondary occlusion of the ureter must have occurred. As the lumen of the ureter is an outgrowth from the existing archinephric (i.e. mesonephric) duct, there must once have been a clear opening. If there had been no archinephric duct, there could have been no mesonephros, but in the two male rats exhibiting a similar abnormality (rats L and M) the epididymis, itself derived from the mesonephros, is well marked.

The ovary and uterus on the defective side were normal, as also were the organs on the right side.

Rat H. Male rat with no left kidney (fig. 7). On the left side there was no trace of kidney or ureter. Midway between the normal renal site and the pelvic girdle lay a small testis only 10 mm. long; the epididymis was below

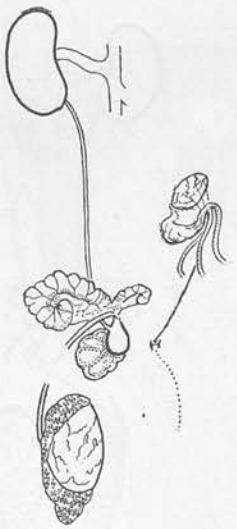


Fig. 7. Male rat H.

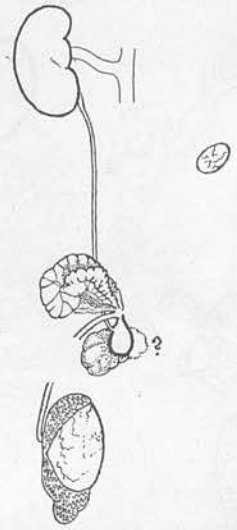


Fig. 8. Male rat I.

this and not contiguous with the testis, and from it there arose three strands which appeared to bury themselves in the abdominal wall; the gubernaculum connecting with the scrotal sac was 55 mm. long. The seminal vesicle on the left side was underdeveloped, measuring only $7{\cdot}5 \times 6{\cdot}5$ mm. and weighing 100 mg. (cf. $28 \times 11{\cdot}5$ mm. and 1·0 gm.—normal).

The right kidney was greatly enlarged and weighed 2·7 gm. (cf. normal litter-mate's kidney 1·6 gm.). The right testis and seminal vesicle were normal. There was much diffuse chromaffin tissue on both sides. A male in the same litter was a typical unilateral cryptorchid with no kidney abnormality.

Rat I. Male rat with no left kidney (fig. 8). On the defective side no renal vessel and no ureter were found. The testis occupied a position a little below the normal site of the kidney and away from the midline; it weighed only 0·2 gm., while that on the right side weighed 0·85 gm. There was a complete absence of ducts on the left side, and no seminal vesicle.

The right kidney was greatly enlarged, as in rat H, and weighed 2·2 gm. (cf. 1·6 gm., the average weight of a kidney of an animal of the same body weight). The seminal vesicle was peculiar in that it consisted of two lobes, the one arising out of the centre of the other.

Rat J. Male rat with an atrophic left kidney (litter-mate of rat I) (fig. 9). A small atrophic mass of tubules about 5 mm. long lay in a pad of fat, and the whole mass was connected to the abdominal vessel by a pedicle of blood vessels which was twisted on itself twice. That these tubules were renal tissue was verified by section, but as these must evidently have been a remnant of the mesonephros there was no ureter. The testis on the same side was abdominal, very small, and lay 30 mm. below the "kidney"; it had neither attachments nor vas. The gubernaculum was represented by a thin fibrous

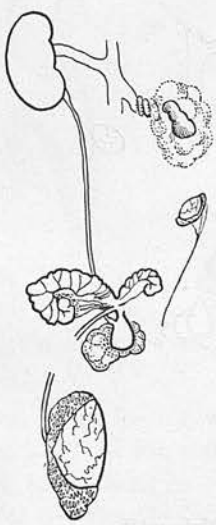


Fig. 9. Male rat J.

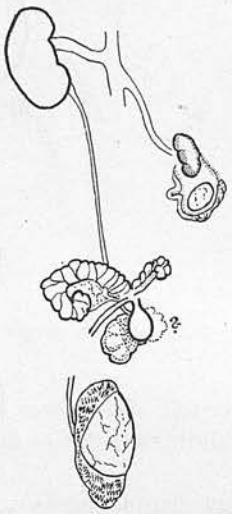


Fig. 10. Male rat K.

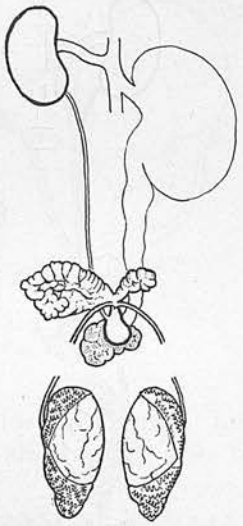


Fig. 11. Male rat L.

cord which disappeared into the inguinal canal. The seminal vesicle was small and, like other underdeveloped vesicles in this series, was quite translucent. The prostate on this side was also very tiny and semi-transparent.

The right side was normal.

Rat K. Male rat with cystic hypoplastic left kidney (fig. 10). On the defective left side a small, soft and amorphous kidney lay about 25 mm. below the normal position. The testis on this side was tiny and had not descended (male 3 months old). There was no connection with the seminal vesicle which was underdeveloped as in rat J, and passing from the small globus major a thin, fibrous strand connected with the outer side of the kidney, i.e. the side remote from the hilum.

Rat L. Male rat with polycystic left kidney (figs. 11 and 12); killed when 7 weeks old, along with its litter-mate in which the right testis was interstitial in position but in which there was no renal abnormality.

Rat L exhibited considerable urinary incontinence. At necropsy the left kidney, which was in the normal position, was found to be almost three times the size of the right kidney and was a mere sac of renal tissue, distended with fluid; the wall was normal in colour; the left ureter was much dilated. By the use of probes and with the aid of the dissecting microscope, it was found that, as in the case of female rat G and male rat M (his litter-mate), an obstruction apparently existed at the point at which the ureter was attached to the bladder at the left of the trigone, just above the neck (fig. 12). Cross-sections taken of the lower portion of the bladder and of the urethra demonstrated microscopically that the left ureter had no opening either into the bladder or directly into the urethra. The portion of bladder wall (?) obstructing the ureteral lumen shared in the general distension of that tract,

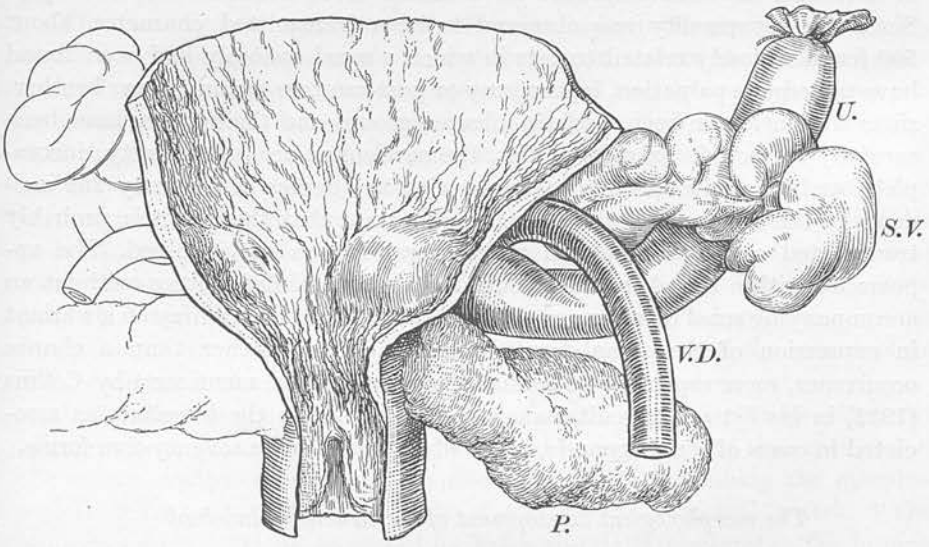


Fig. 12. Male rat L. *P.*=prostate; *V.D.*=vas deferens; *S.V.*=seminal vesicle; *U.*=ureter.

and this resulted in distortion of the neck of the bladder and consequent incontinence of urine. The testes, the vascular system of the seminal vesicles and vasa deferentia and of the bladder circuit were normal; the left lobe of the prostate was smaller than the right. The right side was normal.

Rat M. Born in a subsequent litter to the same parents as rat L. This male had shown signs of incontinence but to a less extent than its litter-mate, rat L. When killed at 5 weeks old, it was found to have a large, translucent, hydronephrotic kidney on the left side. Its appearance, and that of the ureter, which was also greatly distended and kinked, resembled in all respects the condition observed in female rat G already described (see fig. 6). The testes and other genital organs were normal.

Rat N. Kidneys normal but left testis was very much larger than the right

and exceeded any other testis encountered both in weight and in size. The measurements were as follows:

Left testis: 28 mm. long, weighing 3·0 gm.
Right testis: 20 mm. long, weighing 1·5 gm.

This male fathered female rat E already described.

Renal agenesia and hypoplasia as an inherited defect

The pedigree chart (p. 567) strongly suggests that the occurrence of the defect is not a random one. As the defect can rarely be detected externally in females, it is probable that instances of its occurrence in that sex have been overlooked; in males, however, all animals having a single descended testis or testicular underdevelopment have always been submitted to necropsy. Since the abnormality was observed to be a transmitted character, about 500 females closely related to rats in which a renal anomaly had been found have undergone palpation, laparotomy or post-mortem examination; brother sister matings have been made in affected groups and their litters have been carefully inspected. In spite of this, the particulars are, of necessity, incomplete, and it is impossible to calculate with any degree of accuracy the incidence of the abnormalities; one can merely state that the defect is probably transmitted as a recessive and that several factors are involved. The appearance within the chain, of females having genital deformities without an accompanying renal defect, suggests that such abnormalities may be a variant in expression of the same developmental anomaly rather than a chance occurrence, more especially as similar abnormalities are mentioned by Collins (1932) in his list of congenital anomalies described in the literature as associated in cases of renal agenesia, a list which totals some seventy-five forms.

The morphological development of the structures involved

The varying degrees of renal defect would seem to be attributable to the stage at which the arrest of embryological development occurred, e.g. the entire absence of a kidney must belong to an earlier date than the hypoplastic kidneys of rats E, J and K, which, in turn, belong to an earlier stage than those of rat L and also rats G and M.

The regularity with which the left side only was affected is striking and suggests that an inequality exists in the rate of development of the two sides of the body. Gray (1930) has recorded that the right kidney of the common frog always develops more slowly than the left; this asymmetry is not observable after metamorphosis. Cases of lateral asymmetry described in birds (Crew, 1931; Crew and Lamy, 1935) and in man (Potter and Urwick, 1935; Bowman, 1935) have almost entirely related to limb size and to colour. When gynandromorphism was present this was associated with gonadic abnormality. Crew (1931) is of the opinion that the various expressions of asymmetry can

bear a single interpretation, viz. that "aberration in chromosome distribution is the cause of the regional expression of a recessive character" present in a heterozygote. He considers that the autosomal constitution is affected and explains the variations in manifestation as due to differences in time during development at which the loss of the particular autosome or its part occurred, the earlier the loss occurs the greater being the distribution of the recessive character.

In order that the earliest stages of the developmental idiosyncrasy might be observed, litter-mates of rats with renal agenesia were given to Dr Peter Gray (Zoology Department, Edinburgh University), when these were 8–12 days pregnant, for an embryological study of the abnormality, as it seemed possible that the defect would appear in closely related stock. The result of this investigation is not yet available.

The simultaneous occurrence of anomalies in both the genital and urinary systems in the affected animals is not surprising in view of the relation known to exist in the morphological development of the two systems. In those rats in which there is no trace of a urinary system (rats D, F and I) it is manifest that the defect dates to a stage prior to the development of the oviduct and archinephric duct and involves the non-development of either a mesonephros or pronephros. This is compatible with the absence of epididymis and ducts in rat I. In those rats in which the ureter is absent but renal tissue exists along with partial development of a duct system and of the seminal vesicle (rats H, J and K), the developmental arrest must have taken place after the formation of the mesonephros but prior to the formation of the metanephric bud, since the ureter and pelvis of the kidney are not found.

In female rats C and E it would seem that the arrest in the development of the oviduct occurred during the process of back growth of the inner end of the ostium abdominale tubae (Brambell, 1927). In describing the morphological development of the gonads of the mouse Brambell writes: "The Müllerian duct develops, as stated by Felix and de Winiwarter in the human species, as a back growth of the inner end of the ostium abdominale tubae. The similarity in structure of the primordium of the ostium abdominale tubae to the nephrostomes and its similar position in the urogenital ridge slightly anterior to them, suggests that they may be homologous. On this view the ostium abdominale tubae would be a nephrostome which grew and extended posteriorly to form the Müllerian duct." Thus these abnormalities add striking support to the theory, not universally accepted to-day, that the mammalian oviduct develops in a postero-anterior direction.

Since the mesonephros is already well established and the tubules formed anteriorly by the 9th day post coitus (Brambell, 1927), it is manifest that development can be arrested at a very early stage. The existence of some adverse environmental influence upon the developing embryo would have suggested itself had all the members of a litter been abnormal, but in only two instances were defective genitalia encountered in more than one member of a litter.

The occurrence of a male with renal maldevelopment in each of two successive litters born to the same parents suggests that an inherent defect existed in the parent cell, and the ovum itself may have been defective.

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"Microphthalmia and other eye-defects
throughout 14 generations of Rats."

by A.M. Hain.

This paper has been submitted for publication.

MICROPHTHALMIA AND OTHER EYE-DEFECTS THROUGHOUT 14 GENERATIONS OF ALBINO RATS

by

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The albino rats used in this study were obtained originally from the Wistar Institute of Philadelphia and belong to a strain in which microphthalmia was first observed by Dr. Helen Dean King in the F_4 generation extracted from a cross between an albino female of this stock and a wild grey Norway male. The defect occurred only in the "S" strain which formed a small proportion of the main stock. Its inheritance was observed by Dr. King (1931) through eleven generations, and 538 rats having either both or a single eye microphthalmic were obtained in a strain comprising 1884 individuals. The greater frequency of microphthalmia in the American stock is a marked feature since only 154 rats with eye defects have been observed in the Edinburgh branch of Wistar rats in fourteen generations comprising over 4000 rats, and this total includes animals with eyes of normal size but possessing defects such as aniridia, cataract, etc., and also rare cases of anophthalmia and buthalmos. It is probable that

80% of the total affected were microphthalmics, and apparently the defect was generally more pronounced than in the parent stock, that is if the rat illustrated in Dr. King's monograph is to be taken as typical. According to Dr. King, the eye-defect could easily be detected at birth "since a small eye did not protrude from the socket." In the total given here no still-borns are included as it was found that this method of diagnosis, as also the opacity of the lens in such rats immediately or soon after birth, were unreliable criteria of abnormality. We are in agreement that prenatal mortality did not play an important rôle in determining the ratio of normal to abnormal individuals, because litters containing affected were of the average * litter size and generally contained some normals (Table I). However, where a large number of still-born rats occurred in litters containing defectives, it is possible that the abnormality involved proved lethal since still-births are not of common occurrence in * this stock (Table 2). The same may be true in those instances in which a litter consisted of only 2 or 3 rats all of which were defective, since such small litters are comparatively rare (Hain, 1934); six litters of this type were encountered, one of which consisted of 4 rats, four of 3, and one of 2. Moreover 7 rats which had given birth to defectives subsequently had still-born litters, and absorption occurred 14 times.

Growth and Fertility. It was found by Dr. King that "although affected rats grew rapidly and often attained a large size, in vitality and fertility they were definitely below the average of the stock from which they came, and showed a marked susceptibility to pneumonia" This /

This was not observed in the Edinburgh stock. Occasionally the defective rat was smaller than others in the same litter as a youngster, but later the average of the litter was generally attained and sometimes surpassed. Table 3 which gives the weights and ages of a number of affected rats at the time they were killed illustrates this point. The largest rat ever obtained in the Wistar stock - a male weighing 520 gm. when killed - was a microphthalmic, and another was almost three years old when killed. The number living to a great age would probably have been greater had it not been necessary to kill many when only 3 to 6 months old for the histological study of the eyes, which forms a separate investigation.

There is, moreover, nothing to indicate that females with eye-defects were appreciably below the average in fertility. Of 50 ♀♀ which littered, one gave birth to 65 and another to 48 living young (each had six litters); four had between 30 and 40 young, seven between 20 and 30, and twenty had between 10 and 20 young. The average was 7.6 per litter. A random selection of 18 ♂♂ indicates that their fertility was in no way impaired. One of these fathered 137 offspring (of which only 2 had eye-defects), three others were each responsible for 105 to 115 offspring, two for between 65 and 85 each, and the remainder for from 15 to 40 each.

Other Anomalies. No other structural abnormalities were ever found in association with microphthalmia in the American stock of Wistar rats. In the Edinburgh stock renal and genital anomalies including unilateral renal agenesia have been observed in animals related to microphthalmics, but are of much rarer occurrence than microphthalmia (Hain, /

(Hain, 1936). That there is probably a connection between the two defects - microphthalmia and urogenital developmental anomalies - is suggested not only by the fact that the latter are transmitted, but also by their association in man (Collins, 1932). In addition, and for the same reasons, hydrocephaly may be considered as an associated anomaly. It was found in 15 rats, of which 12 were males; five of these were themselves anophthalmic, microphthalmic or buthalmic; two were brothers of an anophthalmic female, six had microphthalmic sisters, and of six one or both parents were microphthalmic.

DATA.

Results of selected matings.

The defect first manifested itself in the British branch of the Wistar stock in 1932 and during the four years that have elapsed since its appearance 154 rats suffering with eye-defects have been encountered in 14 generations, i.e. out of some 5,000 animals. In the pedigree chart defective rats are indicated as solid squares (males) and solid circles (females). In order to show the relationship and antecedents of both parents, it has frequently been necessary to insert an animal twice. In such cases an asterisk refers the reader * to the list attached to the pedigree chart.

In order to study the mode of inheritance of microphthalmia and kindred eye-defects, a certain procedure was adopted as to matings. (a) In the first place it was necessary to secure as many rats with eye-defects as possible. Consequently ♂ Rat III.8 (or 21), the first affected animal, and his brother ♂ III.10 (or 18) were mated as often as possible to different females and especially to rats related to them. /

them. These two males are together responsible for 40 ♂♂ and 39 ♀♀ descendants, the greater number of which can be traced to the normal brother.

(b) Brother-sister matings were observed whenever possible.

(c) Backcrosses, i.e. father to daughter, or uncle to niece, were made on several occasions.

(d) Defective males and females were repeatedly crossed and frequent changes made.

(e) Defectives and recessives were outcrossed with a different strain of rat. As outcrosses, black and white rats of the "hooded" variety (Reading rats) were employed. Not only has microphthalmia not been reported in this variety ϕ but their possession of black eyes made them eminently suitable for the study of a defect which might be associated with albinism only.

* The outstanding feature of Table 4 is the low incidence of affected obtained from ♂ x ♀ and the high proportion of the total obtained from ♂ x ♀ - 132 out of 154. It is impossible to estimate the total number of matings of ♂ x ♀ made, as such comprise by far the largest number in a stock which, during the four years under observation, has numbered more than 2,000 breeding females.

An analysis of the 64 matings which form the last group is given * in Table 5. From this it is clear that in many cases either one parent or both were genotypically abnormal. In 18 cases either both parents or both grandparents had microphthalmia or other eye-defects and in 6 others the defect is traced back three, four or even five generations. In one of the latter - IX. 1 x 2 - no microphthalmia occurred /

ϕ ϕ "Not a single case has occurred in over 6000 rats (16 generations)." — Personal communication from Dr Kon.

occurred in three generations in direct ascent but in the fourth generation back a ♂ mated with a Reading female (V. 1 x 2). It is of interest that its re-occurrence followed the mating of a ♀ with a Reading male (IX. 1 x 2). Two other normals which gave birth to defectives (XIII. 19, 20 and 21) and which were themselves brother and sister, had no microphthalmia in four generations of ancestors but in the fifth generation of ascent two defectives (brother and sister - VII. 22 and 23) mated. All of the four generations consisted of brother x sister matings except in the third generation when a ♀ was outcrossed to a Reading ♂.

Hofmann (1912) is of the opinion that the eye-defect was transmitted chiefly through affected females, and states that matings of affected males with normal females produced very few abnormals. The figures given in Groups 2 and 3 in Table 4 are contrary to this finding, as also those in Table 5; for example, 13 defectives were born to ♂ x ♀ but only 1 to ♀ x ♂, and where ♂ x ♀ matings gave affected it was found that in 16 cases mating occurred between two defective ancestors of the <u>male</u> but only in 4 cases of the female. The occurrence of microphthalmia or other eye-defect among litter-mates of the two parents was, however, shared equally by both parties.

The chief type of mating in Group 4 was between normal brother and sister, viz. 24, and it is remarkable that 47 affected (26 ♂♂, 21 ♀♀) should result from such matings when 49 matings between ♂♂ and normal sisters (Table 4, Group 2) produced only 5 ♂♂, and 42 litters born to brothers and sisters <u>both</u> of which were microphthalmic contained only 3 ♂♂ 2 ♀♀ distributed over three litters.

Two /

Two other types of mating are noteworthy, namely those, numbering nine, in which defectives occurred after mating between a ♂ and a ♀ of the younger generation not necessarily related; and those in which a normal ♀ crossed with a Reading ♂ produced affected. Of the five pairs in which the latter occurred (VI. 25 x 26; VI. 53 x 54; VII. 26 x 27; VIII. 41 x 42; IX. 1 x 2) two are of special interest as in one case - VII. 26 x 27 - the father of the ♀ also was a Reading ♂ and in the other - VIII. 41 x 42 - both the father and the grandfather also were Reading rats.

As Dr. King found that the number of microphthalmic rats increased from 3.45 per cent in the first generation to 43.16 per cent in the ninth generation, an abstract was made of five generations in which brother x sister matings were observed throughout (Table 6). This shows no such increase as was found in the American stock.

It has already been stated that rats with microphthalmia and other eye-defects were, on the whole, equal to the rest of the stock in vitality, fertility and longevity. The female that gave birth to such defectives, however, was generally below the average fertility in that she rarely littered again after producing microphthalmics, or else had still-born young. Of 80 female rats which had litters containing defectives, 61 showed this inability to litter again although the male was changed and mating occurred. The details given in Table 7 of litters born to the remaining 19 females show that within this group frequently normal litters were born to the same parent as had previously produced microphthalmics. The data in the third column of

Table /

* Table 7, as also in Table 8, when studied in conjunction with the pedigree chart, demonstrate the erratic nature of the occurrence of microphthalmia in view of its non-appearance after such matings as are described there. That the same parents sometimes produced normal litters not only after a defective litter but also (as is shown in Table 8) before the birth of microphthalmics, is not to be explained away on the grounds of prenatal mortality of the defective rats, as, with few exceptions, the litters born were of average size.

Reproductive cycle of the female parent. The rareness with which rats which had once given birth to microphthalmics again became pregnant suggested that such animals might possess or develop an abnormal reproductive cycle. Vaginal smears of 18 such females were taken daily for periods varying from 3 to 5 weeks commencing a few months after the birth of the affected litter and before the rat herself was a year old. Only two rats had normal cycles; the remainder had cycles of one of two types - (a) long periods of dioestrus followed by occasional appearances of large epithelial cells about to lose their nucleus (i.e. the pro-oestrous smear), but an absence of the normal oestrous condition; 10 rats, + 2 which came in heat twice but otherwise belonged to this type, formed this group. (b) A persistence of the pro-oestrous type of smear with oestrus occurring rarely if at all (6 rats). The absence of a condition common to all female parents of defectives, as well as the lack of a rigid cycle in rats which are in all respects normal, makes one hesitate to associate microphthalmia with a maternal reproductive mal-adjustment, yet the data as to the subsequent /

subsequent infertility of the female parent of such animals are sufficiently striking to suggest that a physiological basis may be partly responsible for the condition.

Measurements of skulls and eyes. Since one frequently obtained the impression that the skull of the microphthalmic rat was narrower and more tapering than that of the normal control, measurements were taken of the skulls of defective rats of both sexes and were compared with normals and with the data obtained by Hatai (1907) and reported by Donaldson (1915). The skulls were macerated, bleached and dried at room temperature. All the rats were adult and controls were selected of approximately the same body-weight as the microphthalmics. The measurements were made with vernier calipers and followed those taken by Hatai, except in the determination of the fronto-occipital length. This has been measured from the tip of the frontal bone to the end of the occipital bone, without adjustments. The measurements given in
* Table 9 reveal little difference between affected and control rats of either sex and accord with Hatai's data, indicating that the impression of narrowness was apparent and not real. The difference in the weights of the eyes as between microphthalmics and controls is, on the other hand, very marked (fifth column of Table 9). Two of the defective males (VI. 33 and VI. 38), although blind, had eyes of normal size; hence in these two rats the eyes are of normal weight.

It may be mentioned here that Dr. Biggart of the Pathology Department, Edinburgh University, failed to find any abnormality in the pituitary-hypothalamic region of microphthalmic rats submitted to him for examination.

Cytological /

Cytological Study. Eight ♂ rats (III.8, V.2, VI.6, VI.30, VI.33, VI. 40, VI.41, VI.46, and ♂ IV.15) were investigated cytologically; mitosis and meiosis were studied and the chromosome complements were analysed. No irregular chromosome behaviour or structure was encountered in the animals under observation, except in ♂ V.2, which was microphthalmic and epileptic. In this individual occasionally an unpaired chromosome * was seen during meiotic prophase (Fig.1a), metaphase (Fig.1c,d,e), and anaphase (Fig.1f). In some instances one smaller and another longer chromosome were observed lying off the equatorial plate (Fig.1b). Such behaviour strongly suggests that in rat V.2, one chromosome pair is heterozygous for a structural change (translocation, inversion, deletion or duplication) and it is this which is responsible for the 'univalent' and fragment chromosome.

In the other individuals the chromosome complement and chromosome behaviour are normal, indicating that microphthalmia is a genetic abnormality, caused by genetic factors rather than brought about by structural changes in the chromosomes themselves. The structurally abnormal chromosome in ♂ V.2 may have arisen independently and have no causal connection with the morphological abnormality, though it may be responsible for the exaggeration of microphthalmia with epilepsy.

DISCUSSION.

From the preceding data it is manifest that microphthalmia in rats was not associated exclusively with albinism as more than a dozen affected rats were black and white animals and exhibited microphthalmos, buthalmos and anophthalmos.

It /

It is also clear that, since it was impossible to predict the character of the progeny from any given type of mating, the mode of its inheritance cannot be a straightforward one. Although the data cannot be taken to show definitely the existence of a genetic factor or factors responsible for the abnormality, they are consistent with the possibility of a dominant factor with a poor expression which is conditioned by genetic and physiological modifiers. The fact that the defect appears in outcrosses suggests that a dominant is involved.

Summary.

(1) The incidence of microphthalmia and other eye-defects has been examined throughout 14 generations of Wistar albino rats, and the results of various types of mating have been given; defectives are rarely born to defective parents.

(2) The vitality, growth and fertility of affected rats is normal, but a tendency to sterility in the female parent of such animals is noted.

(3) Associated anomalies are reported.

(4) It is probable that microphthalmia possesses both a genetic and a physiological basis.

ACKNOWLEDGMENTS

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TABLE I.

Showing the proportion of affected to normals in litters of various sizes.

Tot. in litter	No. of litters	No. affected rats	No. dead.
13	2	1,5.	0, 3
12	1	3.	3
11	7	4,1,1,1,3,1,1.	-
10	6	1,1,1,1,3,3.	1
9	4	1,4,8,2.	3
8	5	1,1,2,4,4.	-
7	4	2,4,5,1.	-
6	11	1,2,1,2,2,1,4, 1,3,1,1.	2
5	4	1,1,2,1	-
4	5	1,1,1,4,1.	-
3	8	1,1,3,3,3,3,2,2.	-
2	1	1.	-

TABLE II.

Analysis of litters in which defectives occurred and in which the incidence of still-births was high.

No. of rats affected	No. of rats dead	No. of rats. normal.
1	13	-
3	10	2
1	10	2
3	7	-
1	6	1
1	6	1
1	5	6
1	5	5
1	4	4
1	4	-
2	4	3
2	4	-
1	4	-
1	3	-
1	2	2
* 1	-	1
* 1	-	-
* 1	-	-

* It was presumed from the size of the mother late in pregnancy that more young were born but were eaten at birth.

TABLE III.

Showing weights and ages reached by ♂♂ and ♀♀ at the time they were killed.

Weight at death.			Age at death.		
wt. in gm.	No. of ♂♂	No. of ♀♀	Age in days	No. of ♂♂	No. of ♀♀
> 200	-	28	200 - 365 (i.e. 1 yr.)	23	11
> 250	13	7	366 - 549 (i.e. 1½ yrs.)	17	8
> 300	26	2	550 - 730 (i.e. 2 yrs.)	3	10
> 400	13	-	731 - 912 (i.e. 2½ yrs.)	5	8
> 450	3	-			
> 500	1	-	913 -1095 (i.e. 3 yrs.)	1	-

N.B. 1 ♂ was aged 1076 days, 1 ♀ 807 days;

1 ♂ weighed 520 gm., 1 ♀ 362 gm.

TABLE IV.

The Occurrence of Eye-Defect in Relation to the Type of Mating.

Group	Type of Mating.	No. of Litters born to such mating.	♂♂	♀♀	No. of Litters in which ●● present.	Total ●●/Total oo in same litter.
1	♂ x ♀	65	3	5	4	8/506
2	♂ x ♀	122 *	10	3	10	13/903
3	♀ x ♂	26 ϕ	-	1	1	1/169
4	♂ x ♀	?	72	60	64	132/?
		Total	85	69		

Chart numbers of affected in Groups 1, 2 and 3:-

Group 1: VI.3; VI.8,9,10; VII.21,22,23; XIII.12.

Group 2: IV.5; V.10; VI.11; VI.46; VII.3; IX.37; IX.41; IX.47; X.48 to 51; XI.20.

Group 3: VI.4.

* This figure includes (a) 22 matings with outcross females which produced 74 ♂♂ 79 ♀♀; and (b) 21 backcrosses of F_1 ♀♀ from such matings with the defective father, which produced 99 ♂♂, 113 ♀♀ and 1 ♀. Of the total, 46 matings were between a ♂ and his normal sister; 6 were backcrosses with a defective father.

ϕ Including 9 litters from outcross males; of the remainder, 8 were matings between litter-mates and 3 were backcrosses.

TABLE V.

Analysis of ♂ x ♀ matings of Group 4 in Table 4.

Affected relations, etc.	of ♂	of ♀
Parents were ♂ x ♀	8	1
2nd (a) or 3rd (b) generation back were ♂ x ♀	(a) 7 (b) 1	(a) 2 (b) 1
4th or 5th generation back were ♂ x ♀	1	3
No defectives in direct ascent	3	3
Mother was ♀	-	2
Father was ♂	3	4
Bro. and/or Sis. were ●●	20	19
Parent had ●● in another litter	3	-
Parent had ●● litter-mates	-	5
Rat had ●● in F_1 and F_2 to another mate.	7	-
No. of matings bro. x sis.	24	24
" " " ♀ x Reading ♂	-	5
" " " ♂ x ♀ of younger generation.	9	-

N.B. In 6 cases defectives were born to sister of ♀ crossed to the same ♂: total affected born thus was 6 ♂♂, 2 ♀♀.

TABLE VI.

Showing the incidence of Microphthalmia in 4 generations of brother x sister matings.

Type of Mating.	No. of Litters.	♂♂	♀♀	♂♂	♀♀	Tot. affected to Tot. normal.	No. of affected litters.
♂ x ♀	65*	240	266	3	5	8/506	4
F_1 ♂ x ♀	140	484	538	8	4	12/1022	6
F_2 ♂ x ♀	45	139	149	5	3	8/388	5
F_3 ♂ x ♀	22	84	85	1	-	1/169	1
F_4 ♂ x ♀	15	49	44	1	-	1/93	1

* 44 ♀♀ littered; only 11 ♀♀ of the total affected were either not crossed with ♂♂ or had still-born litters to such ♂♂.

TABLE VII.

Litters born subsequent to affected litters.

Female x same male		Female x Reading male		Female x Related male		Female x Different unrelated male.	
♀	Litter: ♂/♀	♀	Litter: ♂:♀	♀	Litter: ♂:♀	♀	Litter: ♂/♀
V.50	4/2	V.24	2/1	IV.33 #	10/6	V.4	3/3
VI.39	1♂/1♀	V.12	3/1	VII.10 ϕ	1/5	V.23	1/1
V.37	-/1♀	V.41	0/6	" †	6/5		
	-/1♀	VI.26	1/0	" †	1/2		
			4/5				
V.22	3/1						
VII.20	3/2	VI.2	4/6	VI.2 ↑	3/6		
	5/3		4/3				
				VIII.15 ¶	5/1		
IX.40	4/2			VII.24 ⊘	11/13		
	4/3			" †	7/6		
IX.29	0/1	IX.29	3/3			IX.29	5/8
XI.18	1/1						
Tot: 11 litters born subsequently to same female x same male yet only 1 ♂ 2 ♀♀ cf. 8 ♂♂ 3 ♀♀ previously.		Tot: 8 normal litters born subsequently to same female x Reading ♂.		Tot: 11 normal litters born to same female x related males (see foot).		Tot: 3 normal litters born to same female x different unrelated ♂.	

N.B. The following had previous litters (see Table VIII.):-

Col. 1: IX.40; Col. 2: V.24; VI.26; Col. 3: VI.2; VIII.15.

♂ Uncle † ♂ son ¶ brother

ϕ ♂ father ↑ ♂ son ⊘ ♂ father (4 litters)

TABLE VIII.

Normal litters born before defective litters.

Female x same male as gave defectives later		Female x bro. or other relation whereas defectives born to unrelated ♂		Female x ♂ whereas ●● born to unrelated ♂		Female x difft. brother ♂ from the one which gave ●●		Others	
♀	Litter ♂/♀	♀	Litter ♂/♀	♀	Litter ♂/♀	♀	Litter ♂/♀	♀	Litter ♂/♀
VIII.10	4/7	V.24 ϕ ♀	3/1	V.6 ♀ x bro.♂	7/3	V.52	2/3	IV.30 * (x unrel. ♂)	7/2
						VI.28	5/3		
VIII.15 (i) x bro. (ii)	2/1 8/4	VII.26 # (i) (ii)	3/3 7/6	V.8 *	2/7	IX.44	3/3	VI.2 † (x bro.)	6/1
IX.28 (i) (ii)	2/4 7/5	XI.12 (i) (ii)	6/2 5/4					VIII.41 (i)x Read.♂.(i) (ii)x unrel.♂.(ii)	7/6 4/1
IX.45 (i) x bro. (ii)	4/5 4/7	X.5 (i) (ii)	7/2 3/4						
IX.40 x bro.♂	5/3	XI.5	3/2						
XI.13	4/3	X.14 ↑	4/3						
XI.21 x bro.	3/5	XI.18	3/2						
VIII.30 (i) x ♂ (ii)	3/4 4/7	VIII.30 ϕ	4/5						
		VI.26 # (N.B. 6 still-born)	0/2						

N.B. The following had subsequent litters (see Table VII)

Col: 1. VIII.15; IX.40; Col: 2. V.24; VI.26; Col: 5. VI.2.

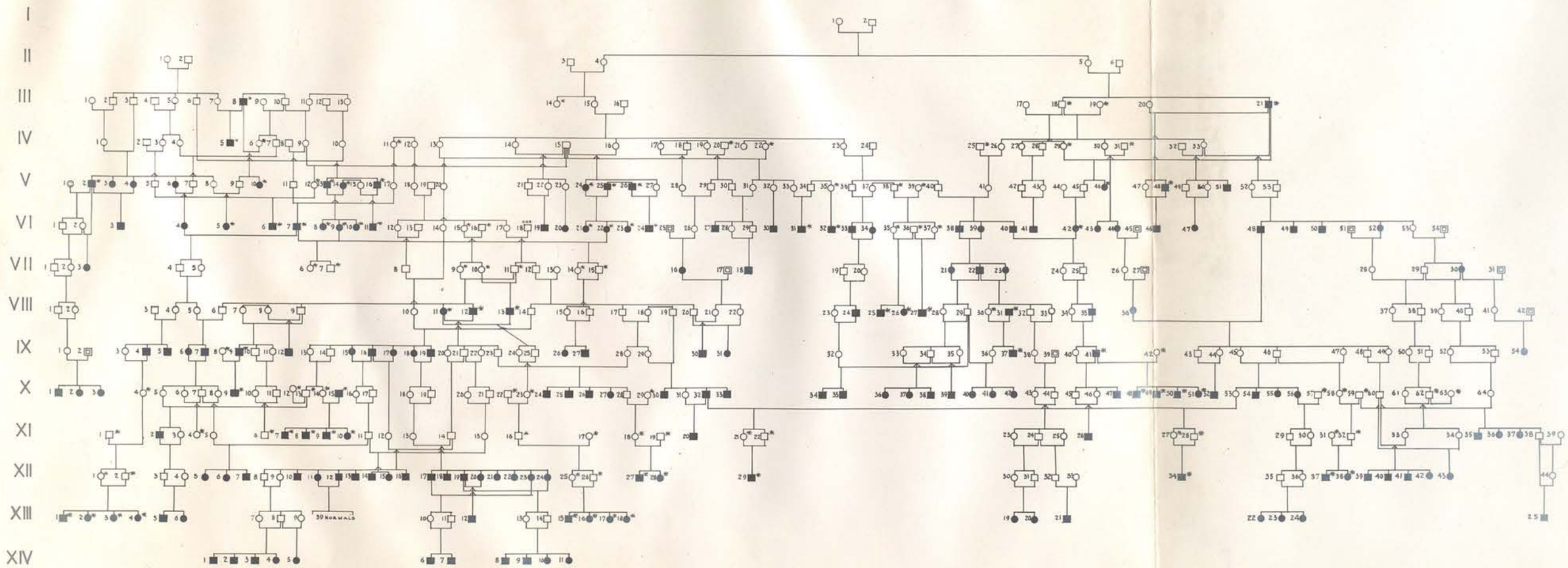
ϕ Defective litter born to unrelated ♂ not ♂
\# " " " " Reading ♂.
↑ x father.
* defectives born to cousin ♂.
† defective born to father (i.e., backcross).

TABLE IX.

Skull measurements of microphthalmic and normal rats compared.

Rat.	Lgth. of cranium mm.	Zygomatic width. mm.	Lgth of nasal bone. mm.	Fronto-occip. lgth. mm.	Squamosal distance. mm.	Height of Cranium. mm.	Eyes. gm.
♂♂							
VI.33 (368 gm.)	48.5	25	19.5	31	16.25	13	0.45
VI.41 (281 gm.)	45	24	18	29	16	12	0.1
VI.38 (337 gm.)	46.25	25	19	30.75	16	12.25	0.340
III.8 & 21 (365 gm.)	48.25	25	19.25	31	16.25	12.25	0.2
V.2 (300 gm.)	47.5	25	19.0	30.8	17	13	0.142
VI.6 & 32 (300 gm.)	46	23.5	17	31.5	15.5	12	0.2
VI.30 (332 gm.)	47.5	25	18.5	31.5	16	12	0.2
VI.40 (397 gm.)	48	25	18.5	32	16	12.5	0.280
VI.46 (338 gm.)	47	24	18.5	30.5	15.5	12	0.270
♂♂ (controls)							
IV.15 (440 gm.)	48.5	26	19.5	31	16	12.25	0.5
♂ 246 gm.	44.5	23.75	17	29.75	16.5	11.5	0.340
♂ 310 gm.	45	24	18	30	15.5	11.5	0.375
♂ 346 gm.	47	25	18	33	16	12.5	0.380
♂ 383 gm.	47.5	25	19.75	30	15.75	12.5	0.360
♀♀							
VI.14 & 24 (264 gm.)	44.25	22.25	17	29	15	11.5	0.150
VI.9 & 22 (192 gm.)	42.5	21.5	16.5	28	14	10.5	0.220
VI.34 (185 gm.)	42.5	21.5	16.5	28.25	15.5	12	0.087
♀♀ (controls)							
V.43 (226 gm.)	45.25	23.5	17	30	16	11.8	-
♀ 181 gm.	40.5	21	15	28	15.25	11.2	0.280
♀ 185 gm.	42.25	21	16	28	15.25	11.5	0.285
♀ 197 gm.	43	22	16.5	28.5	14.75	11.25	0.300
♀ 210 gm.	43.5	22.5	16.2	28	15.8	11.4	0.300

PEDIGREE CHART SHOWING THE INCIDENCE OF MICROPHTHALMIA ETC. IN RATS



⊙ ⊡ = OUTCROSSES .i.e. HOODED RATS.

* = RATS WHICH APPEAR TWICE ON THE CHART : SEE LISTS.

♂ ♀ Duplicate Numbers on Pedigree Chart:-

III.8 = 21
IV.5 = V.48
V.10 = 46
13 = 25
14 = 24
16 = 26
48 = IV.5
VI.5 = 42
6 = 32
7 = 31
8 = 21
9 = 22
10 = 23
11 = 24
VIII.11 = 26
12 = 25
13 = 27
31 = IX.9
IX.9 = VIII.31
37 = X.9
41 = X.15

X.9 = IX.37
15 = IX.41
48 = XI.7
49 = XI.8
50 = XI.9
51 = XI.10
XI. 7 = X.48
8 = X.49
9 = X.50
10 = X.51
XII.27 = 37
28 = 38
29 = 34
XIII.1 = 15
2 = 16
3 = 17
4 = 18

♂ ♀ Duplicate Numbers on Pedigree Chart:-

III.14 = 19

18 = IV.20

19 cousin of 18 and = 14

IV. 6 = 29

11 = 22

20 = III.18

25 cousin of V.2

31 cousin of 30.

V. 2 cousin of IV.25

12 = 35

38 = VI.16

39 = VI.15

VI.15 and 16 = V.39 and 38

36 cousin of 35 & 37 and = VII.11,9,10.

VII. 6 and 7 = 14 and 15.

9, 11, 10 = VI. 35,36,37.

VIII.30 = IX.8

IX. 8 = VIII.30

42 = X.14.

X.4 = XI.17

12 and 13 = 58 and 59.

14 = IX.42

22 and 23 = 62 and 63

29 = XI.4

57 = XI.6

58 and 59 = 12 and 13

XI.1 = 16

4 = X.29

6 = X.57

16 = 1

17 = X.4

18 and 19 = 31 and 32

21 and 22 = 27 and 28

XII. 1 and 2 = 25 and 26

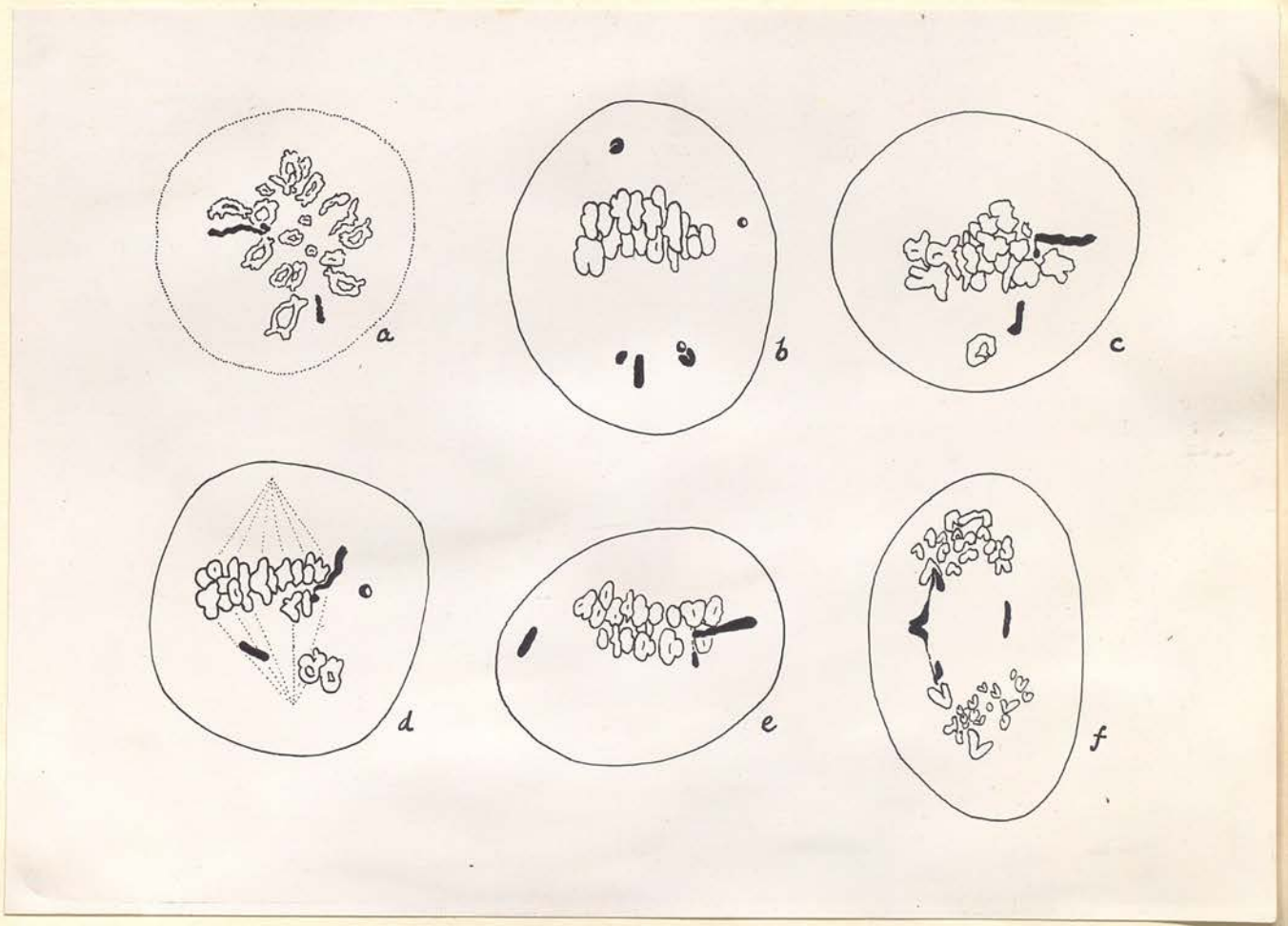


Fig. 1 — CHROMOSOME PICTURES OF ♂ RAT V. 2

DRAWN BY DR. P. CH. KOLLER.