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Pregnancy Maintenance in Hypophysectomized-Oöphorectomized Rats Injected with Estrone and Progesterone.*

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Pregnancy is quickly terminated in the rat if hypophysectomy is performed before day 11, but is not interrupted in the majority of cases if the operation is done after this time.^{1,2} Other experiments^{3,4} have made it seem likely that the pituitary is indispensable in the first half of pregnancy only because of its follicle- and interstitial-cell-stimulating hormones which in the proper ratio and dosage will cause estrogen secretion by the ovaries of an hypophysectomized rat, and the lactogenic hormone which stimulates the hypophysectomized rat's luteal tissue to secrete progesterone. Further support to this proposition has been given by the data submitted in this report.

Experimental. Rats of the Long-Evans strain, 60-70 days of age, in pro-oestrus were caged with males overnight and those showing vaginal sperm on the following morning (day 1) were hypophysectomized† and oöphorectomized (2 were also adrenalectomized) one week later (days 7-8). At the time of oöphorectomy, implantations were checked. All animals received 200 mg of

glucose intraperitoneally 4 times weekly for at least one week postoperatively and were provided with a wet as well as a dry diet. Hormonal injections were begun immediately after operations and continued until the day before autopsy. Estrone in 0.1 cc of sesame oil was injected subcutaneously in a daily dose of 1 μ g (10 I.U.) alone and with 2 daily levels (3 and 4 mg or I.U.) of progesterone, also given subcutaneously in 0.1 cc of sesame oil. Progesterone was also injected alone in daily doses of 3 and 4 mg. Three rats doubly-operated on day 9 received no hormonal treatment. The progressive growth or resorption of the uterine contents was checked daily by gentle palpation and records made of the nature of the vaginal smear. In a few instances where the uteri were palpably empty the rats were sacrificed before the time of normal term; otherwise necropsies were carried out from day 21 to 24. Rats 7 and 14 (see Table I) died at term after having been observed in unsuccessful labor. The other mothers were chloroformed and the young quickly taken from the uterus, stript of membranes, weighed and, if alive, usually given to a foster mother. Approximate figures for gestational span, size of litter and average birth weight for this colony are 22 days, 9 young and 5 g respectively.

Results. The results are listed in the accompanying table. Seven rats hypophysectomized and oöphorectomized on days 7 or 8 of pregnancy and injected daily with 1 μ g of estrone and 4 mg of progesterone carried from 3 to 9 living young to term and, in addition, were found to have from 1 to 6 fetuses in different stages of resorption. One of the 7 rats died on day 22 after having attempted to deliver herself of 6 term-size fetuses. All of the living fetuses seemed to be normal and equalled or surpassed in weight

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¹ Pencharz, R. I., and Long, J. A., *Science*, 1931, **74**, 206.

² *Id.*, *Am. J. Anat.*, 1933, **53**, 117.

³ Cutuly, E., *Endocrinology*, 1942, **31**, 13.

⁴ Lyons, Wm. R., Simpson, M. E., and Evans, H. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1943, **52**, 134.

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TABLE I.
Showing That Pregnancy May Be Maintained in Hypophysectomized-Oöphorectomized Rats by Injections of Estrone Plus Progesterone.

Daily hormonal dose								
Rat	Estrone	Progesterone	Day of Operation	Body wt on day of			Day of Autopsy	Uterine findings
	μg	mg		Mating	Operation	Autopsy		
1	1	4	8	165	204	246	K 23	9 L (48 g), 1 R
2	1	4	8	143	160	198	"	9 L (46 g), 1 R
3	1	4	8	159	184	230	"	5 L (34 g), 5 R
4	1	4	8	144	166	196	"	3 L (20 g), 6 R
5	1	4	7	141	164	210	22	7 L (40 g), 1 R
6	1	4	7	163	183	241	"	6 L (35 g), 2 R
7	1	4	7	153	182	225	D 22	6 RD (32 g), 2 R
8	1	3	7	160	180	214	K 21	7 L, 1 RD
9	1	3	7	180	213	260	"	8 L (35 g), 1 R
10	1	3	7	210	240	292	"	6 L (35 g), 1 RD
11	1	3	7	188	220	260	"	4 L (24 g), 2 RD
12	1	3	7	174	201	243	22	5 L (29 g), 3 R
13	1	3	7	160	191	234	"	6 L (42 g)
14	1	3	8	190	221	259	D 21	9 RD
15	1	3	7	180	212	235	K 22	2 RD, 5 R
16	1	3	7	206	233	198	23	10 R
17	1	3	7	176	189	170	"	No implants
18	0	3	7	178	187	177	"	" "
19	0	3	7	202	234	219	"	11 R
20	0	3	7	205	228	204	"	9 R
21	0	3	7	218	241	232	"	11 R
22	0	3	8	170	182	212	"	10 R
23	0	3	8	188	212	192	"	6 R
24	0	3	8	187	209	212	"	11 R
25	0	4	8	154	179	225	22	1 L, 2 RD, 6 R
26	0	4	8	144	162	186	"	10 R
27	0	4	8	175	192	206	"	8 R
28	0	4	9	172	201	207	"	9 R
29	1	0	9	169	194	151	15	No implants
30	1	0	9	218	236	207	"	" "
31	1	0	9	243	266	210	"	" "
32	0	0	9	170	185	164	24	" "
33	0	0	9	165	202	172	"	" "
34	0	0	9	246	273	220	"	" "

K = Killed. D = Died. L = Living fetuses.

R = Fetuses and placenta being resorbed. RD = Recently dead fetuses.

To the results tabulated may be added those obtained from experiments repeated to confirm old observations. Eight rats hypophysectomized and 4 oöphorectomized within the 6th to 9th days of pregnancy and uninjected all resorbed 24-72 hours post-operatively. Of new interest were 2 rats, hypophysectomized, oöphorectomized and adrenalectomized on the 7th day of pregnancy and injected, daily, with 1 μ g of estrone plus 3 mg of progesterone. One was sustained through day 25 and had 1 recently-dead, and 5 living fetuses and the other, gravid with 7 term-size fetuses, died on day 21. Similar results were obtained with 2 rats, hypophysectomized only on the day of insemination and injected daily with 1 μ g of estrone and 2 mg of purified lactogenic hormone. One was still gravid with 1 recently dead and 4 living fetuses when sacrificed on day 25, and the other died on day 22 without being able to deliver its 7 large fetuses.

the normal new-born of this colony. Some of these have been successfully nourished by foster mothers, but have lagged behind, in size and weight, the normal rats of this colony. Approximately the same results were obtained with the 10 rats similarly

operated and injected daily with 1 μ g of estrone and 3 mg of progesterone. Six of these had from 4 to 8 living young with one to 3 resorbing or recently dead fetuses; one died on day 21 after having attempted to deliver 9 term-size fetuses; 2 had resorbing

fetuses and one had an empty uterus when autopsied on days 22-23.

The 7 doubly-operated rats injected with 3 mg of progesterone and 4 injected with 4 mg of progesterone may be considered as one group since the results did not differ greatly. In only one of the 11 rats was pregnancy maintained until term, and that animal (No. 25) was gravid with one living, 2 recently-dead and 6 resorbing young when autopsied on day 22. With the exception of rat 18, which showed no implants at autopsy, all of the others still bore placentae in various stages of regression, and either no recognizable fetuses or autolysed vestiges. The 3 rats operated on day 9 and injected with 1 μ g of estrone promptly resorbed as did those similarly operated and uninjected.

Discussion. It is not assumed that the dosage and ratio of estrone and progesterone found adequate in maintaining pregnancy in this study will eventually prove optimal, but the measure of success attained with 10 I.U. of the former and 3 and 4 I.U. of the latter has seemed sufficient to warrant reporting at this time. The results may be said to clarify the role played by the pituitary in pregnancy, and to call attention once more to the placenta as an organ probably capable of carrying on the pituitary's gonadotrophic and the ovary's hysterotrophic function, thus making doubly certain that an ample supply of estrogen and progestogen will be available. That the pituitary's main role in the first half of pregnancy is to secrete 3 hormones (FSH, ICSH and lactogenic) which cause the ovaries to form daily equivalents of 1 μ g of estrone and 3-4 mg of progesterone is indicated by the finding that at least during the 7-11-day period when hypophysectomy causes resorption just as quickly as oöphorectomy, estrone-progesterone therapy has proven to be adequate substitution following the double operation. This is especially interesting in light of the emphasis recently placed on the so-called metabolic hormones of the pituitary which few workers would think of as functioning extensively through the mere production of estrogen and progestogen. Before declaring the pituitary hormones, other than the gonadotrophic

triad, completely unessential in pregnancy, a search should be made for these or related substances in the placenta,^{5,6} and the possibility still remains that the pituitary's contribution of even small amounts of these hormones might have been beneficial in increasing the number of living young carried to term in this experiment.

With new emphasis being given to the need of the pregnant rat for both estrogen and progestogen, the experiment of Haterius⁷ wherein he removed from pregnant rats the ovaries and all fetuses but one (leaving the placentae) and maintained pregnancy, might be interpreted as indicating the secretion of both of these hormones by the rat placentae. It is possible that rat 26, of the present series, injected with 4 mg of progesterone alone was able to maintain its one living fetus until day 22 because of estrogen formed by the placentae of this survivor and its eight unsuccessful mates. In the rats injected with progesterone, that hormone alone or in combination with placental estrogen and progestogen was capable of supporting some of the fetuses—other than the sole survivor—for at least a week after operation, judging by the amount of fetal residue observed in some of the rats at autopsy. The striking autopsy finding in many of these cases was the survival of large (though abnormal) placentae with scarcely a trace of fetal tissue. It seems as though progesterone alone may have been partially successful in maintaining the uterine contribution to the gestational apparatus and this is in keeping with the fact that alone it is able to support^{8,9} deciduomata in non-pregnant, oöphorectomized rats.

Summary. Levels of estrone and progesterone have been determined which, when given in combination to rats oöphorectomized and hypophysectomized on day 7 or 8 of pregnancy, will permit most of them to carry

⁵ Newton, W. H., *Physiol. Rev.*, 1938, **18**, 419.

⁶ *Id.*, *Sex and Internal Secretions*, Williams and Wilkins Co., Baltimore, 1939.

⁷ Haterius, H. O., *Am. J. Physiol.*, 1936, **114**, 399.

⁸ Astwood, E. B., *Endocrinology*, 1939, **1**, 49.

⁹ Rothchild, I., and Meyer, R. K., *Physiol. Zool.*, 1942, **15**, 216.

a slightly less than average number of apparently normal young through the usual gestational period. Of 7 rats given 1 μ g of estrone and 4 mg of progesterone daily, one died on day 22 following an attempt to deliver 6 large and 2 resorbing fetuses, and the other 6 when sacrificed on days 22 or 23 were found to have a total of 39 (3 to 9) living young and 16 (1 to 6) fetuses in various stages of resorption. Of the 10 rats given 1 μ g of estrone and 3 mg of progesterone, one died on day 21 without being able to deliver 9 term-size fetuses, 6 when sacrificed on days 21 or 22 were found to have a total of

36 (4 to 8) living young and 8 either recently dead or in various stages of resorption, and the remaining 3 when sacrificed on days 22 or 23 were found to have resorbing implants or no visible-implantation sites. It may be concluded, therefore, that 1 μ g of estrone and 4 mg of progesterone substituted for the ovaries and pituitary during the period of pregnancy between days 7 and 11, and for the ovaries alone from day 11 until term. The pituitary is only necessary during the first half of pregnancy in the rat, and then probably because of its gonadotrophic triad, FSH, ICSH and lactogenic hormone.

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Circulatory Effects from Cyclopropane Administered After Hemorrhage.*

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Clinical observations suggest that patients with recent extensive blood loss have an improvement of the circulation during cyclopropane anesthesia. With the onset of anesthesia, blood pressure and pulse pressure frequently increase and the pulse rate slows. These findings suggested laboratory observations under similar circumstances.

Method. Twenty-two experiments were performed on normal animals (18 dogs, 4 cats). No drugs other than cyclopropane, oxygen and a small amount of procaine were used. Arterial blood pressure determinations were made by cannulating a carotid or femoral artery after procaine infiltration. Hemorrhage was accomplished by direct rapid arterial bleeding until 25 to 30% of the estimated circulating blood volume had been withdrawn in an average of 5.7 minutes.

Cyclopropane was administered intermittently as a 30 to 50% mixture with oxygen, using the to and fro carbon dioxide absorption technic. Induction of anesthesia was

accomplished with a face mask, and maintained by using an endotracheal airway. Depth of anesthesia was determined by the character of the respirations and the activity of the eyelid and swallowing reflexes. When respirations became shallow or ceased in deep anesthesia, they were aided by rhythmic manual pressure on the rebreathing bag to eliminate the effects of hypoxia and hypercapnia. Observations were made in the 4 planes of surgical anesthesia. Cyclopropane administration was started at an average time of 29 minutes following the completion of bleeding and was maintained for an average period of 44 minutes.

In 13 dogs, venous blood samples were taken for hematocrit and plasma specific gravity determinations at 3 points in the experiment: (a) before bleeding (b) prior to the onset of anesthesia (c) at the completion of anesthesia. Hematocrit cell volume was measured in Sanforth-Magath tubes and plasma specific gravity was determined by the falling drop method of Barbour and Hamilton. Electrocardiograms were obtained

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