hours, while 35% gave it in 6 to 9 hours. (4) The detoxication of 1 g of borneol by the 26 subjects was almost complete in 15 hours, the average value at that time being about 96% of that obtained in 24 hours.

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Influence of Lactogenic Preparations on Production of Traumatic Placentoma in the Rat.*

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For several years we have been concerned with the problem of the hormonal factors involved in the establishment and maintenance of pregnancy, hoping ultimately to understand the pituitary factors necessary for nidation and maintenance of pregnancy in the hypophysectomized rat. For the present we have had to limit ourselves to an examination of the factors necessary for maintenance of pregnancy in the precociously matured rat and to an examination of the pituitary factors necessary for the production of *functional* corpora lutea, as judged by the ability to produce deciduomatous tissue in the traumatized endometrium. These studies on the effect of various fractions of the pituitary on placentoma formation will be reported elsewhere[1] *in extenso,* the present paper being limited to the effect of lactogenic preparations.

Evans and Long showed many years ago that the corpora lutea of the normal cyclic rat were non-functional and would not sustain placentomata unless the cycle was prolonged by stimulation of the cervix. They further showed that crude pituitary extracts would also prolong the cycle, and Teel showed that traumatic placentomata could be produced under the latter conditions. It is relatively easy to produce corpora lutea in the ovaries of immature rodents or of hypophysectomized rats with gonadotrophic agents but such corpora lutea like normally occurring cyclic corpora are usually non-functional as judged by the placentoma test.

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1 Evans, H. M., Simpson, M. E., Lyons, W. R., and Turpeinen, K., *Endocrinology,* in press.

Among all the purified pituitary fractions investigated in the last few years by the placentoma test, we have found that the lactogenic hormone is the only one which will lead to functional corpora lutea in hypophysectomized rats. In the earlier report[2] on this subject some doubt remained whether adrenocorticotrophic hormone contaminating the lactogenic preparation might be the effective factor. Since that time lactogenic hormone has been further purified and it elicits no adrenal response even when given in extremely high doses (50 mg doses in 10 days in hypophysectomized rats) so that this doubt has been minimized. It is true that several pituitary factors will, in high doses, and somewhat sporadically, lead to placentoma formation in normal rats, but this effect is probably indirect, through stimulation of the pituitary to secrete lactogenic hormone.

The placentoma test as utilized here is as follows: young mature female rats about 65 days of age, possessing newly-formed lutein tissue, are injected for 5 days with the pituitary substance under investigation. Threads are then inserted in the uterus on the day of the fifth injection, care being taken that the thread actually passes through the endometrium. Injections are continued for the next 5 days and the animals are sacrificed on day 11 of the experiment. In the case of hypophysectomized animals, injections are begun on the day of operation and the procedure is otherwise the same. Under the proper conditions discrete enlargements of the uterus occur at the point where the endometrium is injured. In the 6-day period of growth the enlargements attain a diameter of 0.5-0.6 cm. Microscopically they can be seen to be composed of tissue resembling the maternal part of the placenta.

Table I shows the reaction of hypophysectomized animals injected with lactogenic hormone containing approximately 30 International Units per mg. It will be noted that at the 1 mg level all animals

TABLE I.
Stimulation of Placentomata in Hypophysectomized Rats on Administration of Lactogenic Hormone.
Rats 65 Days Old at Hypophysectomy.

Total dose in 10 days		No. of rats	
mg	IU	Total	Positive
50	1500	4	4
10	300	4	4
5	150	4	2
0	0	13	0

2 Evans, H. M., Simpson, M. E., and Turpeinen, K., *Anat. Rec.*, Suppl. 3, 1938, **70**, 26.

responded; at 0.5 mg daily dosage approximately 50% were positive. The controls receiving no lactogenic hormone uniformly failed to respond to threads passed through the endometrium.

Immature animals, therefore smaller in body weight, in which corpora lutea had been artificially produced by injection of gonadotrophin (29-day-old rats injected subcutaneously with pituitary FSH, 15 RU total dose in 3 days) also formed placentomata around threads, if this treatment was followed by lactogenic hormone. Six rats given 0.5 mg of lactogenic hormone daily and 6 given 0.1 mg daily all showed placentomata, indicating that 0.1 mg was above the MED in this age group. None of the 6 controls, whose ovaries were luteinized, and in which threads were inserted, but which received no lactogenic hormone, responded by placentoma formation. As may be seen in Table II, adult hypophysectomized rats in which corpora lutea were induced by a gonadotrophic injection, also failed to nidate threads if not given supplementary therapy.

The corpora lutea present at hypophysectomy survive for a long period judging by histological appearance. They can, however, only be brought to functional condition by lactogenic hormone if injections are begun immediately after hypophysectomy. Table II summarizes one experiment which illustrates this point. When injections were begun immediately after operation, 1 mg daily doses were effective in 100% of the cases. When a period of 10 days or more—13 in this case—was allowed to elapse between operation and the onset of injection, no placentomata resulted. However, as the lower part of the table shows, when a new crop of corpora lutea was produced a considerable period after operation by the injection of gonadotrophic agents (in this case pituitary FSH (10 RU) and human chorionic gonadotrophin (1 RU) in 3 daily subcutaneous injections), and this priming was followed by lactogenic hormone,

TABLE II.
Influence of Post-operative Period on Stimulation of Placentomata in Hypophysectomized Rats by Lactogenic Hormone.
Rats 65 Days Old at Hypophysectomy.

Post-operative period in days				Total dose in 10 days		No. of rats	
Onset of injection	Threads inserted	Autopsy	No. of days injected	mg	IU	Total	Positive
0	4*	10	10	10	300	4	4
13	17	23	10	10	300	3	0
Corpus luteum formation induced by pituitary FSH and chorionic gonadotrophin, days 10 to 12 post-operative.							
13	17	23	10	10	300	3	3
—	17	23	—	—	—	7	0

*Day of 5th injection.

the newly formed corpora could be brought to functional condition and the animals would nidate threads. The last line of the table shows that these newly formed corpora lutea were unable to support placentomata without supplementary lactogenic hormone therapy.

It is possible to distinguish histologically those corpora lutea which are capable of supporting placentomata. They are large and are composed of large cells, in both respects reminding one of the corpora of pregnancy. The sparse, fat-laden interstitial tissue is also similar to that seen in pregnant rats.

It is of interest to note that the dose of lactogenic hormone found effective in producing placentomata (0.1 mg daily in immature rats and 0.5 mg daily in mature rats) is more than the minimal systemic dose necessary to stimulate the pigeon crop (0.02 mg given subcutaneously daily for 4 days) and less than that necessary to initiate lactation in the estrous guinea pig (1 mg given subcutaneously daily for 4 days).

The lactogenic hormone used is the purest pituitary product prepared in this laboratory, and although it is not crystalline, there is perhaps even stronger evidence for its purity. It has been shown to be homogeneous both by the electrophoretic test (Tiselius) and by solubility tests.[3] The assumption is made here, due to the degree of purity of the preparation, that it is the lactogenic hormone itself which is concerned in inducing the functional state of the corpus luteum. It is true that in certain other instances in the past very minute amounts of contamination in relatively purified hormonal preparations have proven to be the active portions of the product, *i. e.,* the sensitiveness of some biological tests equals or exceeds our best physical and chemical tests for purity.

Astwood[4] has recently published a paper also reporting a pituitary fraction (called "luteotrophin") which brings corpora lutea to a functional state. His test is somewhat different than that here described. Astwood's test consists in injecting 23-30-day-old rats with 2 IU chorionic gonadotrophin daily for 4 days. When estrus occurs, he injects 20 gamma estradiol dipropionate, followed by a maintenance dose of 10 gamma of the same material twice weekly. After 1-3 days of vaginal cornification, the vagina shows the mucification reaction indicative of active corpora lutea. The rats are then hypophysectomized and injected with "luteotrophin" twice daily for 4-14 days. If no estrus occurs in that period (that is, vaginal

[3] Li, C. H., Lyons, W. R., and Evans, H. M., *J. Gen. Physiol.*, 1940, **23**, 433; *J. Am. Chem. Soc.*, 1940, **62**, 2925; *J. Gen. Physiol.*, 1941, **24**, 303; *J. Biol. Chem.*, in press.

[4] Astwood, E. B., *Endocrinology*, 1941, **28**, 309.

mucification is sustained) he considers the test positive for "luteotrophin."

This test is, doubtless, thoroughly satisfactory for measuring lutein function and as far as can be judged, the minimum effective doses for the production of the phenomena described by Astwood and by this laboratory are essentially the same (0.1 mg or less). Although one of Astwood's methods for the preparation of "luteotrophin" is that described by Lyons for lactogenic hormone, he nevertheless has not identified this active factor with the lactogenic hormone, inasmuch as he designates it by a new term. His inability to cause the proliferation of mammary lobules in hypophysectomized rats with some of his luteotrophic preparations apparently forms the basis for his unwillingness to identify this factor with lactogenic hormone. However, as far as we are aware, no one has ever caused proliferation of mammary lobules in hypophysectomized rats with any amount of lactogenic hormone,† nor has any one used this as a method of identifying lactogenic hormone.

The series of responses described by Astwood can perhaps be explained and harmonized with the findings and explanations given here as follows. He luteinizes the immature rat ovary by injection of chorionic gonadotrophin. The injection of estradiol dipropionate then stimulates the pituitary to secrete a substance "luteotrophin" (identified by us with lactogenic hormone) which causes the lutein tissue to secrete progestin. This is indicated by vaginal mucification. After hypophysectomy, unless the luteotrophin is injected the lutein tissue no longer functions in maintaining the mucification, and vaginal cornification ensues from the continued injection of estrin. It is our experience that in the normal adult rat, daily injections of 0.5 mg of lactogenic hormone induce vaginal mucification and maintain it for long periods. It is assumed that here too, the lactogenic hormone is effective through stimulation of already existing lutein tissue to secrete progestin.

Summary. Normally occurring or artificially induced lutein tissue in the rat does not produce progestin, as shown by the placentoma test. The only pituitary preparation which has been shown to stimulate the production of progestin by such lutein tissue is lactogenic hormone. Therefore, besides its classical mammotrophic activity and crop-sac stimulating function, the "lactogenic hormone" is important in activation of the corpus luteum, and for this reason should be considered a part of the gonadotrophic complex.

† We have injected as high as 10 mg daily of purified lactogenic hormone into both normal mature and hypophysectomized female rats without stimulating the proliferation of mammary lobules.