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A Note on the Effect of Theelin, Theelol and the Luteinizing Substance on Reproduction.

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Past investigations have definitely shown that the prolonged administration or single massive doses of the female sex hormone exerts an inhibiting effect on the ovary. By means of ovarian transplants[1] and extracts[2] Haberlandt produced a lasting sterility in rabbits and guinea pigs. These results have been confirmed by Bondi and Neurath[3] and Reiprich,[4] who used ovarian transplants, and by Fellner[5] and Gostimirovic[6] with feminin and menformon-folliculin respectively. The effect on the ovary produced by high dosages of folliculin[7, 8, 9] has been duplicated with theelin by Doisy, Curtis and Collier,[10] who found in the immature rat a complete inhibition of growth of the ovary, degeneration of follicles and inhibition of the development of new follicles. Similar effects have been obtained by Hisaw, Leonard and Myers[11] upon injection of amniotin and by Kunde and coworkers[12] with the female sex hormone from pregnancy urine when administered to immature dogs.

The luteinization of the ovary by anterior lobe substance has been adequately demonstrated. Evans[13] found that the cessation of estrus in the normal rat resulting from the injection of large amounts of saline extracts of beef anterior pituitary glands was associated with a profound luteinization of the ovary and an embedding of the ova in the corpora lutea. This effect has been induced by other in-

[1] Haberlandt, L., *Pflug. Arch. f. des. ges. Physiol.*, 1922, **194**, 235.

[2] Haberlandt, L., *Pflug. Arch. f. des. ges. Physiol.*, 1923, **202**, 1.

[3] Bondi, Josef, and Neurath, Rudolf, *Wien. Klin. Wochschr.*, 1922, **35**, 520.

[4] Reiprich, W., *Arch. Gynakol.*, 1930, **141**, 27.

[5] Fellner, O., *Med. Klin.*, 1927, **40**, 767.

[6] Gostimirovic, D., *Biol. Z.*, 1929, **49**, 24.

[7] Golding, G. T., and Ramirez, R. T., *Endocrinol.*, 1928, **12**, 804.

[8] Mahnert, A., *Zentr. f. Gynakol.*, 1930, **54**, 2883.

[9] Hauptstein, P., *Endokrinol.*, 1931, **8**, 169.

[10] Doisy, E. A., Curtis, J. M., Collier, W. D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 885.

[11] Hisaw, F. L., Leonard, S. L., Meyer, R. K., *Endocrinol.*, 1931, **15**, 17.

[12] Kunde, M. M., D'Amour, F. E., Gustavson, R. G., and Carlson, A. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 122.

[13] Evans, H., Harvey Lectures, 19, 1924.

vestigators.[14, 15, 16, 17, 18, 19, 20] Similar results have been obtained with extracts of pregnancy urine. Thus the injection of Prolan B into adult mice is reported by Zondek[20] to have caused cessation of the estrus cycle and luteinization of the ovaries. Since Prolan B causes all follicles to become luteinized, Zondek believes that ovulation is inhibited and hence a veritable sterilization is produced. In line with this, Engle[21] found that the treatment of immature mice with pregnancy urine resulted in atresia of the follicles and prevention of ovulation by the transformation of the follicle into a corpus luteum with retained ovum. That a sterility is actually induced has been shown very recently by Mandelstamm.[22]

Experimental. Although the effect of these substances upon the ovary seems to be quite amply established, it seemed desirable to determine the permanency of the inhibition produced by daily administration of the crystalline hormones theelin and theelol and also of a purified preparation of the luteinizing substance of pregnancy urine. Four groups of 5 healthy, 120-day-old, female rats with normal cycles were injected for 25 days. The first group was injected daily with 50 mouse units of the luteinizing substance, the second group with 6 spayed rat units[23] of theelol, the third with 6 immature rat units[23] of theelol, and the fourth group with 6 rat units of theelin. Smears were made daily throughout the course of the experiment. The animals remained in good health during the entire period of treatment.

The rats which received the luteinizing extracts were in a continuous state of estrus for the first 5 to 7 days of the treatment, whereupon estrus was in all cases completely inhibited. After this inhibition, which persisted for 5 to 10 days following the cessation of the injections, the reestablished cycles were of greater than normal length due to a long diestrus interval. The animals receiving theelin and theelol stayed in continuous estrus throughout or evidenced very long cycles with extremely short dioestrus intervals.

[14] Parkes, A. S., *Proc. Roy. Soc.*, 1929, **B104**, 171.
[15] Zondek, B., and Aschheim, S., *Arch. f. Gyn.*, 1927, **130**, 1.
[16] Smith, P. E., and Engle, *Am. J. Anat.*, 1927, **40**, 159.
[17] Fels, E., *Wien. Klin. Wochen.*, 1928, **41**.
[18] Loewe, S., *Endokrinol.*, 1928, **1**, 323.
[19] Siegmund, H., *Zentr. f. Gyn.*, 1928, **52**, 1189.
[20] Zondek, B., *Die Hormone des ovariums und des Hypophysenvorderlappens*, 174.
[21] Engle, E. T., *J. Am. Med. Assn.*, 1929, **93**, 276.
[22] Mandelstamm, A., *Zentralbl. f. Gyn.*, 1931, **55**, 3004.
[23] Curtis, Jack M., Doisy, E. A., *J. Biol. Chem.*, 1931, **91**, 647.

The cycles returned to their normal rhythm promptly after the injections were stopped.

At the end of the 25-day injection period, 2 animals from each group were sacrificed for observations of the genital tract. (Table I.) The ovaries of the group injected with the luteinizing principle

TABLE I.

Rat No.	Total Amt. Injected	Period of Injection, Days	Weight of both ovaries, mg.	Follicles	Corpora Lutea	Degree—enlargement of uterus
			Luteinizing Hormone (Mouse Units)			
38	1250	25	372	10 (large to medium)	100-150	enormous (gravid type)
40	1250	25	263	"	"	"
			Theelol (Spayed rat unit = 0.68γ)			
43	150	25	41.1	12 (small)	15	moderate
44	150	25	67.8	10 "	20	"
			Theelol (Immature rat unit = 0.16γ)			
47	150	25	74.4	5 (small)	20	"
51	150	25	43.7	3 "	15	"
			Theelin (Rat unit = 0.33γ)			
52	150	25	60.3	a few (small)	25	"
53	150	25	50.4	"	20	"

were enormously enlarged (5 and 7 times normal), the bulk of the weight obviously was made of lutein tissue although several follicles, varying in size from large to medium, were present in each case. The uteri were livid and very much enlarged, giving the appearance of pregnancy. This excessive luteinization apparently persists for some time for, upon examining the ovaries of rats 37 and 41 twenty-five days after the injections had been stopped and after the appearance of 3 and 2 cycles respectively, it was found that the luteinization was still pronounced although there was evidence of lutein degeneration. In rat 37 no follicles were in evidence while in rat 41 a few very small follicles were observed. When the ovaries of rat 41 were again inspected 40 days after the cessation of injections their appearance was very much the same as on the first examination except for a more marked degeneration of the corpora lutea.

There was little difference in the appearance of the genital tracts of the rats receiving theelol and those receiving theelin. The weight of the ovaries was very nearly normal even though a considerable number of very small and highly congested corpora lutea were present in all cases. The ovaries of the rats receiving the higher dosage of theelol had a greater number of follicles but in all cases their size

was exceedingly small. The uteri were only moderately enlarged.

A recovery period of 29 days was allowed before the males were admitted to the females. Copulation was determined by observing sperm in the vagina and the beginning of pregnancy was taken as the date when such a finding was made. This was confirmed by the absence of further estrus and also by the placental sign on the thirteenth day of pregnancy. The number of males and females in each litter was observed; also the age at which canalization of the vagina took place. The results are recorded in Table II.

TABLE II.

Rat No.	Total Amount Injected	Period of Treatment. Days	Recuperation Period Allowed. Days	Elapsed Time before Copulation Took Place. Days	Length of Gestation Period. Days	Number in Litter	Number Survived	Number of ♀	Number of ♂	Age at Which Vagina Opened Days
Luteinizing Hormone (Mouse Units)										
37	1250	25	29	23	22	2	0	—	—	
39	1250	25	29	28	21	6	6	5	1	46-54-54-55-55
41	1250	25	45	4*	21	8	8	2	6	39-42
Theelol (Spayed rat unit = 0.68γ†)										
42	150	25	29	17	22	10	7	3	4	45-47-49
45	150	25	29	4	22	9	9	3	6	37-39-41
46	150	25	29	9	22	7	7	3	4	42-42-50
Theelol (Immature rat unit = 0.16γ)										
48	150	25	29	4	22	9	9	1	8	39
49	150	25	29	1	22	8	8	4	4	37-38-38-37
50	150	25	29	1	21	8	8	5	3	37-38-38-40-41
Theelin (Rat unit = 0.33γ)										
54	150	25	29	12	22	10	9	1	8	38
55	150	25	29	9	22	11	10	7	3	37-38-38-40-42-45-47
56	150	25	29	9	22	9	9	7	2	43-43-44-44-45-45-45

* Mated 16 days later than No. 37 and No. 39.
† γ —0.001 mg.

It will be observed that, with the exception of the rats receiving the smaller dosage of theelol, 29 days were not sufficient for complete recovery from the effect of the injections for they did not receive the male even though estrus occurred normally as indicated by the smear technique. The animals receiving the luteinizing substance recovered more slowly than did those injected with theelin and theelol. The length of the gestation period in each case was normal.

The young were normal and healthy and the survival was very good. The limited number of animals used in this experiment does not permit any conclusion regarding the ratio of males to females

in the litters. Canalization of the vagina took place at about the same age as it does with the young stock females of our colony.

Discussion. While a greater number of animals treated with both larger and smaller quantities of these hormones for longer and shorter periods of time is desirable, we believe that our data justify certain conclusions. It seems quite definite that the changes evoked by the administration of theelin, theelol and the luteinizing substance do not permanently affect the normal ovarian function. The fact that those animals receiving the smaller amount of theelol mated immediately upon introduction of the males might indicate that a sterilization was induced in the other 3 groups. However, since we have not determined the average length of time that untreated females remain with the male before fertilization takes place, such a conclusion may be unjustified.

Summary. Prolonged administration of either theelin or theelol to adult female rats does not destroy the reproductive function, as is evidenced by their ability to bear and rear normal litters. The treatment with the luteinizing substance from pregnancy urine produces complete inhibition of estrus together with profound ovarian luteinization but does not permanently impair the reproductive mechanism.

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On the Classification of Cells According to Their Inorganic Structure in vitro.*

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One of the greatest difficulties in carrying out any problem *in vitro* is that of establishing a morphological criterion by which the various cell elements composing the growth can readily be classified. When dealing with pure culture strains of "Fibroblasts" and epithelial cells it is a comparatively simple matter to distinguish between the 2 cell types, as they not only exhibit distinct differences in their morphology, but also in their mode of growth. But when

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