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Effect of Swingle's Extract upon Lactation in the Adrenalectomized White Rat.

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Six lactating animals of 200 gm. average weight were included in this group. Three females one day post partum were etherized and kept under normal conditions as controls. One female 4 days, and 2 females 7 days post partum were adrenalectomized. In these mothers the milk supply had developed and the young were thriving. Beginning the day of operation, the 3 animals operated upon received one cc. of Swingle's cortical extract subcutaneously, each cc. being equivalent to 40 gm. of beef cortex. In the control group the milk supply developed normally and the nourishment of the young was without variation from normal limits. In each of the other 3 there was a noticeable decrease in the supply of milk the second day after operation and lactation ceased on the third. The 4-day-old young died on the fourth day after the operation on the mother, and the young 7 days old were moribund on the fourth day, when they were transferred to other mothers for nourishment. The action of this group parallels that of the animals in Experiment II, where the milk supply failed the second to the fourth day with death of the young from the third to the fifth days post partum.

Conclusion. The substance necessary to maintain lactation in the white rat is absent in cortical extract made after the method of Swingle.

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Effects of Adrenal Cortical Extracts upon the Sex Organs of Animals.

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I. *Effect of Swingle extract on maturity in rats.* In view of the known effect of certain adrenal tumors, and possibly hyperplasias, in producing sexual precocity in children, and because of the general impression that mature rats were less susceptible to sarcoma than

younger animals, an attempt was made to produce precocious maturity in rats, with the intention of then testing their relative susceptibility to tumors. One of the methods used was to inject young rats with adrenal cortical extract, prepared after the manner of Swingle and Pfiffner.¹ Four litters of rats were used, half of which were injected daily with extract in doses amounting to 20 gm. of adrenal cortex to 200 gm. of rat weight. The extract was freshly prepared every 10 to 12 days, and was tested on adrenalectomized rats to insure its potency.

Corey and Britton² have reported changes in the ovaries and testes of rats following similar treatment, which they interpreted as a stimulation to sexual maturity. The present groups were watched carefully for signs of maturity (the opening of the vagina and vaginal smears in the females; the behavior of the males in the presence of females in oestrus), and it was found that the controls matured as early, and in some cases earlier, than the injected animals. (Table I.)

TABLE I.

Litter	Age at Start	Weight at Start average	No. Injections average	Weight at Maturity average	Age at Maturity
	Rats	Days	gm.	gm.	Days
T	3	19	17.2	90	117
C	3	19	17.0	92	114
T	4	40	25.0	85.2	135.1
C	3	40	29.6	117.0	143.0
T	4	60	51.6	111.3	104.3
C	3	60	63.3	125.0	123.0
T	4	80	107.0	141.2	114.2
C	4	80	119.5	149.2	116.2
Treated	15				118.0
Controls	13				124.0

T = Treated; C = Controls.

II. *Effect of Swingle extract on egg-laying in chickens.* In May, 1930, I reported a degeneration of the ovaries of chickens following the injection of a watery extract of adrenal cortex.³ It was desirable to know whether Swingle and Pfiffner's lipid extract acted in the same way. Five laying hens were injected every other day with 1 cc. of Swingle extract. These chickens were followed for 49 days, receiving 25 injections. The hens continued to lay regularly, producing on an average of 21 eggs in this period. This is in marked contrast to the experiment reported in which watery extract

¹ Swingle, W. W., and Pfiffner, J. J., *Am. J. Physiol.*, 1931, **96**, 153, 164.

² Corey, E. L., and Britton, S. W., *Science*, 1931, **74**, 101.

³ Connor, C. L., *Arch. Path.*, 1930, **9**, 1296.

was used. This quite constantly produced degeneration of the ovaries with immediate cessation of ovulation.

III. *Effect of various extracts on the oestrus cycle in rats.* Watery extracts of adrenal cortex were injected in comparatively large doses into 13 female rats which were having oestrus cycles at regular intervals. From 10 to 15 daily injections of 2 cc. each were given. This was made by macerating adrenal cortex from beeves in an equal amount of Ringer's solution and filtering the liquid through a Seitz filter. It was always used fresh. With the exception of one rat, this treatment caused the oestrus cycles to cease. In this one rat (Rat 4, Table II), the dioestrus period was considerably prolonged. In 6 rats (8, 9, 10, 11, 12, 13) injections were stopped,

TABLE II.
The Oestrus Cycle of Rats Following the Injection of Various Substances.

A. Ringer's solution extract of adrenal cortex, 2.0 cc. daily, 10-15 days.	
Rat	
1.	---ooo---oo---oo---oIo-----C-----
2.	---oo---ooo---oo---ooI-----C-----oo-----o
3.	oo---oo-Ioo---oo-----C-----
4.	---oo---oI---oo---o---C---o---oo---oo
5.	o---oo-I---oo-----C-----oo---
6.	---oo---I---oo-----C-----oo-
7.	---oo---Ioo-----C-----
8.	---Ioo---C-----oIo---oo---C-----
9.	---oIo---C-----oIo-----C-----o
10.	---I-----C-----oIo---oo---C-----o
11.	oooI-----C-----oIo-----C---o-----
12.	oI---oo---C-----oI-----C-----oo-
13.	-Ioo---oo---C-----oIo-----oo---C-----oo-
14.	---oo---ICo---oo---oo---oo---o
15.	---oo---oo-IC---oo---oo---ooo
16.	---oo---ooIC---oo---oo---ooo
17.	oo---oo---ICoo---ooo---ooo
B. Protein (veal broth), 2.0 cc. daily for nine days.	
18.	---oo---Ioo---oo---C---oo---oo
C. Adrenalin (1.0 cc., 1-100,000 sol. 5 days; 1-50,000, 7 days).	
19.	oo---ooI---ooX-----oC
20.	---oo---I---Xooo---oC
21.	---ooI---X-----ooC
22.	---oo---I---X-----C
23.	---oo---I---ooX---oo---oC
24.	---oo---I---ooX---oo---C
25.	---oo---I---X----- (died, pneumonia)
26.	---ooo---I---oo---X---ooo---C
27.	---oo---I---ooX---ooo---C
Swingle Extract (1 cc. daily for 7 doses)	
28.	---oo---oo---I---oo---Coo---oo---
29.	---oo---oooI---oo---C---ooo---ooo
30.	---ooo---I---oo---C---oo---ooo
31.	---oo---I---oo---oo---oo---

In the above table, o means oestrus, dash (-) means dioestrus; I means injections started; C means injections ceased; X indicates when the dose of adrenalin was doubled; IC—only one injection was given to this group.

and after an average interval of 13 days, the cycle was resumed. Further injections caused a second cessation of oestrus which persisted until from 4 to 10 days after the last injection. Four rats (14, 15, 16, 17) were given only 1 dose (2 cc.) and no effect was produced. Rat 18 was injected repeatedly with 2 cc. of veal broth without affecting its cycle. Nine rats (19, 20, 21, 22, 23, 24, 25, 26, 27) were given 5 injections of 1 cc. each of a 1-100,000 solution of adrenalin. Then the dose was doubled for 8 daily injections. This caused no effect in 6 rats. In one, oestrus did not take place. Another rat had no cycle. This one died on the 13th day of pneumonia. Four rats (28, 29, 30, 31) were injected daily for 7 days with 1 cc. (representing 30 gm. of adrenal cortex) of an extract made after Swingle's method. This caused no change in the sex cycle.

From these experiments we can conclude that the adrenal cortex contains a substance which inhibits the activity of the gonads of mature animals and that, while the lipoid extract of Swingle contains the principle necessary to maintain life, it does not contain the sex-affecting portion of the adrenal gland.

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A Method for Studying the Microscopic Structure of Bacterial Colonies.

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The study of bacterial colonies has in recent years been stimulated by investigations of microbic dissociation. The existence of variant strains, variously developed, which differ in pathogenicity, in biochemical reactions, in antigenic structure, and in colonial characteristics can no longer be doubted. Furthermore, almost no attempts have been made to explain the mechanism whereby bacterial cells pile up to produce colonies varying widely in gross morphology. The relation of pleomorphic bacteria, and of organisms having abnormal staining characteristics, to the development of cells into masses containing tremendous numbers and representing a considerable number of generations is largely unknown. Technical difficulties are responsible for many of these omissions. The ordinary