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I hereby recommend that the thesis prepared under my supervision by Charles Parry Kraatz entitled A possible role of the eosinophile leucocytes in the endocrine complex of the female rat. be accepted as fulfilling this part of the requirements for the degree of Doctor of Philosophy

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A POSSIBLE ROLE OF THE EOSINOPHIL LEUCOCYTES IN THE ENDOCRINE COMPLEX OF THE FEMALE RAT

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by

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The experimental production of pseudopregnancy in white rats has been accomplished in two general ways: (a) Single stimulation of the cervix of the uterus, this, by initiating events leading to follicular inhibition and luteal activity, producing the typical early gestational picture in the uterus; (b) Repeated hormone administration, effecting the ovarian and uterine changes presumably by a more direct action.

Goat anti-rat thymus serum, non-specific and generally hematotoxic (Goldman and Kraatz, unpublished), injected intraperitoneally into 14 adult virgin female rats in doses of $\frac{1}{4}$ to $\frac{1}{2}$ cc. daily, resulted in 10 pseudopregnancies. The uteri of three of these pseudopregnant females were traumatized by the insertion of threads and responded with formation of macroscopic placentomata. All rats receiving doses of $\frac{1}{2}$ cc. and the smaller rats receiving $\frac{1}{4}$ cc. doses became pseudopregnant after having one or two normal cycles; while daily injections of 1 cc. of normal goat serum continued for three weeks did not affect the cycles.

The possibility that the gonadotropic hormone content of the immune serum was responsible for the effects described is doubtful since it was found that the serum had no influence on the ovaries or the maturing of young females. Furthermore, single intraperitoneal injections in 47 animals of $1\frac{1}{2}$ to 3 cc., given at procestrus or early oestrus, resulted in 27 pseudopregnancies, nine being verified by securing positive decidual reactions. The failures may be attributed to the fact that this is apparently a "threshold reaction". Successive bleedings of the goat produced sera of varying titer and, where $1\frac{1}{2}$ cc. of a given lot would fail to evoke pseudopregnancy, 2 cc. of the same lot would

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succeed. The pseudopregnancies uninterrupted by autopsy ranged from 12 to 17 days in length with an average of 13.81 days. Neither normal goat serum nor goat anti-rat liver serum in single injections of 3 cc. affected the cycles. Occasionally, slight weight losses were noted following injections, the maximum being 4.9% of body weight in a rat injected daily, but recovery of initial weight was always completed after five days from the first injection and the animals gained from that point.

Similar doses of the anti-thymus serum in late oestrus or post-oestrus lengthened the following dioestrous period to 3 or 4 days, while single injections during dioestrus produced a continued full oestrus of 3 or more days in 8 out of 14 animals. The ovaries of two of these when sectioned showed follicular cysts.

Thus, an anti-thymus serum, primarily affecting blood cells in the adult rat, was found to produce a heavily luteinized ovary by a single injection during prooestrus, and, in some cases, a cystic ovary by a single injection in dioestrus. It appeared probable that the prooestrus injections initiated a chain of events comparable to those producing normal pseudopregnancy.

The most constant effect of serum treatment was eosinophilia. Animals injected daily were found to have eosinophil leucocytes in the circulation during pseudopregnancy ranging from 6.5 to 11.5% of the total white count, while controls receiving larger amounts of normal serum for the same length of time had 3% or less. Single injections resulted in a slight eosinophilia within 24 hours, the condition becoming pronounced in 48 to 72 hours and persisting for at least

4 days (Table 1).

The only generally accepted belief regarding the eosinophil leucocytes is their proliferative response to foreign proteins in the circulation. Daily leucocyte counts were made on female rats of various ages and stages of sexual activity. Differential counts of 500 white cells each (1000 in immature animals) were made in a longitudinal direction on Wright-stained smears. The product of the eosinophil percentage and the white cell count for that day resulted in a figure representing the number of eosinophil cells per cubic millimeter of blood. Averages of these figures, based on differential counts of 127,500 cells, are used in Table 1 and Figure 1.

(Insert Table 1 and Figure 1)

Only the counts for regular 4-day oestrous cycles are included in the figures for normal adult females in table 1. Additional counts of slightly aberrant cycles showed the lowest eosinophil number sometimes before prooestrus in the case of an abbreviated oestrus, or after prooestrus in the case of lengthened oestrus, but a rise always occurred following oestrus. Individual animals maintained levels of eosinophil fluctuation relatively constant in successive cycles, but differing from the levels of other individuals. In general, the level rises with increase in age. Thus, the rats in the pseudopregnancy determinations, being young, showed lower levels in the normal cycles than the average.

Excepting the constant oestrous condition, the significant differences in the eosinophil number under various conditions parallel the probable variations in luteinizing hormone liberated from the anterior lobe of the pituitary gland under those conditions: i.e., low in immature animals, with a sudden rise at maturity; a rise during oestrus, contributing during this rise to the process of ovulation as suggested by Hisaw, Fevold, Foster and Hellbaum (1934); a rise during pseudopregnancy resulting in the formation of functional corpora lutea. Regarding constant oestrus, divergent opinions exist. Parabiosis experiments lead DuShane, Levine, Pfeiffer and Witschi (1935) to believe that the condition is due to lack of luteinizing hormone. On the other hand, Smith and Engle (1934) find ovarian cysts resulting from implantations of anterior lobe containing probably both follicular stimulating and luteinizing hormones, while injections of castrate urine containing only the follicular stimulating factor do not produce this effect. A high, relatively static balance of hormonal influence on the ovary as the causative factor in continued oestrus is indicated by the results of both groups. In the present experiments, the production of pseudopregnancy and cystic ovaries by a common treatment and the high eosinophil number in constant oestrus subscribe to this concept.

The observations above led to attempts to assay the eosinophil leucocytes of the female rat qualitatively for luteinizing hormone. Isolation of these cells has never been accomplished, but white cell suspensions of relatively high and low eosinophil content were compared in respect to their power to augment the action of follicular stimulating hormone in immature female rats. Suspensions of high eosinophil concentration were obtained from the citrated blood of pregnant, serum-injected or constant-oestrous rats by repeated centrifuging and washing three times with physiological saline solution.

Low concentrations were similarly obtained from non-pregnant and saline-injected adults. Greater eosinophil concentration was sometimes obtained by the use of hypotonic (0.75%) saline in washing, while hypertonic (0.9%) solution tended to have an opposite effect.

The follicular stimulating hormone used was a 60% alcohol-soluble fraction of an aqueous pyridine extract of acetone-dried pig anterior lobe, prepared after the method of Fevold et al. (1933).

Subcutaneous injections of follicular stimulating hormone-plus-white cells were made twice daily into 22-day old rats for three days, the animals being autopsied on the fifth day. Ovaries were dissected free and weighed. There was considerable variability in the response of different litters to the control doses of like amounts of hormone, but, within each litter, the results were fairly constant. $ZnSO_4$, augmentative in action (Maxwell, 1934), injected in doses of 10 mg. in combination with follicular stimulating hormone into 4 rats from 3 different litters, although resulting in ovaries which ranged from 66 to 118 mg. in weight, showed fairly uniform augmentation percentages of 433, 464, 500 and 503 over littermate control ovaries. Therefore the percent of increase in ovarian weight over littermate hormone-injected controls rather than gross ovarian weight has been used in Table 2. The 12 controls used had a range of 12.4 to 22.6 mg. in ovarian weight with an average of 18.02.

(Insert Table 2)

The response is apparently proportional to the number of eosinophil leucocytes injected. Group 1, receiving on the average 319% more eosinophils than group 2, demonstrated a 274% greater ovarian response, while receiving 53% more white blood cells and 6% more total blood cellular material. The small difference in number of white cells injected can hardly be responsible for the increased augmentation in group 1, since ovarian response does not increase proportionally as the amount of a non-hormonal substance administered is increased, e. g. casein (Saunders and Cole, 1936). Evans, Simpson and Austin (1933) achieved augmentation with 3 cc. doses of rat blood serum, greater from non-pregnant than pregnant females. Eosinophilia, provoked by blood-group antagonisms, is indicated by these results, especially since pregnant blood is believed to be higher in luteinizing hormone content than non-pregnant. Blood-group reactions, too, are probably responsible for the wide ranges shown in Table 2, but this effect should be common to both groups, 1 and 2. Therefore, the difference in response is probably due to a difference in content of luteinizing hormone.

A number of non-specific substances have displayed augmenting properties when administered to rats in conjunction with purified or unfractionated pituitary extracts. Extracts of male urine, milk, egg white, lemon, horse thyroid and beef liver (Hellbaum, 1936); various blood sera and urines (Evans et al., 1933); formed elements of beef blood (Casida, 1936); casein and egg albumen (Saunders and Cole, 1936); serum from non-pregnant mares (Cole and Hart, 1934); tannic acid (Fevold et al, 1933); and $ZnSO_4$ (Maxwell, 1934) have proven effective in evoking this phenomenon. The tentative explanation, a delayed absorption of follicular stimulating hormone, may account for a portion of the augmentation, but, in view of the huge increases observed in non-hypophysectomized animals in response to non-hormonal substances, these have probably initiated events resulting in a steady liberation of luteinizing hormone from the living pituitary.

As shown by Lane and Hisaw (1934), the pituitaries of immature rats can be provoked to liberate luteinizing hormone by uterine cervical stimulation and by administration of oestrin. It seems believable that the unifying mechanism of the action of various injected substances may be a stimulation to eosinophilia, these cells providing an increased amount of luteinizing hormone in the circulation. An eosinophilia, persisting for some time after treatment, is expected in the case of foreign protein injections. Cold, Guilbert and Goss (1932) found gonadotropic mare serum with a high protein content as effective in a single dose as in divided doses, suggesting again the initiation of some mechanism such as that proposed, rather than direct hormonal action. The failure of $ZnSO_4$ and casein in combination to act additively in augmentation (Saunders and Cole, 1936) points to the identity of method of their action. The common pharmacological action of $ZnSO_4$ and tannic acid in the precipitation of proteins hints at the same mechanism. Preliminary experiments in this laboratory indicate a rise in eosinophils after injections of $ZnSO_4$ either alone or in combination with follicular stimulating hormone into immature females, while injections of hormone alone tend to lower the eosinophil number.

In view of the evidence presented, it seems credible that the concentration of eosinophil leucocytes in the circulation of the female rat may be an indication of luteinizing activity; and further, that the cells themselves may find a role in the control and transport of the luteinizing hormone.

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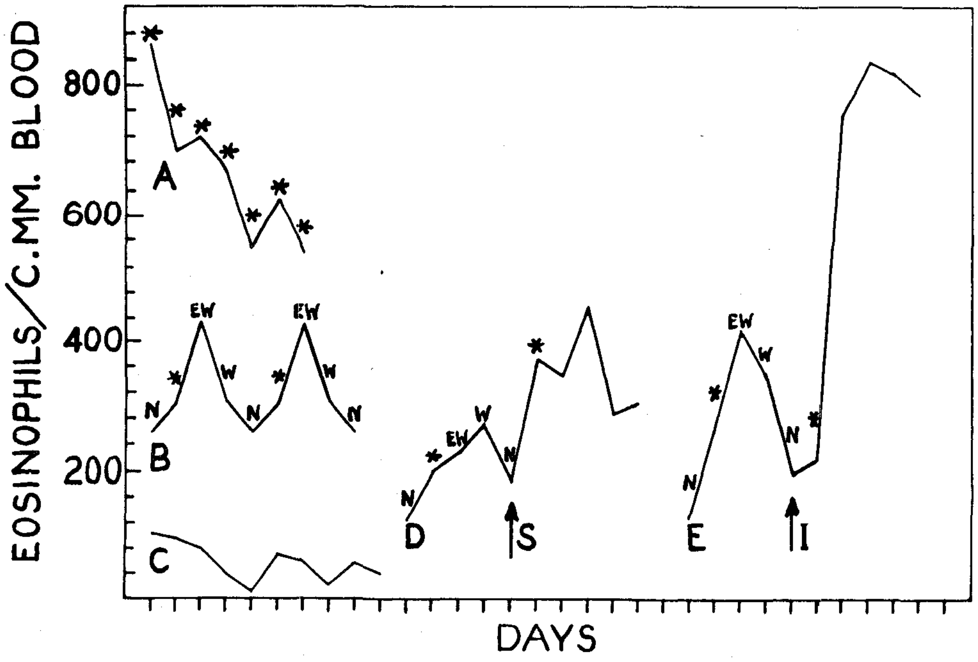

Fig. 1. Graph of figures in Table 1, showing daily variations in concentration of eosinophil leucocytes under differing conditions. A, rats in constant oestrus. B, rats experiencing normal cycles. C, immature rats. D, pseudopregnancy induced by sterile copulation at (arrow) S. E, pseudopregnancy induced by serum injection at (arrow) I. Stages of cycle indicated by symbols: N - prooestrus; * - oestrus; EW - postoestrus; W - dioestrus.

TABLE 1.

Condition	Smear (vaginal)	Eosinophil %	Eosinophils per c.mm.
Normal cycles (21 cycles in 15 rats)	Prooestrus	1.705	257±31.8
	Oestrus	2.015	307
	Postoestrus	2.5	432±28.2
	Dioestrus	1.88	305
Normal pseudopregnancy with preceding cycle (7 rats - see Note)	Prooestrus	0.95	125
	Oestrus	1.34	206
	Postoestrus	1.6	233
	Dioestrus	1.64	270
	Prooestrus	1.5	188
	(Sterile copulation)		
	Oestrus	2.26	373
	Pseudopregnancy (2)	2.34	346
	Pseudopregnancy (3)	2.16	457
	Pseudopregnancy (4)	1.7	285
	Pseudopregnancy (5)	2.4	306
Serum pseudopregnancy with preceding cycle (4 rats - see Note)	Prooestrus	0.9	127
	Oestrus	1.73	266
	Postoestrus	1.8	423
	Dioestrus	1.86	352
	Prooestrus	1.5	197
	(Injection)		
	Oestrus	1.775	217
	Pseudopregnancy (2)	3.55	755
	Pseudopregnancy (3)	4.0	841
	Pseudopregnancy (4)	2.7	822
	Pseudopregnancy (5)	3.3	786
Immature females (10 rats)	None	0.59	59± 6.6
Adults with cystic ovaries (3 rats - 7 days each)	Oestrus (constant)	4.41	666±44.4
50-day old littermates			
2 mature - 5 days each	Cycles	1.16	240±26.6
2 immature - 5 days each	None (closed)	0.18	45± 9.7

NORMAL CYCLES: Difference between prooestrus and postoestrus 175±45.1

NORMAL PSEUDOPREGNANCY: Average of 5 days before stimulation 200±15.3
Average of 5 days after stimulation 347±17.6
Difference 147±23.3

TABLE 1 (continued).

SERUM PSEUDOPREGNANCY:	Average of 5 days before injection	305±25.4
	Average of 5 days after injection	705±21.0
	Difference	400±33.0

Note: In the pseudopregnancy determinations, the animals being young show, in general, lower levels of eosinophil fluctuation in their normal cycles than those in the averages for all ages given above.

TABLE 2.

	Hormone plus	Animals	A.	B.	C.	% Augmentation Range	% Augmentation Average
1.	Over 1 million E.	11	50.85	.177cc.	2.154±.223	47-246	148.2±16.1
2.	Under 1 million E.	9	33.2	.167cc.	0.513±.053	0-63	39.6± 5.46
	Difference				1.641±.23		108.6±17.0

A. Average of millions of white cells received by each animal.

B. Average volume of blood elements (packed by centrifuging) received by each animal.

C. Average of millions of eosinophil cells received by each animal.