Prostigmin on the Ovaries and Uteri of Albino Rats.* (17414)

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Prostigmin is a parasympathomimetic drug that exerts some of its effects through its ability to inhibit cholinesterase and enhance the action of acetylcholine. Soskin, Wachtel, and Hechter[1] showed that prostigmin will start the menstrual flow in the non-pregnant, but would have no effect in the pregnant human. On this basis they proposed its use as a pregnancy test. This idea had its beginning in 1939 when Reynolds[2] reported that estrogens caused a transient increase in the amount of acetycholine in the uterus of rats, and that the uterus thereby becomes hyperemic. Since that time several authors[3-9] have reported using prostigmin as a valuable test for pregnancy and treatment of 'nervous' amenorrhea in the absence of organic dysfunction. In a subsequent paper Taubenhaus and Soskin[10] attempted to explain this effect of prostigmin from a study of the hypothalamic region and the release of luteinizing hormone. They offered evidence that prostigmin applied directly to the pituitary will cause a release of LH and a transient corpus luteum formation.

Since this drug is now commonly used it seemed desirable to know more about its action on the reproductive system of the female. The present report deals with such a study in albino rats.

Procedure and results. Female rats of the Wistar strain were used. Prostigmin was injected twice daily. The daily dose for all small rats was .04 mg and for mature females 0.1 mg given in physiological salt solution in dilution of 0.1 mg of prostigmin per cc. Five groups of young female rats are shown in Table I. Group 1 consisted of a group of immature virgin females which weighed approximately 45 g at the start. The control animals received 1.5 units of gonadogen† daily, and the test animals also received the same amount of gonadogen plus 0.04 mg of prostigmin‡ daily. Gonadogen was given in order that the immature uterus would be in a hyperemic state at the time prostigmin was given and would, therefore, be more like the premenstrual endometrium of the human than would the uterus of the untreated immature rat. After 5 days of treatment, the animals were sacrificed, the uteri and ovaries were weighed and desiccated for a period of 8 days, 4 days at 80°C followed by 4 days in a desiccator containing calcium chloride. The weights of these fresh organs are shown in Table I. Both ovaries and uteri are heavier in the prostigmin injected rats. Statistical analyses, using the mean difference divided by the standard error of the difference, are shown under "significant ratio" (S.R.) Any figure in this column (Table I) should be at least 3 in order to say that a significant change in weight has occurred between two means. In the present study ovaries and uteri of the prostigmin injected rats are compared with controls. In Group I the "significant ratio" was 2 for the ovaries and the same

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† Gonadogen was kindly supplied by the Upjohn Company.

‡ We are indebted to Hoffman-La Roche for the Prostigmin.

TABLE I.

Procedures				Ovaries			Uteri		
Group	No. of rats	Kind of inj.	Autopsy body wt, g	Wt in mg	S.R.	Odds	Wt in mg	S.R.	Odds
				Immature females					
1	20	G.	63	139	2.0	2-1	136	2.0	20-1
	20	G. & P.	57	156			151		
2	10	S.	81	23	1.2	7-1	68	2	10-1
	10	P.	81	19			55		
				Immature females: ovariectomized					
3	21	St.	103	—			250	3.5	120-1
	21	St. & P.	102	—			272		
				Mature females					
4	16	S.	147	51	1.0	4-1	186	3.0	60-1
	16	P.	140	53			202		
5	19	S.	206	70	2.7	100-1	410	1.5	4-1
	19	P.	198	81			449		

The wet wt of the ovaries and uteri are shown. G = Gonadogen, P = Prostigmin, S = Saline controls, and St = Stilbestrol.

S.R.—Significant ratio which is based on S.D. (stand. dev.) = $\sqrt{\frac{\Sigma d^2 f}{n}}$; S.E.—(standard error) = $\frac{\text{S.D.}}{\sqrt{n}}$ and standard error of the difference = $\sqrt{(\text{S E}_1)^2 + (\text{S E}_2)^2}$.

for the uteri.

Some investigators[11,12] believe that in cases of paired data the method of Student[11] should be used. This method uses the standard deviation and by tables[11,12] the odds that such results will occur again can be determined. We have analyzed the data by this method as shown in Table I. It will be seen that the odds for Group 1 are 2-1 for the ovaries and 20-1 for the uteri. In contrast to this, Group 2 shows that prostigmin alone did not enlarge the uterus of the immature rat, in fact the mean weights of ovaries and uteri were somewhat smaller after prostigmin as compared with saline (0.9%) controls (Group 2 Table I). In other words the uterus must be in a congested state before the effects of prostigmin can be detected.

Group 3 consisted of ovariectomized virgin female rats in which the controls received 0.25 mg stilbesterol daily while the test animals received 0.25 mg of stilbesterol plus 0.05 mg prostigmin daily. Treatment was begun 4 or 5 days after operation and was continued for 7 days. The animals were then similarly sacrificed and the uteri weighed and disiccated. This group was designed to test prostigmin on the hyperemic uterus in the absence of ovaries. It will be noted that the increase in weight of these uteri was at least as much as that found in Group 1 where the ovaries were present. The figures of 250 and 272 for the weights on the uteri give a difference of 8.8%. The "significant ratio" of 3.5 shows that this weight difference is significant and when analyzed by Student's method the odds are 120 to 1, which are the largest in Table I.

These experiments with immature females lead us to consideration of prostigmin studies in sexually mature female rats. These data are shown in Groups 4 and 5 (Table I). The only difference between these two groups is the duration of injection which was 7 and 30 days in Groups 4 and 5 respectively. Both

11 Student, *Biometrika*, 1908, **6**, 1.

12 Love, H. H., *J. A. Soc. Agronomy*, 1924, **16**, 70.

groups received 0.1 mg of prostigmin daily.

Although the ovarian weights were unchanged in the seven day group and only slightly larger in those injected thirty days there was an increase in the percentage weight of the uteri in both Groups (4 and 5, Table I) which showed an analysis to have a "significant ratio" of 3 and 1.5 in Groups 4 and 5 respectively (Table I). Since uterine weights are more variable than those of the ovaries these "significant ratios" for the uteri are all the more interesting.

Pathology-histological examination§ showed no consistent change in the ovaries. The uteri of the castrated prostigmin rats (Group 3) show the most changes; there were more round cells, edema and inflammatory cells, both polynuclear leucocytes and lymphocytes, than in the controls. The changes were almost completely confined to the submucosa. In the castrated controls both polynuclear leucocytes and lymphocytes are present but not numerous. Some edema and inflammatory cells were found in the submucosa cell groups including the controls. No changes in epithelial or musculature structure were observed in the prostigmin injected rats. The difference between the prostigmin and control animals is minor and pathologically there is no marked alteration.

Discussion. The ovaries of prostigmin injected rats were apparently in good condition as seen histologically and by the estrous cycles. Twenty additional rats injected twice daily with 0.5 mg of prostigmin at each injection showed no significant change in estrous cycles as compared to saline injected controls. We were impressed by the fact that when the background was a mature uterus, induced by age as in Groups 4 and 5, or in hyperemic state brought about by estrogens Groups 1 and 3, prostigmin further increased the weight. This is also evident after corrections are made for differences in body weight and after desiccation. Thus in Group 3 the uteri in the prostigmin injected rats were 8.8, 9.9 and 9.2 percent heavier in the uncorrected, corrected for body weights and desiccated tissues respectively. This clearly shows that the increase in weight of the uterus after prostigmin was not due to edema.

Summary. Prostigmin was given to young rats in daily amounts of 0.04 to 0.1 mg. The ovaries were slightly increased in weight. The uteri if mature as the result of age or estrogens showed increase in weight following injections of prostigmin and these changes were present after desiccation. Estrous cycles showed no significant change from normal. Cellular elements of inflammatory cells were more abundant in the submucosa after prostigmin. On the whole, pathological changes were not marked; therefore, from the pathological viewpoint effects of prostigmin are minor as far as changes in the ovaries and uterus are concerned.

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Essential Hypertension. Therapeutic Trial of Veriloid, a New Extract of *Veratrum viride*.* (17415)

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Veriloid (Coe Chemical Company), a purified alkaloidal fraction of *Veratrum viride*,[1]

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