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Factors Influencing Ovarian Response of Normal and Hypophysectomized Rat to Pregnant Mare Serum.*

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Fluhmann^{1, 2} states that the ovarian response produced by pregnant mare serum (PMS) is independent of the period over which the dose is distributed. He also adds that PMS is a less effective ovarian stimulant than either sheep anterior lobe or chorionic gonadotropic fractions presumably of equal potency. The former observation that the ovarian weight produced by a given level of PMS is independent of the time interval over which the dose is distributed has been confirmed, but the relative ineffectiveness of PMS was surprising in the extreme, and it is with this phase of the problem that the present communication is concerned.

Fluhmann used normal animals only and all injections were given subcutaneously.† There is considerable evidence that the method of injection may markedly influence the response to gonadotropic hormones. It is also now generally recognized that the pituitary of the intact animal may modify the ovarian response. For these reasons, one group of animals was injected subcutaneously and a second intraperitoneally. Both normal as well as hypophysectomized rats were tested.

One hundred and forty-five rats, 23 days old, of the Long-Evans strain, were divided into groups as indicated in Table I. The gonadotropic preparation used was Gonadin,‡ a commercial extract of PMS standardized to contain 200 R.U. (Cole-Saunders) in each cc of solution. Previous experience with this preparation showed that the actual unitage was higher than the labelled unitage. The stock solution, therefore, was restandardized on normal animals. One unit as here defined is the minimum amount of hormone, which when injected in a single dose intraperitoneally into immature rats

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¹ Fluhmann, C. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **41**, 313.

² Fluhmann, C. F., *Endocrinology*, 1939, **25**, 193.

† Personal communication after the present studies had been completed. In the original publications of Fluhmann, the mode of injection was inadvertently omitted.

‡ The pregnant mare serum preparation (Gonadin, lot No. x534) was generously supplied for this study through the courtesy of Mr. D. Wonder of the Cutter Laboratories, Berkeley, California.

TABLE I.
Effect of Dose, Subcutaneous and Intraperitoneal Injection on the Ovarian Response of Normal (23 days at onset of injection) and Hypophysectomized Rats (23 days at operation and 31 days at onset of injection) to Pregnant Mare Serum.

Group	No. of animals	Method of administration	Daily dose, R.U.	Total dose, R.U.	Avg wt of ovaries, mg	Avg wt of uterus, mg
I. Normal	3	Intrap.	2	6	73.2	202.0
		Subcut.	2	6	35.0	240.0
II. Hypd.	3	Intrap.	2	6	36.0	94.5
		Subcut.	2	6	17.5	28.5
III. Normal	36	Intrap.	2	12	180.3	203.7
		Subcut.	2	12	57.3	228.8
IV. Hypd.	15	Intrap.	2	12	226.6	150.4
		Subcut.	2	12	19.6	157.0
V. Normal	9	Intrap.	6	24	221.1	138.0
		Subcut.	6	24	237.1	138.0
VI. Hypd.	8	Intrap.	6	24	277.7	138.0
		Subcut.	6	24	250.0	136.0

23 days old, causes the formation of a small crop of medium large follicles and one to 2 small corpora lutea at the end of 96 hours. As an additional precaution to assure uniform dosage, the dilution of the daily dose was prepared from the restandardized solution just prior to injection to avoid loss of potency on standing.

The daily dose, contained in 0.25 cc, and given in a single injection, was 2 or 6 units. The periods of injection were 3, 4 and 12 days. Injection of the normal animals was started on the 23rd day of life. An interval of 8 days intervened between hypophysectomy and the onset of treatment in the operated groups. This was done to permit utilization of endogenous hypophyseal hormones circulating in the tissues at the time of hypophysectomy. Thirty-six hours after the last injection, the animals were sacrificed, the uteri and ovaries dissected free of extraneous tissues, weighed separately and fixed for subsequent studies.

The results secured by the 2 methods of injection are briefly summarized in Table I. Groups I and II served as pilot experiments and formed the basis for subsequent studies. It will be seen that subcutaneously administered PMS was a less effective ovarian stimulant in both normal and hypophysectomized rats. Intraperitoneally administered PMS was twice as effective as subcutaneous in both groups, but the response in the normal animal was twice as great as that secured in the hypophysectomized. The uterine weights were not significantly affected by the route of injection in the normal animal, but in the hypophysectomized intraperitoneal injection was approximately 4 times as effective as subcutaneous.

Decisive as these experiments are, it was felt, nevertheless, that the

number of animals studied was too small to be crucial. Similar experiments, therefore, were repeated on larger groups injected over a more extended period of time. These disclosed the same enhanced effect with intraperitoneal administration. It will be noted that at a 24-unit level, 2 units on 12 successive days (Groups III and IV), the gravimetric ovarian response of the normal and hypophysectomized series injected intraperitoneally showed no significant difference. The ovaries of the 2 groups, normal and hypophysectomized, injected subcutaneously with the same total dose over a similar period of time, were markedly smaller than those treated intraperitoneally. In the normal animals the intraperitoneal route was approximately 3 times as effective as subcutaneous administration. The average ovarian weight of the former was 180.3 mg as compared with 57.3 mg in the latter. This difference in ovarian growth resulting from the 2 methods of treatment was still more striking in the hypophysectomized series. The average ovarian weight of the intraperitoneally injected rats was 222.6 mg as compared with 19.6 mg in the subcutaneously treated rats. Within each group, normal and hypophysectomized, receiving 2 units daily for 12 days, the uterine weights did not vary significantly with the method of treatment.

It must be admitted that the present work does not permit a direct comparison with that of Fluhmann. Fluhmann's preparation was standardized in Cutter units and the daily dose was given in 2 injections. The preparation used here was standardized somewhat differently and the daily dose was administered in a single injection. Nevertheless the data presented in this report would seem to offer a partial explanation for Fluhmann's findings in that they suggest that the apparent lack of effect of PMS which he reports might have been related to the route of administration.

While no complete explanation for the enhanced effect of intraperitoneal administration can be given at this time, the observation of Russel and Cori,⁸ who found that the glycogenolytic effect of epinephrine is diminished in the hypophysectomized rat when given subcutaneously but not when administered intravenously, would seem to be relevant here. This they attribute to impaired subcutaneous absorption. It would seem likely, therefore, that the rate of absorption may also be important in determining the efficacy of PMS in the hypophysectomized rat, and to a lesser degree in the normal animal. The decreased subcutaneous effect in the hypophysectomized rat is not surprising, if it be remembered that hypo-

⁸ Russel, J. A., and Cori, G. T., *Am. J. Phys.*, 1937, **119**, 167.

physectomy is followed by a lowered metabolic rate, a fall in blood pressure and diminished circulation.

But the rate of absorption cannot be the only factor involved, for it will be seen in succeeding experiments that on augmentation of the daily dose subcutaneous administration was equally as effective as intraperitoneal.

In a recent paper, Evans, *et al.*,⁴ state that the effectiveness of PMS is independent of the route of administration. The problem was, then, to reconcile the preceding results with the conclusion of Evans, *et al.* An answer to this was sought in groups V and VI listed in Table I. It will be noted that when the same total dose, namely 24 units, was distributed over a 4- instead of a 12-day period, subcutaneous administration was just as effective as intraperitoneal in both normal and hypophysectomized rats. The only outstanding difference between the 2 groups was the smaller uterine weights in animals injected over the shorter period. Fluhmann reports a similar progressive increase in uterine weight with PMS as the period of injection was prolonged.

At first sight it may seem that the results secured with groups V and VI contradict the thesis advanced earlier that subcutaneous administration is less effective than intraperitoneal. The paradox, however, is apparent rather than real. Other experiments now under way, and to be reported elsewhere, suggest that the effects produced by PMS depend on the size of the initial dose, whether it is given in a single or divided dose and on the route of administration. When relatively large doses are given, the discrepancy between subcutaneous and intraperitoneal injection is obscured. That this may indeed account for the conclusion of Evans, *et al.*, is strongly suggested by the present investigation.

Summary. A study of the ovarian response of the normal and hypophysectomized rat to PMS showed that subcutaneous administration is less effective than intraperitoneal. With small doses, 6 units distributed over 3 days and given at a 2-unit level in a single injection, the intraperitoneal route was twice as effective as subcutaneous in both normal and hypophysectomized. In the normal animal, PMS was twice as effective as in the hypophysectomized. The uterine response was not significantly affected by the mode of injection in the normal animal, but in the hypophysectomized intraperitoneal injection was 4 times as effective as subcutaneous. When the same daily dose is given for 12 days intraperitoneal injection was 3 times as effective as subcutaneous in the normal animal

⁴ Evans, H. M., *et al.*, *Univ. Calif. Pub. Anat.*, 1936, **1**, 237.

and 11 times as effective in the hypophysectomized. At this level, intraperitoneally administered PMS yielded ovaries of approximately the same weight in both groups. When the same total dose, namely 24 units, was distributed over a 4-day period, subcutaneous injection proved equally as effective as intraperitoneal in both normal and hypophysectomized rats. As a tentative explanation, it is suggested that the rate of absorption may be one of several factors involved in this mechanism.

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Effect of A.T.10 (Dihydratachysterol) on Various Types of Experimental Rickets in Rats.

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A.T.10 (dihydratachysterol) is, according to Holtz,¹ its discoverer, a reduction product of irradiated ergosterol. It has been claimed that it has a special capacity to raise the blood serum calcium concentration. Because of this remarkable property it has been used extensively in the treatment of parathyroid tetany. Albright, Bloomberg, Drake and Sulkowitch² have reviewed the literature and have studied the effects of this agent in comparison with vitamin D in cases of idiopathic hypoparathyroidism. They concluded that vitamin D has two fundamental actions: it increases the amount of calcium absorbed from the gastrointestinal tract and also the amount of phosphate excreted in the urine. A.T.10 acts similarly, but to a different degree; it has less effect on calcium absorption and greater effect on the excretion of phosphate in the urine.

A.T.10 has been reported not to cure experimental rickets in rats, except perhaps in toxic doses.³ We have been unable to find a detailed account of Holtz's experiments on experimental rickets. We surmise that he used the classical high-calcium-low-phosphorus type of ricketogenic diets. If A.T.10 has the action described by Albright *et al.*, it is logical to postulate that it would be particularly unfavorable to the healing of rickets caused by low-phosphorus diets

¹ Holtz, F., Gissel, H., and Rossmann, E., *Deutsche Z. f. Chir.*, 1934, **242**, 521.

² Albright, F., Bloomberg, E., Drake, T., and Sulkowitch, H. W., *J. Clin. Invest.*, 1938, **17**, 317; *Ibid.*, 1939, **18**, 165.

³ Harnapp, G. O., *Monatschr. f. Kinderh.*, 1935, **63**, 262.