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Gonadotropic Response to Subcutaneous and Intraperitoneal Injections of Urine Prolan in White Rats.

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Investigations by Collip[1] and Evans[2] showed that hypophyseal gonadotropic hormone is much more effective by subcutaneous than by intraperitoneal administration. With reference to gonadotropic hormone derived from pregnant mare serum (PMS), Evans[2] showed that it was just as effective intraperitoneally as subcutaneously. Later Pencharz[3] demonstrated that intraperitoneally PMS was 2 to 3 times more effective than subcutaneously, the degree of effectiveness, however, depending upon the number of injections.

As to the effectiveness of intraperitoneal and subcutaneous administration of pregnancy urine prolan, Evans[2] reported: "Pregnancy prolan, which contains no antagonist, is distinctly less effective intraperitoneally than subcutaneously. Here the difference seems to be due merely to faster absorption by the intraperitoneal route with consequent increased rate of excretion by the kidney or destruction by the tissues." The following experiments are intended to demonstrate that, provided a certain experimental procedure is used, it is possible to make pregnancy urine prolan develop its full effect also in the intraperitoneal route.

For the experiments, 72 infantile female rats, 3-4 weeks old and weighing 20-30 g were used. They received prolan by various methods, by a single injection, or 3 times at intervals of 24 hours, or 6 times during 40 hours, either intraperitoneally or subcutaneously. Sixty hours after the first injection we began taking vaginal smears every 12 hours, and 120 hours after the first injection the animals were killed and their ovaries inspected.

Two prolan preparations were tested in this way: (1) "Purified prolan" (PP) which contained 333,333 RU per g. (2) Crude prolan (CP) which contained 1000 RU per g. The results of these experiments are shown in Table I.

As may be deduced from the experiments there is no fundamental

1 Collip, *Canad. Med. Assn. J.*, 1936, **34**, 458.

2 Evans, H. M., Korpi, K., Pencharz, R. I., and Simpson, M. E., *Univ. Calif. Publ. Anat.*, 1936, **1**, 237.

3 Pencharz, R. I., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 525.

difference between the effectiveness of purified prolan (PP) and crude prolan (CP). We shall only compare the results read from the estrous reaction (APR I):

One single injection of prolan was approximately 10 times (in case No. 24 at least 6 times) as effective subcutaneously as intraperitoneally (Nos. 2, 6, 20, 24). When, however, the injections were given in 3 portions the subcutaneous route was only twice as effective as the intraperitoneal one (Nos. 9, 10, 26, 27). When the same amount of prolan was, however, distributed over 6 injections there was no longer any difference between intraperitoneal and subcutaneous administration (Nos. 15 and 32).

Taking the corpus luteum reaction (APR III) as a standard almost the same values were obtained and it even seemed that with 6 intraperitoneal injections (*Cf.* No. 16) the APR III could be produced with smaller doses intraperitoneally than subcutaneously.

Experiments Nos. 15 and 32 represent the routine method of prolan standardization (Zondek[4]) in terms of estrous units using 6 injections. If this experimental technic is adhered to it is therefore irrelevant whether the standardization is performed with subcutaneous or intraperitoneal injections.

Discussion. It was possible to verify the results obtained by Evans[2] that subcutaneous administration of pregnancy urine prolan proved to be more effective than intraperitoneal, provided only 1 to 3 injections were given. When, however, the same amount of prolan was given in 6 portions, both methods were equally effective. We are inclined to attribute these results to the more rapid absorption of the hormone from the intraperitoneal deposits, as explained by Evans.[2] After only one to 3 injections it is very rapidly absorbed and its effectiveness considerably impaired. Only when using 6 injections over 40 hours the absorptional conditions in the peritoneum become equal to those in the subcutis, so that optimal utilization of prolan is possible.

When standardizing small amounts of prolan we had, moreover, the opportunity of studying the following phenomenon, which has been described for PMS by Hamburger:[5] Distribution of 1 RU of prolan over several subcutaneous injections produces a less marked gonadotropic reaction than the administration of one single dose. With larger doses, however, this does not hold true. This phenomenon may be explained by the fact that, by splitting up a threshold dose

[4] Zondek, B., *Z. Gebh. and Gyn.*, 1926, **90**, 378; *Med. Klin.*, 1927, No. 13; *Hormone d. Ovariums u. d. Hypophysenvorderlappens*, Springer, Berlin, 1st ed., 1931; *cf.* Zondek, B., and Aschheim, S., *Klin. Wschr.*, 1927, **6**, No. 6.

[5] Hamburger, Ch., *Quart. J. Pharm. and Pharmacol.*, 1938, **11**, 186.

TABLE I.

Exp. No.	Prolan	Injections	Doses		Gonadotropic reactions (APR) after subcutaneous injection			Gonadotropic reactions (APR) after intraperitoneal injection		
			Weight	RU*	I Estrous	II Follicle hemorrhage	III Corp. lut.	I Estrous	II Follicle hemorrhage	III Corp. lut.
1	PP	1 x	1 gamma	0.3	—	—	—	—	—	—
2			1.5 "	0.5	+	—	—	—	—	—
3			3 "	1	+	—	—	—	—	—
4			6 "	2	+	—	—	—	—	—
5			9 "	3	+	—	—	—	—	—
6			15 "	5	+	—	—	+	—	—
7	PP	3 x	1 "	0.3	—	—	—	—	—	—
8			1.5 "	0.5	—	—	—	—	—	—
9			3 "	1	+	—	—	—	—	—
10			6 "	2	+	—	—	+	—	—
11			9 "	3	+	—	—	+	—	—
12			15 "	5	+	—	+	+	—	+
13	PP	6 x	1 "	0.3	—	—	—	—	—	—
14			1.5 "	0.5	—	—	—	—	—	—
15			3 "	1	+	—	—	+	—	—
16			6 "	2	+	—	—	+	—	+
17			9 "	3	+	—	+	+	—	+
18			15 "	5	+	—	+	+	—	+
19	CP	1 x	0.5 mg	0.5	—	—	—	—	—	—
20			1 "	1	+	—	—	—	—	—
21			1.5 "	1.5	+	—	—	—	—	—
22			3 "	3	+	—	—	—	—	—
23			4.5 "	4.5	+	—	—	—	—	—
24			6 "	6	+	—	—	—	—	—

25	CP	3 x	0.5	''	0.5	—	—	—	—	—	—
26			1	''	1	+	—	—	—	—	—
27			1.5	''	1.5	+	—	—	+	—	—
28			3	''	3	+	—	—	+	—	—
29			4.5	''	4.5	+	—	+	+	—	++
30			6	''	6	+	—	+	+	—	++
31	CP	6 x	0.5	''	0.5	—	—	—	—	—	—
32			1	''	1	+	—	—	+	—	—
33			1.5	''	1.5	+	—	—	+	—	—
34			3	''	3	+	—	—	+	—	—
35			4.5	''	4.5	+	—	+	+	—	++
36			6	''	6	+	—	+	+	—	++

*According to Zondek's definition[4] 1 RU is the smallest amount of prolan which given in 6 injections spread over 36 hours to a 3-4-week-old female, white rat weighing 20-30 g produces the estrous reaction (APR I).

(as 1 RU certainly is) several subthreshold doses are produced which cannot induce the gonadotropic response in the ovary.

Summary. Comparing the gonadotropic response of the organism to subcutaneous or intraperitoneal administration of pregnancy urine prolan the following results were secured: (1) If the minimum dose is split up into 6 portions intraperitoneal and subcutaneous administration are equally effective. (2) If the minimum dose is split up into 3 portions the intraperitoneal route is approximately half as effective as the subcutaneous one. (3) If the minimum dose is given in one injection the intraperitoneal administration is about 1/10 as effective as subcutaneous. (4) Using the subcutaneous standardization method with minute doses as *e.g.*, 1 RU (estrous effect) the gonadotropic effect of 6 subthreshold doses is less marked than that of one single threshold dose.

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Cheilosis Successfully Treated with Synthetic Vitamin B_6.

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Aykroyd and Krishnam[1] observed that the incidence of angular stomatitis (sores in the corners of the mouth, perleche, cheilosis) coincided with a deficiency of some factor or factors of the vitamin B_2 complex.

More recently Sebrell and Butler[2] produced these lesions in 10 out of 18 women subsisting on a riboflavin-deficient diet. The lesions developed in a period of 94 to 130 days. They failed to respond to nicotinic acid but responded to riboflavin, the complete healing requiring from 5 to 58 days. Sebrell and Butler[3] added more evidence to their previous findings and these have been confirmed by others.[4, 5, 6]

1 Aykroyd, W. R., and Krishnam, B. G., *Ind. J. Med. Res.*, 1937, **24**, 707.

2 Sebrell, W. H., and Butler, R. E., *U. S. Pub. Health Rep.*, 1938, **53**, 2882.

3 Sebrell, W. H., and Butler, R. E., *U. S. Pub. Health Rep.*, 1939, **54**, 2121.

4 Sydenstricker, V. P., Geeslin, L. E., Templeton, C. M., and Weaver, J. W., *J. A. M. A.*, 1939, **113**, 1697.

5 Spies, T. D., Vilter, R. W., and Ashe, W. F., *J. A. M. A.*, 1939, **113**, 931.

6 Jolliffe, N., Fein, H. D., and Rosenblum, L. D., *New Eng. J. Med.*, 1939, **221**, 921.