HISTOCHEMICAL STUDIES OF GLYCOGEN IN THE RAT OVA OVULATED BY THE GONADOTROPHIN INJECTION

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(Received June 7, 1960)

Introduction

In a previous paper (5), the author reported that there are only glycogen-laden ova in the oviduct of the rat, without response to the presence of both glycogen-free and glycogen-laden ova in the Graafian follicles. In the present investigation, he has made a histochemical examination of the glycogen in the ova ovulated into the oviduct by gonadotrophin treatment, to ascertain whether the appearance of glycogen in the ova ovulated by such treatment is as consistent as that in the naturally ovulated ova.

Materials and methods

Twenty-eight adult female rats of about six months of age, exhibiting a regular estrous cycle, were treated with a single subcutaneous injection of pregnant mares' serum (PMS) at the stage of diestrus, and after six hours with human chorionic gonadotrophin (HCG). The PMS used in this study was Anteron (Schering Co. Ltd.) and the HCG Prymogonyl (Schering Co. Ltd.). The amount of PMS and HCG injected into each rat is given in Table 1. The animals were sacrificed after 24 hours of HCG injection, when all the vaginal smears showed estrus.

All the oviducts were fixed in neutral alcohol-formalin solution, embedded in paraffin and serially cut at 15μ. All the sections containing ova were selected by a microscopical examination, and stained by the periodic acid-Schiff method (PAS). The identification of glycogen was made by means of the salivary test (37°C, 1 hr).

Results and discussion

1. The number of ova ovulated by the gonadotrophin injection.

The number of ova ovulated into the oviduct by the PMS and HCG treatments is given in Table 1. In this table are also given the amounts of PMS and HCG injected.

Table 1. The number of ova ovulated after injections of varying amounts of PMS and HCG.

Rat No.	Body weight in g	Amount of gonadotrophins injected (i.u.)		Number of ova ovulated		
		PMS	HCG	Left tube	Right tube	Total
1	158.5	30	20	5	2	7
2	171.0	60	20	4	5	9
3	149.0	60	20	7	4	11
4	205.0	100	20	5	4	9
5	163.5	100	20	9	7	16
6	191.5	30	40	5	5	10
7	202.0	30	40	3	6	9
8	195.5	30	40	9	2	11
9	190.0	60	40	9	3	12
10	194.5	60	40	0	3	3
11	170.0	60	40	8	6	14
12	186.0	100	40	4	10	14
13	192.0	100	40	4	6	10
14	178.0	100	40	3	3	6
15	168.5	300	100	9	5	14
16	173.5	300	100	2	5	7
17	195.0	300	100	4	3	7
18	181.0	300	100	5	4	9
19	178.5	300	100	6	5	11
20	197.0	Natural ovulation		3	4	7
21	203.5	〃		5	7	12
22	222.0	〃		5	6	11
23	207.0	〃		6	3	9
24	178.0	〃		5	5	10
25	200.0	〃		4	4	8
26	200.0	〃		5	4	9
27	194.0	〃		7	5	12
28	195.5	〃		5	6	11

As shown in Table 1, a single subcutaneous injection of PMS at diestrus followed after six hours by HCG always induced ovulation in every rat, the number of tubal ova being three at minimum and 16 at maximum. The number was not affected by the amount of PMS or HCG injected. The number of naturally ovulated ova was seven at the minimum and 12 at the maximum, hence, there is no difference in the number of ovulated ova between the artificial ovulation by gonadotrophins and natural ovulation.

All the ova were discovered from the ampullae of oviducts. They were oval in shape and surrounded by a large number of follicular cells which

tightly stuck to each other (Fig. 1).

It is generally known that the adult rats and mice, compared with rabbits,

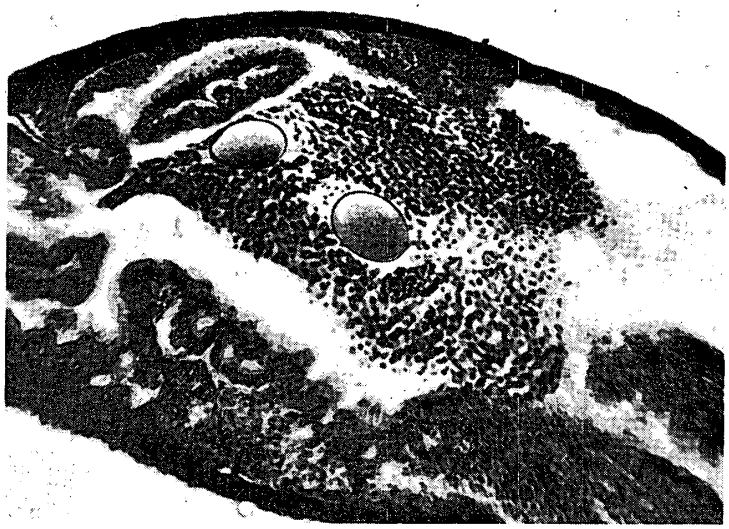

Fig. 1. Glycogen in the ova ovulated into the oviduct by gonadotrophin injection. PAS method. ×100.
The tubal ova surrounded by a large number of follicular cells contain a large amount of glycogen closely packed in the cytoplasm.

are less susceptible to the induction of estrus, ovulation and pregnancy by treatment using gonadotrophins, though there are a few reports on their superovulation. Smith and Engle (8) and Engle (3) reported that daily implants of anterior pituitary tissue into mature rats and mice induced mating 12 hours after the second treatment, ovulation of up to 49 ova, and implantation of 19 to 29 embryos on the ninth or tenth day of pregnancy. Fowler and Edwards (4) reported that the injection of 3 i.u. PMS and varying amounts of HCG 40 hours later into mature female mice selected at random with regard to their estrous cycle induced superovulation, the number of ova ovulated being 24 on an average when treated with 1 i.u. HCG injection, and 21 with 3 i.u. HCG, and 24 with 6 i.u. HCG. However, many investigators (1, 2, 6 and 7) reported that ordinary ovulation was induced in mature pregnant and nonpregnant females by anterior pituitary implantation or by gonadotrophin. In the present investigation, also, no superovulation was observed when treated by gonadotrophins, showing three at the minimum and 16 at the maximum.

2. The amount of glycogen in the ova ovulated by the gonadotrophin treatment.

The amount of glycogen in the ova ovulated by gonadotrophin injection was compared with that in the naturally ovulated ova, and the results are given in Table 2.

As shown in Table 2, all the ova ovulated in both the gonadotrophin

treatment and natural ovulation contained a large amount of glycogen, closely packed in the cytoplasm (Fig. 1).

Table 2. The amount of glycogen in the ova ovulated by the gonadotrophin injection, and that in the naturally ovulated ova.

Treatment-groups	No. of rats	No. of ova ovulated	Amount of glycogen			
			−	+	++	+++
Gonadotrophin injection	19	189	0	0	0	189
Natural ovulation	9	89	0	0	0	89

From the fact just stated, it was proved that the ova must be fully loaded with glycogen when they are ovulated, whether the ovulation is natural or caused by gonadotrophin treatment.

3. The number of glycogen-free and glycogen-laden ova in the ovaries treated by the gonadotrophin.

To ascertain the fluctuation in the number of glycogen-free and glycogen-laden ova in the ovaries caused by the gonadotrophin injection, left ovaries from six rats, three of which were treated by gonadotrophin injection and others not treated, were fixed in neutral alcohol-formalin solution, embedded in paraffin, cut serially at 7μ, and stained by the PAS method. The number of ova in the primary, secondary and Graafian follicles were counted, and the results are given in Table 3.

Table 3. The number of glycogen-free and glycogen-laden ova in the ovaries.

Follicles	Rat No. / Ova	Gonadotrophin treatment					No treatment				
		1	2	3	Average	Ratio	4	5	6	Average	Ratio
Primary follicles	Glycogen-free ova	33	22	96	50	93%	87	42	92	74	100%
	Glycogen-laden ova	8	2	1	4	7	0	1	0	0	0
	Total	41	24	97	54	100	87	43	92	74	100
Secondary and Graafian follicles	Glycogen-free ova	3	12	35	17	16	13	17	8	13	14
	Glycogen-laden ova	77	89	98	88	84	93	81	66	80	86
	Total	80	101	133	105	100	106	98	74	93	100
Sum total		121	125	230	159		193	141	166	167	

As shown in Table 3, both the glycogen-free and glycogen-laden ova were found in the gonadotrophin-injected ovaries as well as in the non-injected ovaries, showing no change in the ratio of the glycogen-free and glycogen-laden ova.

From the present investigation, it is concluded that there are both the glycogen-free and glycogen-laden ova in the treated ovaries, coinciding with those in natural condition, and also that the amount of glycogen in the glycogen-laden ova ovulated by gonadotrophin injection is as much as that in naturally ovulated ova.

Summary

The results obtained in this investigation are as follows.

1. A single subcutaneous injection of PMS at diestrus followed by HCG after six hours always induced ovulation in every rat without exception, the number of tubal ova being three to 16.

2. All the ova ovulated by gonadotrophin injection contained a large amount of glycogen, closely packed in the cytoplasm.

3. Both glycogen-free and glycogen-laden ova were found in the gonadotrophin-injected ovaries, as well as in the non-injected ovaries.

Acknowledgment

The author wishes to express his hearty thanks to Prof. Dr. Y. Toryu for his valuable guidance and criticism through the course of this work.

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