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Glycogen Content in the Mouse Oocytes

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생쥐 난자의 Glycogen함량

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적 요

생쥐 여포난자를 배양하면서 감수 분열 각 단계에서의 난자내 glycogen 함량의 변화를 Microspectrophotometer를 사용하여 조직화학적방법으로 조사하였다.

난자의 성숙이 진행됨에 따라 PAS 반응은 감소하며, 퇴화중인 난자의 glycogen 함량은 현저히 적었다. 난자내 glycogen은 난자의 성숙을 유도하는 역활을 나타내며, 핵막이 붕괴하기 전에 glycogen이 소모되면 난자의 퇴화를 일으킨다.

본 실험의 결과 난자내 glycogen은 감수분열의 진행에 있어서 중요한 요인이 됨을 알 수 있었다.

INTRODCTION

Earlier studies have shown the existence of glycogen in the oocyte cytoplasm (Goldman, 1912; Deane, 1952) and in the preimplantation embryonal cells (Thomson and Brinster, 1966; Wong and Dickson, 1969; Smith and Wilson, 1971; McReynold and Hadek, 1972; Parkening and Soderwall, 1974). In our recent observations (Cho and Yoon, 1975) it has been found that mouse follicular oocytes or those remained at the dictyate stage during cultivation have a strong PAS reaction which is known as a tool to detect glycogen, and the intensity of PAS staining is decreased as meiotic resumption is progressed.

It has been understood that some carbohydrates or certain kinds of nucleotides such as nicotinamide adenine dinucleotide (NAD) supplemented to the medium well support the mouse oocyte maturation (reviewed by Biggers, 1973). However, no particular attention has been paid to the relation between the endogeneous glycogen and the meiotic resumption of the oocytes.

The present studies were planned to investigate the role of glycogen in the oocyte as a series of researches on the mechanism of oocyte maturation.

MATERIALS AND METHODS

Mouse oocytes were obtained from the ovaries of 3~4 week old A-strain mice bred randomly in our laboratory. The ovaries were taken out and transferred to the Dulbecco's phosphate buffered saline (PBS) to wash and trim fats and blood clots out from the ovarian surface.

The cleaned ovaries were placed on a culture dish containing modified Krebs-Ringer bicarbonate solution (Biggers *et al.*, 1971) and the matured ovarian follicles were punctuated with a fine needle (Gauge #27) under a dissecting microscope to expell the oocytes out. Then the denuded oocytes from the cummulus cells but with a clearly visible germinal vesicle (GV) were collected and washed several times in the medium.

The microtube culture method (Cho, 1974) was mainly adopted in the present studies. Ten-15 dictyate oocytes were introduced into 10 μl medium previously set in a microtube (50 mm length, i.d. 1 mm), and incubated with 5% CO_2 in fully moistened air at 37°C for 20 hours. The details of the prepararion for the culture tube were previously described (Cho, 1974).

At the end of the culture period, the oocytes were collected in the medium in a watch glass and washed several times with 0.154 M saline. To induce PAS reaction to the eggs, the method of Thomson and Brinster (1966) was mainly adopted and the detailed description of the procedures was already noted (Cho and Yoon, 1975). Slides carrying processed oocytes were mounted with Cargile oil (refractive index; 1.561) and covered with a coverglass (Thickness No.1) for photometry.

The relative glycogen-Feulgen dye content in the oocyte was determined with a microspectrophotometer (Olympus Model A-4) at 500 mm and 560 mm to measure homogeneous glycogen content in the ovum. The relative amount of glycogen was calculated by multiplication of 1,000 of the figure of the Patau's formula.

RESULTS

All oocytes freshly collected from the follicles and those which kept germinal vesicle during the culture showed a similar intensity for the PAS-reaction. As the meiotic division progressed, the glycogen-Feulgen dye content was gradually decreased (Table 1, Fig.1). Polar bodies showed relatively strong PAS-reaction than the proper cytoplasm of the oocyte at metaphase II (MII). Fragmented ova (Fig. 2, ACT.) showed a strong positive reaction to the PAS treatment as that of the dictyate oocyte. Generally degenerating oocytes were weakly reacted to the PAS (Table 1, Fig. 2, DEG.).

Younger follicles which were composed of an oocyte and several intact follicle cells had shown a positive reaction at the initial state. However, when they were

Table 1. Relative amount of Glycogen-Feulgen-Dye-Content (GFDC) in the mouse oocytes after cultures in the plain medium

Ova stage	No. of ova	Mean GFDC±S.E. (arbitrary unit per oocyte)
Dictyate	15	2456±60
Metaphase I	15	1422±15
Metaphase II	20	1360±19
Polar body	10	84±18
Degenerating ova	15	805±21
Activated ova	3	2371±45

cultured for 20 hours the follicle cells became weaker to the PAS-reaction but the oocyte itself kept stronger.

Comparing the intensity of the PAS-reaction of the MI or MII oocytes induced in the culture with that of the ovulated oocytes, most of which were already at the MI or MII, it was found that they showed quite a similar pattern.

DISCUSSION

It has been demonstrated that mouse oocytes require energy sources such as pyruvate, oxaloacetate, lactate, phosphoenolpyruvate and glucose to induce meiotic resumption (reviewed by Biggers, 1973). On the other hand, Bae and Foote (1975) reported that amio acids such as glutamine and proline stimulated the rabbit follicular oocytes. However, the significance of endogenous glycogen in the oocytes has not yet been investigated with regard to the meiotic resumption except our recent report (Cho and Yoon, 1975).

Cho and Yoon (1975) found that the oocytes with intact germinal vesicle showed the strong PAS-reaction and that the oocytes which once consumed glycogen in the presence of dbcAMP regained glycogen immediately upon the removal of the inhibitor, and started the meiotic resumption. We also found that the content of glycogen in the oocytes gradually decreased as their meiosis progressed. These results strongly suggest that the endogeneous glycogen is essential for the nuclear maturation of the oocytes. The fact that most of those oocytes starting degeneration before the breakdown of the germinal vesicle show weaker response to PAS-reaction might be one of the evidences that the low level of the glycogen concentration in the oocytes eventually leads to degeneration.

Based upon the fact that the amount of glycogen in the superovulated ova was very small and increased during preimplantation as revealed by the enzymatic quantitative methods (Stern and Biggers, 1968; Snyder *et al.*, 1971; Ozias and Stern, 1973), and the fact that the MI or MII oocytes either superovulated or cultured similarly showed less intensity in the PAS reaction might be another

evidences to support the significance of the role of glycogen in inducing meiosis since the initial state of those oocytes generally manifested a strong response to the PAS treatment.

When we compare the glycogen content in the oocytes determined by Stern and Biggers (1968) with that determined by us by means of microspectrophotometer, we can calculate the relative amount of the glycogen in the oocytes at various conditions as seen in the Table 2. With this table, the difference of the amount of glycogen would be assumed those amount of glycogen which might be used by the oocytes during meiotic division.

Table 2. Comparison of the Glycogen-Feulgen-Dye contents with the relative quantitative amount of glycogen in mouse oocytes

Stage of oocytes	Arbitrary unit of the glycogen content represented by PAS-reaction	Glycogen content (mμg/oocyte)
Dictyate	2456	0.194
Metaphase I	1422	0.1135
Metaphase II	1360	0.108
Polar body	84	0.006
Degenerating ova	805	0.064
Activated ova	2371	0.188
Ovulated ova (unfertilized)	1391	0.11~0.03*

*Stern and Biggers (1968)

The glycogen content in the arrested oocytes in the younger follicles was constant and that of surrounding follicular cells was lost during the culture. This might support the assumption that the follicular cells would be active in the carbohydrate metabolism and supply the products to the oocytes, as stated by other investigators (Biggers *et al.*, 1967; Donahue and Stern, 1968; Cross, 1973).

Thus we could infer that the failure of the consumption of glycogen by the oocytes consequently results in arrested state. This finding could also be another evidence for the glycogen participation to the oocyte maturation division.

SUMMARY

The glycogen content of the oocytes at the various stages of meiotic division induced during culture was determined by a microspectrophotometer. The PAS intensity decreased gradually as the meiotic resumption progressed. The amount of glycogen was also decreased in the degenerated ova.

It is concluded that the glycogen consumption is necessary for the meiotic resumption and that the glycogen loss while the germinal vesicle is intact seems to lead degeneration. These results suggest that the endogenous glycogen is important to support meiosis.

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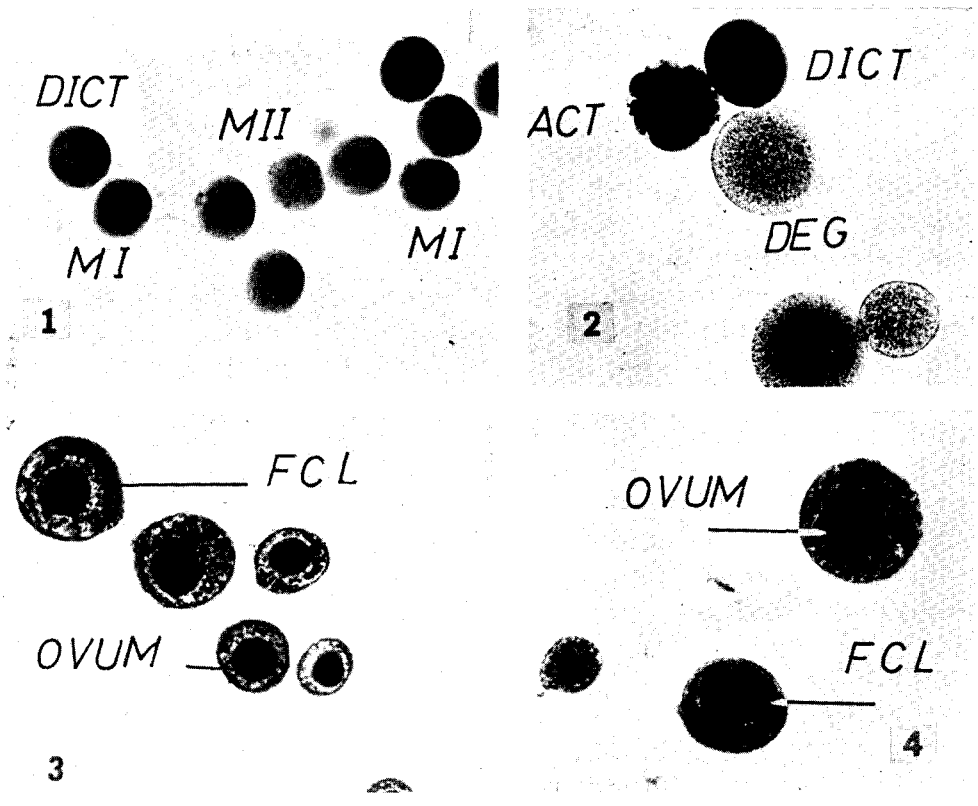

Fig. 1. The oocytes showed a gradual decrease in the PAS-intensity during the meiotic maturation.

Fig. 2. The fragmented oocyte activated in the intact ovary showed a strong reaction to PAS, and the degenerated ova of various size showed a week reaction to PAS.

Fig. 3. The follicles cultured *in vitro* for 20 hours showed a weak reaction to PAS.

Fig. 4. The follicles isolated from the intact ovaries showed a strong reaction to PAS.

Dict, Dictyate oocytes; MI, Oocytes at metaphase I; MII, Oocytes at metaphase II; Act, Fragmented oocytes; DEG, Degenerating oocytes; FCL, Follicle cell layer.