CONNECTION BETWEEN PERMEABILITY OF THE OVARIAN FOLLICLE AND ITS ATRESIA

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The permeability of the follicles of sexually immature noninbred rats was studied by intravital contact fluorescence microscopy. Fluorescein and trypaflavine did not penetrate into the ovum of the primordial follicles but they penetrated rapidly into the ovum and follicular fluid of developed follicles. Since development of the follicles in the sexually immature animal invariably ends in atresia, it is concluded that the process of atresia is connected with disturbances of the barrier function of the follicular epithelium.

KEY WORDS: atresia of follicles; permeability of follicles; fluorescein.

Evidence in support of the existence of the hypothetical blood-follicle barrier in the ovary has recently been published [1-3]. For example, the use of intravital contact fluorescence microscopy of the ovary in sexually mature rats has shown that acidic and basic fluorochromes neither penetrated into the ovum of the primordial follicle nor passed beyond the theca interna of some large ripening follicles, but they easily penetrated into atretic follicles. Some workers, who consider that local changes are the cause of atresia, have postulated that a disturbance of the barrier functions of the system of follicular epithelium–oocyte are the basic mechanisms of development of the complex process of follicular atresia [1]. Particularly active atresia of the follicles is observed before the onset of puberty. This period of the animal's life is characterized not only by mass development of follicles, but also by the fact that this development terminates, not in ovulation, but in biologically predetermined atresia. In this connection the study of the barrier functions of the follicle at various stages of its development under conditions followed by atresia could be of theoretical and practical importance.

The object of the present investigation was to study connections between the character of permeability of the follicle and the process of its atresia. For the intravital study of ovarian follicular permeability in the sexually immature animal the method of intravital contact fluorescence microscopy was used. This technique was used previously with success to study follicular permeability in the sexually mature animals [2].

EXPERIMENTAL METHOD

Experiments were carried out on sexually immature noninbred albino rats weighing 15-25 g, in two series. In series I, 40 animals were used. An intraperitoneal injection of 0.5 mg/kg fluorescein as the 0.1% solution was given to one animal 30-40 min before the investigation and the other received 0.5 mg/kg trypaflavine, also as the 0.1% solution. The animal was anesthetized 30-40 min after injection of the fluorescein by intraperitoneal injection of thiopental sodium in a dose of 30 mg/kg body weight; when sleep developed (3-5 min) laparotomy was performed through a lumbar incision and the ovary was drawn into the wound by traction on the uterine cornu (without injury to the ovary or disturbance of its blood supply). The ovary, continuously irrigated with warm (37°C) Ringer's solution, was examined in the ml-2B luminescence microscope with the aid of the OLK-2 attachment and a series of contact objectives. The objective was taken up to the surface of the ovary until complete but gentle contact was made and microscopic examination of the ovary was then carried out in different "optical section" by changing the system of focusing.

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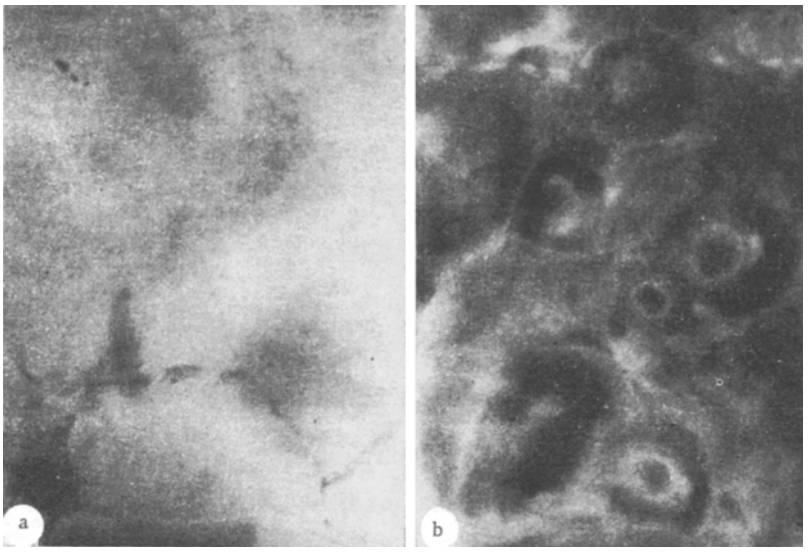

Fig. 1. Fluorescence of ovary of sexually immature rat: A) in light of intrinsic fluorescence; ovary is seen as weakly fluorescent body, thin capillaries can be seen in optical section, no structural details of the ovary can be distinguished; B) the same place in the ovary 12 min after injection of fluorescein: many follicles can be clearly distinguished in a thin optical section, and three of them (right) are seen in optical section passing through the oocyte; a brightly fluorescent follicular fluid and oolemma can be seen against the background of a moderately fluorescent theca and granulosa. Objective 10×, photographic enlargement 200×.

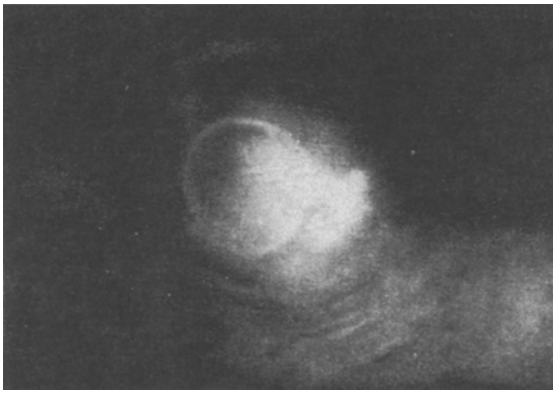

Fig. 2. Ovary of sexually immature albino rat 40 min after intraperitoneal injection of fluorochrome: to the right of the brightly fluorescent ovum a small cavity filled with equally brightly fluorescent follicular fluid can be seen inside a small follicle. Photographed taken with FS filters, 3.5 mm thick, to give a more demonstrative result. Objective 25×, photographic enlargement 200×.

In the experiments of series II (10 animals), laparotomy was first performed on the animals, the ovary was examined in the light of its intrinsic fluorescence, after which the fluorochrome was injected intraperitoneally in the same dose and the ovary was examined continuously under the microscope for 30-60 min. The animals were then killed.

EXPERIMENTAL RESULTS

On intravital contact microscopy in the light of its intrinsic fluorescence the ovary appeared as a pale green body, very weakly fluorescent, on which dark webs of blood vessels could be observed in differ-

ent optical sections. No structural elements of the ovary or of its follicular system could be distinguished in the sexually immature animal in the light of intrinsic fluorescence. A different picture was observed after injection of the fluorochromes. For example, after the injection of fluorescein the brightest pale green fluorescence was given by the cytoplasm of the covering epithelial cells and the poorly developed ovarian stroma. Details of the vascular network, in which the blood flow was visible, could be clearly distinguished (living erythrocytes do not take up the fluorochrome and thus appear dark). By changing the depth of focus, the structural details of the follicular system of the ovary could be clearly distinguished. To begin with, many primordial follicles were seen in the superficial layers of the ovaries. Their appearance was as follows: after staining with trypaflavine the dark, nonfluorescent oocyte was surrounded by a yellow fluorescent border of nuclei of the follicular epithelial cells; after staining with fluorescein a continuous border of brightly green fluorescent cytoplasm of the follicular cells could be seen. During the period of observation (40-60 min) the fluorescent ovum of the primordial follicle could not be seen in any of the animals. On intravital contact fluorescence microscopy an enormous number of follicles, starting to develop, could be seen in the optical sections. It was possible to distinguish follicles whose development was shown only by an increase in size of the oocyte and the appearance of stratification in the surrounding follicular epithelium. Later, the theca externa and theca interna appeared in the follicles, in which a cavity was formed and increased in size. In all animals without exception the developing follicles, irrespective of their stage of development, showed the following features; on the first signs of development of the follicle the fluorochromes used in the experiments penetrated to the oocyte and the brightest fluorescence was observed in the oolemma and the follicular fluid (Fig. 1). The brightness of fluorescence of the oocyte and follicular fluid in some very large follicles was much greater than that of the granulosa and theca (Fig. 2). For that reason, in experiments on all the animals the patterns observed were uniform in type and the only difference was in the color of fluorescence which depended on the fluorochrome used: in the developing follicles the brightest fluorescence was given by the theca interna, the follicular fluid, and the oolemma of the ovum; less bright fluorescence was given by the follicular epithelium and cytoplasm or nucleus of the oocyte. No developing follicles were seen in which the oocyte was not stained with the fluorochrome.

In the experiments of series II, which began with microscopic examination of the ovary in the light of its intrinsic fluorescence, after which a fluorochrome was injected into the animal, the following results were obtained. After intraperitoneal injection of the fluorochrome it appeared in the plasma, passed along the blood vessels of the ovary, and 4-6 min after injection it penetrated instantaneously through the walls of the blood vessels and stained the surface epithelium and stroma cells of the ovary and the theca external and theca interna of the large follicles intensely. After a few minutes the injected fluorochrome began to accumulate in the follicular fluid and ovum. All structural details of the follicular system of the ovaries could be distinguished 10-12 min after intraperitoneal injection of the fluorochrome as a result of the appearance of excited fluorescence in them. No increase or decrease in fluorescence of the objects studied in the ovary, perceptible by the eye, could be observed during the period of investigation (40-60 min) after the injection of the above-mentioned doses of fluorochrome. This suggests that high concentrations of the fluorochromes accumulating in the follicular fluid and ovum appeared 6-10 min after the fluorochrome had entered the blood stream.

This investigation thus showed that during the intravital study of permeability of the ovarian follicles of the sexually immature animal the acidic and basic fluorochromes used in the experiments did not penetrate to the ovum of the primordial follicles, for it was held up in the follicular epithelial cells, but in the developed follicles it penetrated freely to the ovum. The results suggest that the barrier function differs in the follicular epithelial cells of the primordial and developed follicle of the sexually immature animal. This hypothesis appears all the more probable because previous investigations by the writers, using the same experimental method, on sexually mature animals showed that not only the granulosa, but also the theca interna [2], in the developing follicle possesses a well-marked barrier function.

LITERATURE CITED

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