DEMONSTRATION OF THE TISSUE – BLOOD BARRIER IN THE OVARY BY INTRAVITAL CONTACT FLUORESCENCE MICROSCOPY

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The penetration of acidic and basic fluorochromes into the follicles of the ovary was studied by intravital contact fluorescence microscopy. After intravenous and intraperitoneal injection of acriflavine and fluorescein into anesthetized rats in doses of 0.05-5 mg/kg the dyes did not penetrate into the cavity of the developing follicle and did not stain the sex cell. In some follicles slight penetration of acriflavine into the granulosa cells was observed after its injection. Since the fluorochromes were held up in the region of the theca interna and follicular epithelium it is concluded that a tissue–blood barrier exists in the ovary (blood-follicular barrier), and that the cells of the theca interna and follicular epithelium play a major role in its formation. Follicles undergoing atresia have no barrier.

Many recent investigations have been devoted to the discovery of new and the study of already known tissue–blood barriers in mammals. The presence of such a barrier has recently been shown in the testis [8] and its role has been studied under normal and certain pathological conditions [1, 6, 11, 13]. Attempts have been made to discover a tissue–blood barrier in the ovary [9, 10, 12], but no definite conclusions have yet been drawn. For example, in a study of the rate of penetration of various substances (proteins, ions) and the formation of their maximal concentrations in the follicular fluid, appreciable differences have been found in the rate of penetration of substances from the plasma into the follicular fluid and much lower concentrations were formed than in the plasma [9, 10, 12]. However, this fact was not interpreted as evidence of the existence of a tissue–blood (blood-follicular) barrier in the ovary. It must be pointed out that investigations of the follicular fluid evidently cannot help to find the answer to this question. The trophic requirements of the maturing ovum are known to be satisfied by the granulosa cells and direct contact does not exist between the follicular fluid and the ovum. Such contact can arise for a short time only after rupture and, possibly, atresia of the follicle. For that reason the method of studying the permeability of the follicle for various substances can provide a reliable intravital control of the state, not so much of the follicular fluid as of the state of the theca interna, the granulosa cells, and, of course, the ovum itself. These requirements are met most closely by the technique of intravital contact microscopy, previously used with success to study the blood–testicular barrier [1]. The choice of substances for use in this method is also of great importance in experiments to detect tissue–blood barriers; for instance, the discovery of the blood-testicular barrier for the first time was made possible by the use of fluorescent compounds [11, 13], and the subsequent use of fluorochromes in conjunction with intravital contact microscopy made observations on the state of the blood–testicular barrier possible under normal and some pathological conditions [1, 6].

The writers therefore used the method of intravital contact fluorescence microscopy, on which advice was given by E. M. Brumberg, to demonstrate a tissue–blood barrier in the ovary.

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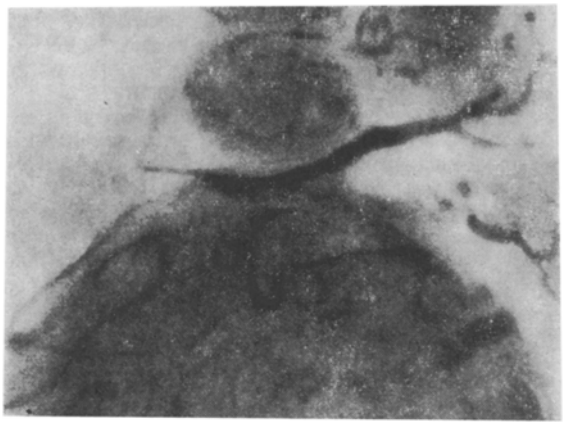

Fig. 1. Ovary of rat 30 min after intraperitoneal injection of 0.1% fluorescein solution. Against the background of the brightly and diffusely luminescent stroma a dark, large, developing follicle and two smaller follicles can be seen; the vascular network also appears dark, for the living erythrocytes do not take up the fluorochrome. Intravital microscopy, OI-30 and OLK-2. Contact objective 10 × 0.40, ocular 4×, subsequent photographic enlargement up to 160×.

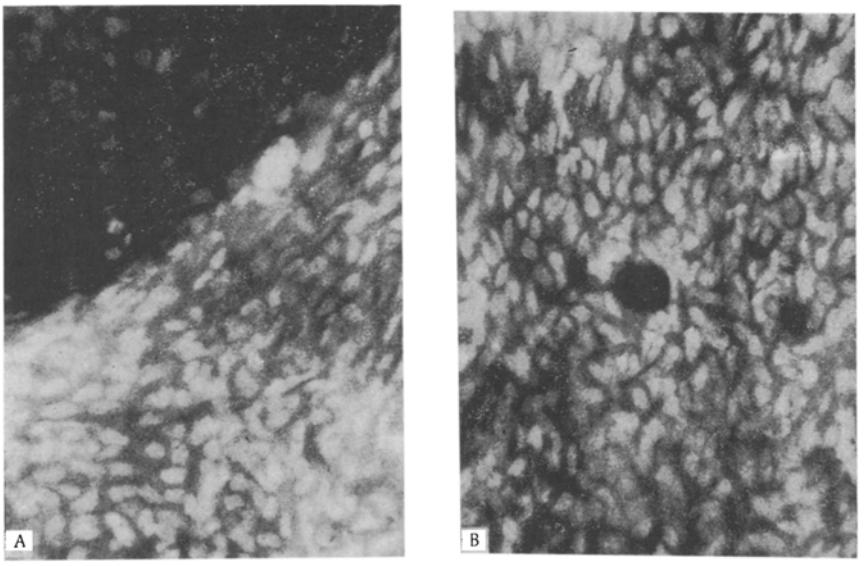

Fig. 2. Ovary of a rat 30 min after intraperitoneal injection of 0.1% acriflavine solution: A) peripheral zone of large ripening follicle; nuclei and cytoplasm of cells of ovarian stroma brightly fluorescent, granulosa cells very palely stained, acriflavine does not penetrate into cavity of follicle. B) Primordial follicles visible in "optical section" (in center of film) and from surface (at right border): ovum does not contain fluorochrome, granulosa cells palely stained, very bright fluorescence of cells of theca and ovarian stroma. Intravital microscopy, OI-30 and OLK-2. Contact objective 25 × 0.75 and ocular 4×, subsequent photographic enlargement to 400×.

EXPERIMENTAL METHOD

Experiments were carried out on 54 sexually mature noninbred female albino rats weighing 100-240 g. Solutions of acidic and basic fluorochromes, namely 0.01 and 0.1% solutions of fluorescein and a 0.1% solution of acriflavine in doses of 0.05 and 0.5 ml (0.5-5 mg/kg body weight respectively), were injected intravenously or intraperitoneally into the experimental animals. The rats were anesthetized by intraperitoneal injection of thiopental sodium in doses of 75-100 mg/kg body weight. After the onset of deep sleep laparotomy was performed on the animal and intravital contact microscopy was carried out on the ovaries (without damage to the tissues) by means of the OI-30 luminescence attachment or a modified MLK-1 luminescence contact microscope; the ovaries were studied by contact microscopy not only in the light of luminescence, but also in incident light (20 rats as the control). The principle of the design of these instruments and the opportunities provided by their use have been described elsewhere [2-5, 7], and they are also mentioned in descriptions issued by the manufacturer (Leningrad Optico-Mechanical Combine).

EXPERIMENTAL RESULTS

On intravital contact microscopy of rat ovaries in incident light all details of their histological structure could be clearly distinguished: a rich vascular network around the follicles, a theca-like stroma, primordial and ripening follicles. In the ripening follicles, by changing the focus, it was possible to distinguish the granulosa cells, the cavity of the follicle, and the ovum itself. In the light of fluorescence (without injecting fluorochrome into the animal) a weak, diffuse bluish-green fluorescence could be seen in the stromal and granulosa cells of the ovary, together with bright orange-yellow intrinsic fluorescence in some cells of the stroma and in the cells of the corpus luteum.

After injection of 0.1 and 0.01% fluorescein solutions the picture observed was virtually identical, differences being found only in the brightness of fluorescence. Fluorescein, injected intravenously, appeared instantaneously in the plasma of the vascular network of the ovary; if injected intraperitoneally it appeared in the vascular network of the ovary for 2-3 min, in a rather lower concentration. In both cases a few seconds after its appearance in the plasma of the vascular network the fluorescein penetrated through the walls of the capillaries and stained the cytoplasm of the ovarian stromal cells diffusely. However, it did not penetrate into the follicular cells, the follicular fluid, and the ovum of the ripening and primordial follicles by the end of the period of observation (40-50 min), as a result of which these structures appeared as dark formations against a light background (Fig. 1). The cells of the theca interna accumulated fluorescein in a rather smaller quantity than the rest of the stroma (Fig. 1).

After intravenous injection of 0.1% acriflavine solution into the rats the dye appeared instantaneously in the blood plasma bathing the ovary, passed quickly (within a few seconds) through the wall of the capillaries, the small arterioles, and veins (staining the nuclei and cytoplasm of their cells), and during the first 2-3 min of observation it stained the nucleus and cytoplasm of the cells of the ovarian stroma intensely. Acriflavine, however, penetrated only slightly, before the end of the period of observation (40-50 min), into the follicular fluid and it did not penetrate into the ovum of the ripening follicles. The cells of the follicular epithelium either stained very palely, and only in the outer layer, or they contained no acriflavine whatever (Fig. 2A). Acriflavine likewise did not penetrate into the primordial follicles and did not stain the nucleus and cytoplasm of the sex cells, although the surrounding follicular cells under these conditons were sometimes stained (Fig. 2B). The cells of the theca interna always fluoresced more brightly than the stroma surrounding them. The same picture was observed after intraperitoneal injection of acriflavine.

The results of intravital fluorescence contact microscopy thus show that acidic and basic fluorochromes, if injected into the blood stream, virtually do not penetrate into the primordial and ripening follicles (except those undergoing atresia) and they do not stain the cytoplasm of the sex cells brightly. This observation suggests that a barrier exists between the female sex cells and the blood and that it is formed by follicular cells, including cells of the theca interna of the ripening follicle. By contrast with other barriers (such as the blood–brain barrier) the blood vessel wall evidently plays no part in the formation of the blood–follicular barrier. Recent investigations [1] by intravital contact microscopy have shown that the blood vessel wall likewise does not play a significant role in the formation of the blood–testicular barrier. Consequently, this feature is evidently common to the sex glands and it may perhaps be explained by the special nature of their function. Further investigations in this direction will provide a fuller explanation of the biological role and importance of the blood–follicular barrier.

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