develop castrate smears, but developed the smear characteristic of mice bearing cortical tumors within a few cycles after lengthening of the cycle (within 30-50 days after the last litter the vaginal smear picture typical of cortical adenoma appeared).

Histologic study of the ovary revealed that at one year of age Graafian follicles were present in the ovaries of all stocks except the NH. (The reason for lack of cycles in the F strain is not clear). The evidence suggests that physiologic castration may be a factor in the development of adenomas in the NH females. It is of interest that the smear typical of tumor formation appeared before there was histologic evidence for a well developed tumor. Sections of the vagina showed extreme cornification. It is probable that the histologically unaltered NH adrenal may secrete estrogen in quantity sufficient to stimulate the female reproductive tract.

When only the ovaries were removed from 6 tumor-bearing mice, the vaginal smear picture was unaltered. When the adrenals alone were removed‡ (ovaries remaining) then the vaginal smear became of castrate type within 5 days (7 cases). This constitutes proof that the adrenal tumors were the primary source of estrogen.

‡ Animals maintained on aqueous cortical extract—Upjohn.

The effect of gonadotrophic hormone was studied on the same mice (adrenalectomized or ovariectomized). One hundred and ten international units (5 units daily for 22 successive days) of pregnant mare serum§ elicited no change in the castrate smear of the adrenalectomized NH females, indicating the refractoriness of the ovaries to gonadotrophic hormone (the ovaries of older mice of other stocks were readily stimulated). The same amount of this hormone, when given to 3 ovariectomized mice (with adrenal tumors) induced a change in the vaginal smear picture: (1) the quantity of secretion was increased, and (2) the ratio of cornified to non-cornified cells was altered from 25:75 to at least 50:50. The number of leukocytes remained the same. It would appear that gonadotrophic hormone can influence sex-hormone secretion of an adenoma of the adrenal cortex.

Summary. The occurrence of spontaneous estrogen-secreting tumors of the adrenal cortex can be correlated with early cessation of ovarian activity in the NH stock. Estrogen is probably secreted by the adrenal cortex even preceding adenoma formation. Gonadotrophic hormone enhanced estrogenic secretory activity of cortical adenomas.

§ Gonadogen—Upjohn.

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Early Effect of X-rays on Ovaries of Normal and Adrenalectomized Rats.*

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In a previous paper[1] we described the changes occurring in the rat ovary during the first few hours after irradiation with X-rays.

These alterations are characterized by the presence of numerous pycnotic nuclei of the follicular cells of the granular layer of the follicle.

We are able to distinguish between pycnotic nuclei induced by irradiation and those normally found in certain ovarian follicles, by showing that pycnotic nuclei of granular cells

* Aided by a grant from the British Empire Cancer Campaign.

1 Halberstaedter, L., and Ickowicz, M., *Radiology*, 1947, **48**, 369.

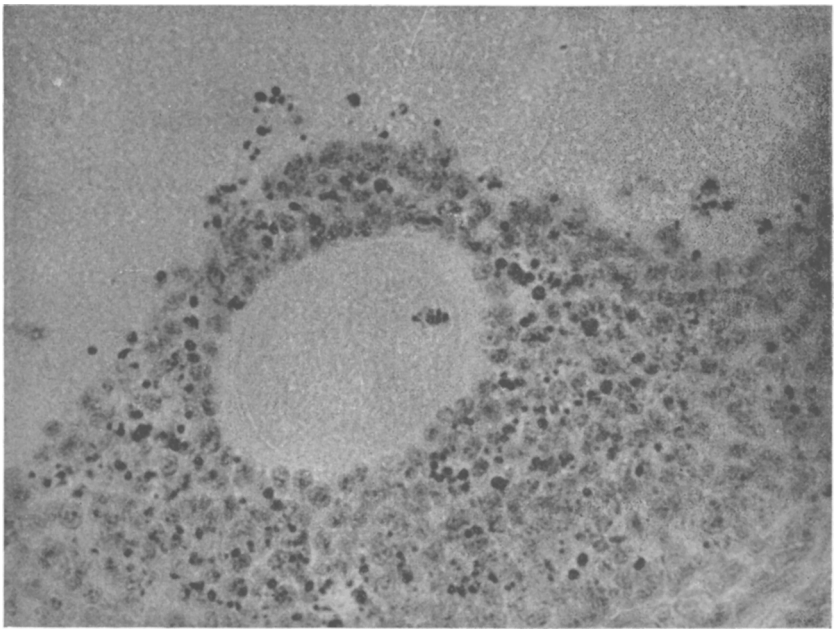

FIG. 1.
Adrenalectomized rat. Graafian follicle with numerous pycnotic nuclei. The nucleus of the ovum is in mitosis. × 640.

in the ovaries of non-irradiated rats occur only in follicles in an advanced stage of development, and whose ovules are already dividing, whereas after X-irradiation numerous pycnotic nuclei are also found in very young follicles and in those whose ovules are in the resting state. In studying the earliest ovarian lesions induced by irradiation it is therefore necessary to examine the entire organ microscopically by means of complete serial sectioning, in order to determine the condition of the ovule.

In addition, we have observed that during the first few hours after irradiation ovarian lesions are limited particularly to follicular cells, while no lesions are to be seen in the other elements of the ovary.

The object of the present study was to determine the effect of adrenalectomy on pycnosis of the follicular cells of the rat ovary during the first few hours after X-ray irradiation.

Methods and Technique. Our experiments on the ovaries of highly inbred albino rats (weighing approximately 150 g) fall into the following 3 categories:

A. Adrenalectomized rats. These animals were killed 24 hours after double adrenalectomy.

B. Irradiated rats. These animals were irradiated over the abdominal region with 2000 r and were killed 4 hours after irradiation.

C. Adrenalectomized and irradiated rats. These animals were adrenalectomized and were irradiated over the abdominal region with 2000 r 24 hours later. They were killed 4 hours after irradiation.

Irradiation was delivered by a Machlett X-ray tube operated on a multivolt apparatus of 150 K.V., Al. 0.5 mm, distance 30 cm, intensity 100 r/minute. The ovaries were fixed in Bouin's fluid. Complete serial sections were prepared and stained with iron hematoxylin and eosin, according to the method of Masson.

Histological Description. The following microscopic description is based on the composite data for each group of rats, with special emphasis on pycnotic nuclei of the follicular cells and their relationship to the state of the ovule.

A. *Adrenalectomized rats.* Follicles at differ-

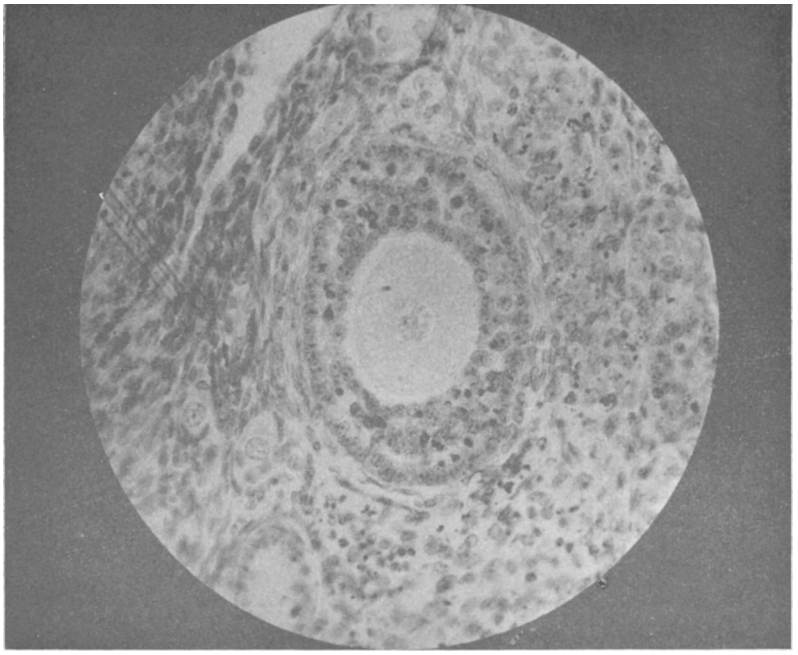

FIG. 2.
Rat irradiated with 2,000 r. Young follicle with pycnotic nuclei. × 450.

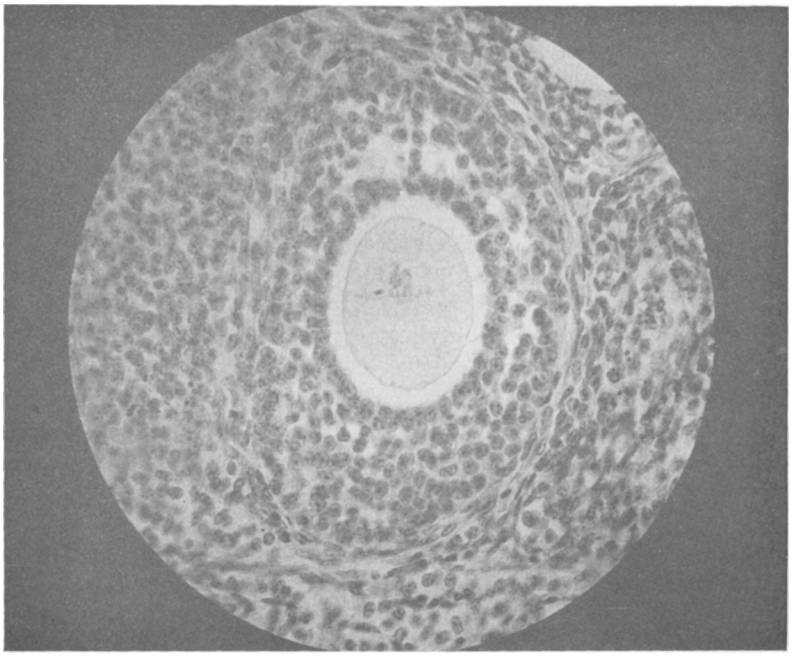

FIG. 3.
Rat adrenalectomized and irradiated with 2,000 r. Young follicle without pycnotic nuclei. × 450.

ent developmental stages were found: Graafian follicles, as well as stratified and young ones. The Graafian follicles of varying stages may be divided into 2 groups: a) Those with pycnotic nuclei in the granular layer, and with a dividing ovule (Fig. 1); and b) those without pycnotic nuclei or with very few in the granular layer and with a resting ovule.

We have, however, come across one ovary with Graafian follicles containing resting ovules, but with a considerable number of pycnotic nuclei in the granular layer. Such follicles are very unusual. The presence of pycnotic nuclei in the granular layer of young follicles (from primary to stratified follicles) is likewise exceptional. On the contrary, mitoses are frequently seen in the granular layer of young follicles.

Furthermore, no changes associated with adrenalectomy have been observed in the other ovarian elements, such as the atresic follicles, corpora lutea, blood vessels or the general stroma. Thus, the ovaries of adrenalectomized rats 24 hours after the operation do not differ significantly from normal rat ovaries.

B. *Irradiated rats.* Microscopic examination 4 hours after X-ray irradiation revealed extensive pycnotic lesions of the follicular cells in all advanced as well as in young follicles (Fig. 2). The number of atresic follicles seems to have increased. However, very rarely, some Graafian follicles appear to be resistant to X-rays; but these are exceptional cases and do not alter the general picture of the massive pycnotic lesion observed in the ovaries 4 hours after irradiation with an adequate dose.

C. *Adrenalectomized and irradiated rats.* Microscopically no pycnotic nuclei were seen in cells of the granular layer in young follicles (Fig. 3) and in the majority of Graafian follicles containing resting nuclei. As in the ovaries of normal rats, numerous pycnotic nuclei were seen in follicles with dividing ovules. However, we have also observed pycnotic nuclei in certain follicles containing resting ovules in the ovaries of one rat. But these lesions were, nevertheless, infinitely less pronounced than those observed in irradiated rats without previous adrenalectomy. No changes were seen in the other ovarian elements.

Discussion. Our experiments demonstrate that adrenalectomy inhibits the appearance of pycnotic lesions in the rat ovary 4 hours after X-ray irradiation. These observations are analogous to those obtained with lymphatic tissues and described by Leblond and Segal[2] and by Halberstaedter and Ickowicz.[3]

The small cells in the granular layer of the ovary appear to react as do lymphocytes. We are as yet unable to tell just how far this analogy may be drawn, since our experiments deal only with pycnotic lesions due to X-ray irradiation. Actually, observations on lymphocytes have shown that these cells are extremely sensitive not only to X-rays but also to a number of harmful agents, both chemical and physical[4] and even to so simple a trauma of the animal organism as an incision of the skin.[5] In any case our experiments clearly demonstrate that under certain conditions, such as X-ray irradiation for example, the adrenal glands influence the pycnotic reaction of the cells of the granular layer of the ovary.

An important point brought to light by our experiments is the fact that adrenalectomy inhibits pycnosis of the granular cells of the ovary even after direct irradiation of the organ. This constitutes a special phenomenon, since in the case of lymphocytes adrenalectomy seems to influence the ensuing pycnosis caused only by the indirect effect of the irradiation.[2,3] So far we have not yet elucidated the question of the influence of adrenalectomy on the indirect effect of irradiation on the ovary.

Summary. The pycnotic lesions of the cells in the granular layer of the ovarian follicles of rats 4 hours after X-ray irradiation are inhibited by previous adrenalectomy.

[2] Leblond, C. P., and Segal, G., *J. Roentgenol. and Rad.*, 1942, **47**, 302.

[3] Halberstaedter, L., and Ickowicz, M., *Radiologia Clinica*, 1947, **16**, 240.

[4] Selye, H., *J. Clin. Endocrin.*, 1946, **6**, 117.

[5] Ickowicz, M., *Radiologia Clinica*, 1947, **16**, 231.