

8754 C

Comparative Studies of Gonadotropic Hormones.* 4. Ovaries and Hypophyses of Rats in Chronic Experiments.

C. F. FLUHMAN.

From the Department of Obstetrics and Gynecology, Stanford University School of Medicine.

In a previous report¹ some significant differences in 3-week experiments were noted between rats given a gonadotropic preparation made from human pregnancy blood and rats injected with an extract of sheep anterior pituitary material. The present study deals with the changes resulting in the ovaries and hypophysis from the use of such hormones for periods varying from 2 months to one year.

Both preparations were made in the same way, by extracting with a sodium acetate-acetic acid buffer, pH 4.2 to 4.5 and reprecipitating with ethyl alcohol, according to the method previously described.² In all cases the experiments were begun when the rats were 20 to 23 days of age. A single daily injection of 0.25 cc. of each extract was used, and the controls consisted either of small numbers of litter-mates or larger groups of rats of the same age.

Sheep Anterior Pituitary Extract. When given to immature rats once daily for 5 days, the sheep anterior pituitary extracts produced an average increase of ovarian weight varying from 70 to 267%. In 63 days, the average increase in the ovarian weight of 6 rats was 153% (body weight 135 gm., ovaries 0.114 gm.), and after 94 days the ovaries of 3 animals had an average increase in weight of only 57%, while the anterior pituitary gland gave an increase of 44% (body weight 174 gm., ovaries 0.107 gm., hypophysis 0.013 gm.). The continued daily administration of the extract resulted in a further decrease in the size of the ovaries, while the hypophysis remained larger than normal. After 150 days the ovaries of 5 rats gave a 47% diminution in weight as compared to the controls, while the hypophysis showed a 27% increase (body weight 190 gm., ovaries 0.031 gm., hypophysis 0.014 gm.). Five rats injected for 179 days gave an average diminution in ovarian

* Supported in part by a grant from the Rockefeller Fluid Research Fund of Stanford University School of Medicine. The sheep anterior pituitary glands were supplied by Messrs. Parke, Davis & Company, through the courtesy of Dr. E. A. Sharp.

¹ Fluhmann, C. F., *Am. J. Physiol.*, 1933, **106**, 238.

² Fluhmann, C. F., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 1193.

weight of 37%, while the hypophysis increased 66% (body weight 206 gm., ovaries 0.042 gm., hypophysis 0.016 gm.).

The histologic examination of the ovaries and anterior pituitary glands presented some interesting changes. The ovaries of the rats injected for 63 days showed the characteristic picture of small corpora lutea, lutein cysts, and developing graafian follicles. In 94 days they gave essentially the same findings, but the anterior hypophysis showed a marked increase in the number of basophilic cells with many vacuolated "castration cells". After 150 and 179 days the changes in the anterior lobe were present to a more marked degree, while the ovaries had undergone a pronounced atrophy, which was also noted in the uterus and vagina. The ovaries generally contained a few atretic follicles but no corpora lutea, and the stroma showed fibrous tissue growth with hyaline degeneration.

Pregnancy Blood Extract. The administration to female rats of a chorionic gonadotropic hormone prepared from blood of pregnant women resulted in an increase of ovarian weight without the subsequent marked diminution in size which has been reported from the use of similar extracts made from pregnancy urine or placentae (Zondek,³ McPhail,⁴ Collip *et al.*,⁵ Wolfe⁶). During the first 175 days of this experiment a dilution of the extracts was used, which in 5 days induced an average increase of 130% in the weight of the ovaries of 20 immature rats, but subsequently the preparations were employed at a dosage which produced an average 5-day increase of 250% in 7 young rats. The average increase in ovarian weight of 11 rats injected for 28 days was 400% and in the weight of the hypophysis, 25% (body weight 75 gm., ovaries 0.071 gm., hypophysis 0.004 gm.); in 59 days the ovaries of 7 rats showed increases of 200% and the hypophysis 80% (body weight 103 gm., ovaries 0.137 gm., hypophysis 0.009 gm.); in 90 days, 9 rats gave an average increase in the weight of the ovaries of 289% and the hypophysis of 48% (body weight 152 gm., ovaries 0.240 gm., hypophysis 0.012 gm.); and in 193 days the ovaries had increased 343% in weight while the hypophysis gave the same average weight as the controls (body weight 188 gm., ovaries 0.232 gm., hypophysis 0.013 gm.). Four rats were injected daily for 272 days, and owing to the wide variations found in organ weights it seems more accu-

³ Zondek, B., *Die Hormone des Ovariums und des Hypophysenvorderlappens*, Julius Springer, Berlin, 1931.

⁴ McPhail, M. K., *J. Physiol.*, 1933, **80**, 105.

⁵ Collip, J. B., Selye, H., Thomson, D. L., and Williamson, J. E., *Proc. Soc. Exp. Biol. and Med.*, 1933, **80**, 590.

⁶ Wolfe, J. M., *Anat. Rec.*, 1935, **63**, 1.

rate to quote the gross weights of each animal instead of using the average for the whole group. The body weights were 235, 248, 232, and 251 gm., the ovaries 0.091, 0.312, 0.337, and 1.137 gm., and the hypophysis 0.018, 0.031, 0.033, and 0.018 gm., while 2 controls gave an average body weight of 204 gm., ovaries 0.066 gm., and the hypophysis 0.011 gm. The injections were continued for 365 days in 2 animals. One (No. 25) became ill toward the end of the experiment and weighed only 190 gm., while the ovaries weighed 0.205 gm., and the hypophysis 0.008 gm. The second rat (No. 37) weighed 253 gm., ovaries 0.236 gm., and hypophysis 0.034 gm., as compared to the average of 2 littermate controls with body weight 242 gm., ovaries 0.068 gm., and hypophysis 0.014 gm.

The histologic examination of the ovaries of the rats injected for 272 days or less showed the presence of developing follicles, lutein cysts, large corpora lutea, and a hypertrophy of the stromal cells. A characteristic finding was the occurrence of relatively large cysts lined with connective tissue, which increased in number as the experiment progressed. The ovaries of the rats injected for one year contained many such cysts, the stroma was atrophic, there was an extensive round-cell infiltration and many phagocytic cells containing blood pigment. The enlarged anterior lobes showed changes apparently comparable to those found by Severinghaus,⁷ Wolfe *et al.*,⁸ and Wolfe⁶ following the injection of pregnancy urine, namely, an increase of chromophobes and a decrease in eosinophiles, but careful cell-counts were not conducted. The uteri of 2 rats injected for 272 and 365 days respectively showed an extensive metaplasia of cylindrical epithelium to squamous epithelium. The mucosa of the vaginae of rats treated for 193 days or longer underwent extensive vacuolization and the lumina were lined with tall, stratified, mucified cells.

Summary. The daily administration for periods of 2 to 6 months of a sheep anterior pituitary extract to female rats resulted in a marked atrophy of the ovaries after a preliminary increase in weight. This change was associated with an increase in the weight of the hypophysis and the appearance of many "castration cells" in the anterior lobe.

The continued daily injection of a gonadotropic preparation made from blood of pregnant women produced a marked increase in ovarian weight which was maintained for as long as one year. The hypophysis also showed a marked increase in weight which was

⁷ Severinghaus, A. E., *Anat. Rec.*, 1934, **60**, 43.

⁸ Wolfe, J. M., Ellison, E. T., and Rosenfeld, L., *Anat. Rec.*, 1934, **60**, 357.

associated with histologic changes affecting the chromophobes and eosinophilic cells.

8755 P

Cytological Studies of Biopsied Pernicious Anemia Bone Marrow During Relapse.

OLIVER P. JONES. (Introduced by C. M. Jackson.)

From the Hematological Laboratory, Department of Anatomy, University of Minnesota.

In the past we have reported that in pernicious anemia marrow during relapse there is a pathologic hemoglobiniferous series, the megaloblastic, which is not related to the normoblastic or definitive red cell series.¹ The only thing these two series have in common is their ancestral cell, the myeloblast. The present report deals with certain observations which were made while the above mentioned work was in progress and takes into consideration pathologically altered neutrophils and megakaryocytes found in pernicious anemia marrow.

The present investigation was carried out upon nine bone marrows from patients with pernicious anemia during relapse. Six were prepared by smearing freshly aspirated sternal marrow and three were dry imprints (abklatsch). All preparations were stained with May-Grünwald-Giemsa combination of Pappenheim. The best morphological detail of marrow cells was elicited by the dry imprint method, consequently these preparations were studied more intensively than films of aspirated marrow.

Studying large neutrophils (macropolycytes) revealed that their entire life history is quite different from that encountered in normal bone marrow. As early as the leukoblast stage the nucleus has assumed a polymorphic shape as indicated by peculiar protuberances and invaginations. During the promyelocyte stage the nucleus may be shaped like a giant "stab" form. In other words, nuclear development and maturation has preceded that of the cytoplasm to such an extent the large pathologic neutrophils have skipped myelocytes and metamyelocyte stages. This particular point has been previously mentioned by Tempka and Braun.² Generally the arrange-

¹ Jones, O. P., *Anat. Rec. (Suppl.)*, 1934, **58**, 23; *ibid.*, 1935, **61**, 57.

² Tempka and Braun, *Folia Haematol.*, 1932, **48**, 355.