

accord with those reported by Mazer and Mazer⁹ and by us¹ where treatment was begun in slightly older but still immature rats. The development of tubal lesions in rats injected from birth is of interest in connection with the observations of Green and Ivy¹⁰ and Hamilton and Gardner¹¹ who found pyometra and distention of the vagina in rats subjected to an androgenic stimulus during the last third of intrauterine growth.

Conclusions. 1. Testosterone propionate injected into female rats from the day of birth arrests follicular development beyond the stage of the antrum and prevents corpus luteum formation. 2. These findings extend our previous observations on adult and 21-day-old rats, and it is therefore concluded

⁹ Mazer, M., and Mazer, Ch., *Endocrinology*, 1939, **24**, 175.

¹⁰ Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 200.

¹¹ Hamilton, J. B., and Gardner, W. U., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 570.

that testosterone in the doses used prevents luteinization of the rat's ovary at any age. 3. When treatment is initiated on the day of birth, there is an arrest of follicular development and a partial transformation of the follicles into seminiferous tubule-like structures, not observed in our previous study with older rats. 4. Inflammatory lesions representing various stages of salpingitis, mesosalpingitis, and periovarian cellulitis appeared in about 40 per cent of the experimental animals. 5. The formation of tubal abscesses, caused by secondary bacterial invasion, is preceded by the stimulation of secretory changes in the tubal epithelium.

Since the paper was submitted for publication, a preliminary report by H. B. Hale and C. K. Weichert appeared in the March issue of these *PROCEEDINGS* (1944, **55**, 201), in which the authors report on ovarian tumors in adult rats following prepuberal administration of estrogens. Grossly, these tumors resembled those reported by Shay *et al.*⁶

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A Method of Blood Replacement in the Rat.

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In the course of experiments in this laboratory it became necessary to remove a large proportion of the blood of a rat and to replace it rapidly with other blood. Preliminary experiments showed that the readily accessible vessels in the rat, such as the jugular, femoral, and tail veins, are not suitable for the rapid withdrawal and replacement of blood. It was found, however, that the inferior vena cava is a very satisfactory site for the performance of this operation, and that rats recover rapidly in spite of the surgical procedure which is necessitated by the use of this vessel.

Method. The rat (wt. 150-250 g) is anesthetized, the abdomen is incised, and the inferior vena cava is exposed. A 20-gauge hypodermic needle which has been bent at an angle of 45° about 0.5 cm from the tip is inserted into the vessel and is held firmly in

position by means of a clamp. The rat is heparinized (25 units per 100 g body wt) by attaching to the needle a syringe containing heparin solution (Connaught Laboratories, Toronto; 1000 units of heparin per ml), drawing 1 ml of blood into the syringe and then reinjecting it. An amount of blood not exceeding 5 ml is then drawn into the syringe. The syringe is replaced by another containing the same volume of heparinized blood and this is then injected. After the replacement process has been carried out the required number of times, the vessel around the site of entry of the needle is grasped with smooth blunt forceps and the needle is carefully withdrawn. A few drops of a solution of thrombin*

*The writer wishes to thank Parke, Davis and Company for a gift of a supply of thrombin.

in saline are then placed on the bleeding point and the pressure of the forceps is gradually released. Under these conditions bleeding from the vessel can readily be prevented. The incision in the abdominal wall is then closed with plain No. 00 catgut. Surgical silk is used to close the skin incision and these sutures are removed 7 days later.

Application of the method. The procedure described above was first studied with 6 rats (wt range 158-250 g, average wt 199 g). In each experiment 5 ml of blood were withdrawn and reinjected several times at short intervals. After showing small losses in weight during the 48 hours following the operation the rats gained steadily in weight.

A second series of experiments with 6 rats was performed. In each experiment 4 withdrawals of 3 ml of blood were made over a period of 4 minutes. After each withdrawal 3 ml of pooled heparinized blood from normal rats of the same strain were injected. The average red cell counts of these animals on the 7th and 14th days after the operation did not differ significantly from those found on the 6th and 2nd days before the operation. The rats recovered rapidly and the internal organs appeared normal on gross examination when the animals were killed 14 days after the operation. The average weight of the rats was 160 g at the time of operation and 14 days later it was 200 g. A control series of 6 rats increased in average weight from 161 g to 195 g during the same period.

The average blood volume of the rat has been reported as 4.3 ml,¹ 6.13 ml,² and 7.4 ml³ per 100 g of body weight. In the present

work experiments using the Evans blue technic gave values of 6.7 ml and 6.5 ml of blood per 100 g of body weight for a male and a female rat weighing 150 g and 170 g respectively. After four 3-ml portions of blood from each of these animals had been withdrawn and had been replaced by blood from other rats by means of the procedure described above, the concentration of dye in the plasma indicated that 72% of the original blood had been replaced in both cases. The calculated replacements based on the determined blood volumes are 76% and 72% respectively.

In addition to experiments of the type which have been described it has been found possible to use the method in the performance of cross-transfusion experiments. In such experiments the same amount of blood is drawn from two animals simultaneously, the syringes are interchanged and the blood is injected.

Summary. A method of rapid blood replacement in the rat is described in which the inferior vena cava is used as the channel of withdrawal and replacement of the blood. The results of experiments in which this method was applied are described.

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¹ Griffith, J. Q., and Campbell, R., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 38.

² Scott, J. M. D., and Barcroft, J., *Biochem. J.*, 1924, **18**, 1.

³ Went, S., and Drinker, C. K., *Am. J. Physiol.*, 1929, **88**, 468.