cryptorchids bearing functional ovarian grafts have essentially normal pituitaries although many of the basophiles show evidence of degranulation. This degranulated condition of the basophiles is more pronounced in castrates and normals receiving daily injections of estrin. These findings confirm the earlier work[7, 8] concerning the effect of estrin on the basophiles of the rat hypophysis.

The changes in the guinea pig hypophysis in castrate, cryptorchid, and estrin-injected animals are seen to be of the same character, although less marked, as those found in the rat. This study serves as a basis of interpretation for the results following ovarian transplantation in guinea pigs of both sexes.

8197 P

Presence of Anti-Pernicious Anemia Principle in Normal Human Urine.*

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In a previous communication[1] we reported a significant modification of the pigeon method of Vaughan, *et al.*,[2] for the laboratory assay of anti-pernicious anemia preparations and showed that normal human urine contains a principle which significantly increases the reticular material in the red blood cells of the pigeon in a manner analogous to the anti-pernicious anemia principle present in liver. At the same time, we reported the preparation of a urinary extract which had a similar effect. In order to test for the identity of the urinary and liver principles, we indicated our plan to study the effect of the urinary extract in pernicious anemia. Due to the well-known present difficulty in securing pernicious anemia patients suitable for assay work, this preliminary report deals with the results obtained with the extract in one patient.

7 Nelson, W. O., *Anat. Rec.*, 1933, **55**, 70 (suppl.).

8 Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 452.

* This work was aided by a grant from the Therapeutic Research Committee of the American Medical Association.

1 Wakerlin, G. E., Bruner, H. D., and Kinsman, J. M., *Proc. Am. Physiol. Soc.*, 1935, pg. 136.

2 Vaughan, J., Muller, G. L., and Zetzel, L., *Brit. J. Exp. Path.*, 1930, **11**, 456.

The extract was prepared by concentrating urine to a small volume *in vacuo* at a temperature of 37°C., adding sufficient 95% ethanol to give a concentration of 70%, concentrating the resulting filtrate *in vacuo* to a small volume at 37°C., adding sufficient 100% ethanol to give a concentration of 95%, collecting and drying the precipitate, and finally dissolving it in physiological salt solution containing 0.5% phenol. Enough saline solution was used to give a 1:20 ratio for the volumes of the extract and the original urine respectively, although a smaller amount of the solvent would have sufficed. This method is basically that commonly employed in the preparation of parenteral liver extracts.

The volume of this urinary extract necessary to increase significantly the reticular material of the pigeon is approximately equal to that for one commercial parenteral liver extract (3 cc. equivalent to 100 gm. of liver), slightly less than that for another (2 cc. equivalent to 100 gm. of liver), and definitely less than that for a third (20 cc. equivalent to 100 gm. of liver). Blood pressure studies on the rabbit showed the urinary extract to contain a somewhat lower concentration of depressor substances than the parenteral liver extracts just mentioned. The rabbit was found to tolerate a dosage of 5 cc. per kilo of the urinary extract administered intravenously. Larger amounts were not used. Subcutaneous and intramuscular injections of the extract (previously tested for sterility) into the rabbit produced no gross evidence of chemical inflammation.

The patient† employed in this study was first admitted to the Louisville City Hospital in September, 1933, when 45 years of age, with typical clinical and laboratory findings of pernicious anemia, inclusive of early cord changes. Oral liver therapy induced a regression of his signs and symptoms with an improvement in his blood picture from 1,600,000 R.B.C. and 47% Hb. (Sahli) to 3,200,000 R.B.C. and 68% Hb. by the eighteenth day. A maximal reticulocytosis of 23% was observed on the eighth day of therapy. During his subsequent 20 days in the hospital, the patient's R.B.C. and Hb. remained essentially unchanged despite a daily treatment regimen of 500 gm. of liver by mouth, supplemented by 3 cc. of liver extract (equivalent to 100 gm. of liver) intramuscularly. Although oral liver therapy was continued for a time following discharge from the hospital, a subsequent observation period of 2 months in the Outpatient Department failed to show any signifi-

† The patient was made available for study through the courtesy of Dr. John Walker Moore of the Department of Medicine of the University of Louisville School of Medicine.

cant change in the blood picture. The patient, therefore, was less responsive than the average to liver treatment.

For 12 months prior to his return to the hospital in April, 1935, the patient had taken no form of anti-pernicious anemia treatment. On this admission a blood study showed R.B.C., 2,650,000; Hb. (Newcomer), 63%; reticulocytes, 0.6%; and W.B.C., 3,500. Largely on the basis of the comparative dosage data obtained from the pigeon assay of the urinary extract cited above, the patient was given a total of 14 intragluteal injections of 3-6 cc. of the urinary extract (each equivalent to 60-120 cc. of urine) during a 35-day period of treatment. The injections were well-tolerated locally and generally. The individual doses and their spacing are recorded in Fig. 1. Iron and ammonium citrate, 6 gm. daily, was also ad-

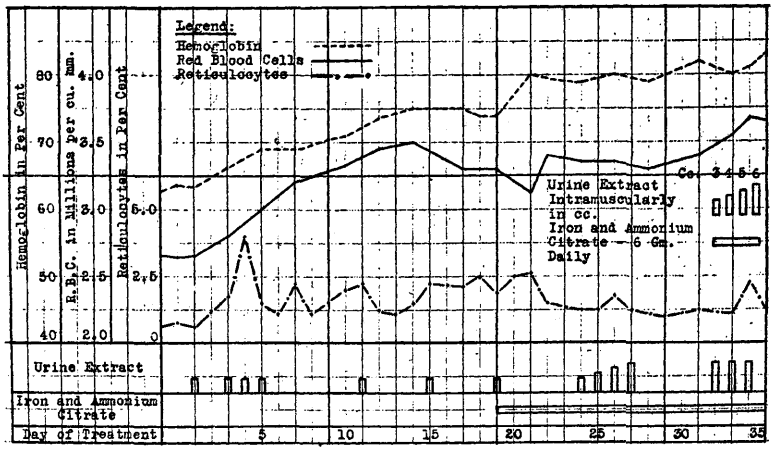

FIG. 1.

The effect of injections of the urinary extract on a patient with pernicious anemia.

ministered during the latter part of the treatment period in view of the patient's tendency to oscillate about a subnormal blood level.[3] Iron alone, of course, is well-known to be without effect in pernicious anemia. No other therapy was given. As seen from Fig. 1, there was a gradual and slightly irregular increase in the R.B.C. and Hb. to levels of 3,640,000 and 83%, respectively by the thirty-fifth day of treatment with the urinary extract. It should be noted that these levels were definitely higher than those previously attained with liver therapy. The W.B.C. also showed a gradual increase to 6,100. A maximal reticulocyte count of 4% occurred on the fourth day. This mild reticulocytosis is, of course, usual with initial

[3] Beebe, R. T., and Lewis, G. E., *Am. J. Med. Sc.*, 1931, **181**, 796.

R.B.C. counts approaching 3,000,000. Coincidentally with the change in his blood picture, the patient was subjectively and objectively improved.

Verification of these results in additional and more suitable pernicious anemia patients with lower blood findings will be attempted as soon as they are available. Arrangements have also been made for trial of the extract in two clinical laboratories. The preliminary data presented, however, suggest that the anti-pernicious anemia principle is present in normal human urine, apparently in sufficient amounts to warrant consideration of the latter as a commercial source for the principle.

8198 C

Absence of Follicle-Stimulating Hormone in Pituitaries of Young Pigeons.

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Earlier studies of Riddle[1, 2] showed that in young pigeons and doves the growth rate of testes is very low during a period of several weeks after hatching; and that during this period of slow testis growth a very rapid body growth is almost completed. Reckoning age from the beginning of embryonic development the curves obtained for testis growth[2] showed that in common pigeons the testes begin a marked increase in growth rate at 2.3 months (1.7 mo. after hatching); in ring doves this spurt in testis growth occurs at about 3.0 months (2.5 months after hatching). The slow growth of testes during the period of rapid body growth was interpreted as evidence that dove and pigeon pituitaries—unlike those of mammals—do not release the gonad-stimulating hormone until the more advanced ages mentioned above; also that the more advanced age at which the testes begin their spurt of growth probably coincides with the initiation of F.S.H. secretion in the bird's pituitary. This interpretation seems to be further supported by the unmatched sensitivity of the immature dove testis to true follicle-stimulating hormone (F.S.H.) administered to these birds. In other words, the great sensitivity of

1 Riddle, O., *Am. J. Physiol.*, 1928, **86**, 248.
2 Riddle, O., *Endocrinology*, 1931, **15**, 307.