parenteral administration, was only 2% that of testosterone.

Kochakian(2) reported that androstanolone was about as androgenic as testosterone propionate in castrated mice. He used a very limited number of doses. The renotropic effects of the two compounds were also similar. Barnes *et al.*(9), using rats, calculated a myotrophic potency of 21.8% and an androgenic potency of 13.2%, thus giving a ratio of 1.6. Again our corresponding figures are slightly higher or 58% and 30% respectively, but the ratio is similar, 1.9. Barnes *et al.* also reported that the cyclopentyl-propionate of 19-nortestosterone was about 1.5 times as potent as testosterone propionate as an anabolic agent and ascribed to it an anabolic/androgenic ratio of 9.3. Our results show 17-ethyl-19-nortestosterone to have a myotrophic potency of 104% and an androgenic potency of only 7% giving an anabolic/androgenic ratio of 15, thus showing the widest separation of effect hitherto reported.

Summary. The androgenic and anabolic effects of testosterone, testosterone propionate, methyl testosterone, methyl androstenediol, androstanolone and 17-ethyl-19-nortestosterone (Nilevar) were compared. The greatest androgenic responses were obtained with testosterone propionate while the most marked anabolic effects were observed with either testosterone propionate or 17-ethyl-19-nortestosterone. Compared to testosterone propionate, methyl androstenediol and androstanolone showed a small increase in the anabolic: androgenic potency ratio but a much more marked increase was observed with 17-ethyl-19-nortestosterone.

1. Kochakian, C. D., *Endocrinology,* 1941, v28, 478.

2. ———, *Am. J. Physiol.,* 1946, v145, 549.

3. McGavack, T. H., Weissberg, J., and Pearson, S., *J. Am. Geriatrics Soc.,* 1954, v2, 489.

4. Pearson, S., Weissberg, J., and McGavack, T. H., *ibid.,* 1954, v2, 26.

5. Saunders, F. J., and Drill, V. A., *Endocrinology,* 1956, v58, 567.

6. Eisenberg, E., and Gordan, G. S., *J. Pharmacol. and Exp. Therap.,* 1950, v99, 38.

7. Hershberger, L. G., Shipley, E. G., and Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 175.

8. Gordan, G. S., Eisenberg, E., Moon, H. D., and Sakamoto, W., *J. Clin. Endo.,* 1951, v11, 209.

9. Barnes, L. E., Stafford, R. O., Guild, M. E., and Olson, K. J., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 35.

10. Henderson, E., and Weinberg, M., *J. Clin. Endo.,* 1951, v11, 641.

Received December 18, 1956. P.S.E.B.M., 1957, v94.

Effects of Age, Castration and/or Steroids upon Hematocrit in Male Rats.* (23040)

JEROME A. GRUNT, WILLIAM H. KNISELY† AND ROGER J. BERRY
(Introduced by J. W. Everett)
Departments of Anatomy and Medicine, Duke University School of Medicine, Durham, N.C.

The effects of age, castration and steroids on the blood of male rats have been studied previously. It has been reported(1) that hemoglobin content rose from 50th to 150th day of life. Several investigators(2-4) have shown that gonadectomy causes mild, transient anemia; these workers and others(2-7) reported that this anemia could be repaired by subcutaneous injections of androgen. Estrogen has been reported to have a moderate (4) or no(7) effect on male rat blood. A recent review article discusses erythropoietic stimuli in additional species(8).

The micro-method of Strumia, Sample and Hart(9) permits hematocrit determinations with very small amounts of blood, thus allowing multiple determinations on a single ani-

* This investigation was supported in part by research grant from Duke University Council on Research.

† This work was done during tenure of Research Fellowship of Am. Heart Assn.

TABLE I. Group Organization and Data.

Group No.	Treatment	Age (days)	No. of animals	Hematocrit
I	Intact animals, untreated	54- 73 m 65 *	22	45.2 ± .4†
II	*Idem*	74-107 m 83.5	22	49.3 ± .5
III	Castrates otherwise untreated	74-102 m 85.9	22	45.3 ± .6
IVa	Self-controls, before castration	60- 87 m 73	16	46.3 ± .7
IVb	" , 14 days after castration	74-101 m 87	16	42.4 ± 1.0
V	Castrates inj. with sesame oil alone	77-102 m 87.7	8	47.3 ± 1.7
VI	Castrates inj. with sesame oil containing 500 μg testosterone propionate	75-108 m 90.1	10	50.1 ± .6
VII	Castrates inj. with 500 μg aqueous testosterone	89- 97 m 92.9	5	49.0 ± 1.6
VIII	Intact, inj. with sesame oil alone	77-102 m 84.4	7	51.6 ± 1.3
IX	Intact, inj. with sesame oil containing 500 μg testosterone propionate	76- 84 m 81.2	10	51.3 ± 1.4
X	Intact, inj. with sesame oil containing 50 μg estradiol benzoate	76- 90 m 82.1	12	48.5 ± 1.6
XI	Castrates inj. with sesame oil containing 50 μg estradiol benzoate	76-101 m 86.7	7	46.8 ± .6
XII	Neonatally castrated animals, otherwise untreated	84-111 m 96	15	50.9 ± 1.0

* m = mean. † Stand. error of mean.

mal. Using this method we investigated the effects of age, castration and/or steroids upon hematocrits of male rats.

Materials and methods. Male rats of locally inbred Osborne-Mendel strain were utilized. Hematocrits were done on blood from cut terminal tail veins or small femoral tributaries. The capillary tubes were held nearly horizontal and one end placed in freely-flowing blood. One drop of blood fills 4 tubes, and very little blood was lost in determining an hematocrit. Tubes were centrifuged at 17,500 rpm for 2 minutes and read on a Drummond microhematocrit reader. The average of a set of 2-6 individual capillary tubes was considered to be the *hematocrit;* 140 hematocrits were thus calculated from 530 individual tubes. There was very little spread among individual tube values; in 18 sets the extreme variation was more than 2.5 points, in 75 sets the extreme variation was from 2.5 to 1, and in 47 sets the extreme variation was 1 point or less. Twelve groups of animals were studied, based upon age and/or treatment. The groups included intact rats of different ages, animals castrated at different ages, intact and castrated animals injected with sesame oil (S.O.) alone, or with S.O. containing hormones,‡ and castrated animals injected with a water-soluble testosterone.§ Litter mates were placed in different categories when possible. In a group called *self-controls,* hematocrits were determined at castration and again 14 days later. Animals castrated within first 24 hours of life are called *neonatal castrates.* Those castrated as adults are referred to as *castrates;* in these 2 weeks elapsed between gonadectomy and onset of injections. When injections were made, 0.5 cc of fluid was given subcutaneously on 7 consecutive days. Specific treatment of each group is indicated in Table I. Mean hematocrits were calculated for each group, comparisons made between groups, and data analyzed by paired-group method with determination of Σd^2; the level of significance was obtained from the Table of probability (10).

Results. Table I presents the groups, their age ranges, mean ages, number of animals in each, mean hematocrit values and standard errors of the means.

The hematocrit increased with age; mean

‡ Testosterone propionate was supplied by Ciba Pharmaceutical Products, Inc. Estradiol benzoate by Schering Corp.

§ Water-soluble testosterone, 17-β, diethylaminoethyl-carbonate hydrochloride was synthesized by Ciba Pharmaceutical Products, Inc. and supplied through the generosity of Dr. C. H. Sullivan. An additional amount was generously supplied by Dr. Albert Segaloff of Alton Ochsner Medical Foundation.

in older Group II was significantly higher than in younger Group I ($P<.01$).

Castration depressed the hematocrit; mean of untreated castrates, Group III, was significantly lower than that of intact Group II ($P<.01$). In self-controls, Group IV, the mean after castration was lower than prior to gonadectomy ($P<.01$).

Both androgens restored the hematocrit after castration, the mean of castrates in Group VII, injected with water-soluble testosterone, was significantly higher ($P<.01$) than that of untreated castrates in Group III and not significantly different from that of intact animals in Group II. The mean of Group VI, castrates injected with testosterone propionate in S.O., was not significantly different from that of intact animals in Group II or intact animals given S.O. alone, Group VIII.

The hematocrit of neonatal-castrates, Group XII, was not significantly different from that of intact adults in Group II, and was significantly higher than that of castrates, Group III ($P<.01$).

It is uncertain whether S.O. alone caused an increase in the hematocrit. The mean in intact animals of Group VIII given S.O. is higher than that in untreated Group II ($P<.05$) and the mean of Group V, castrates given S.O., is higher than that of untreated Group III, but not significantly.

Discussion. Within the 50-115 day life span in these experiments, the hematocrit of intact male rats increased with age. These results parallel a reported rise in hemoglobin from 50th to 150th day(1).

The hematocrit response following castration and subsequent androgen therapy confirms previous findings(2-7). The approximate 4-point fall after castration in self-controls is especially significant in light of predictable increases suggested by the results in Groups I and II.

In both intact and castrated groups treated with estradiol benzoate mean hematocrits were lower than corresponding untreated groups, although the differences were statistically non-significant. These results parallel Korenchevsky and Hall's(7), who found that estradiol benzoate-butyrate ester did not alter hematocrits of castrated males. However, it has been reported that estrogen caused a drop in red cell volume(4).

No reference was found regarding hematocrits of adult neonatally castrated rats. Hematocrits of these neonatal castrates, taken at 84-111 days, were not significantly different from those of intact animals and were thus significantly higher than those of animals castrated as adults and examined 14 days later. These results may confirm Korenchevsky and Hall's findings(7) that 100-150 days after castration hematocrits did not differ from those of intact animals. These findings of Korenchevsky and Hall and our own, may indicate secretion of extragonadal androgen.

There was a tendency for both intact and castrated animals injected with S.O. alone to have higher hematocrits than their controls. These results are equivocal but interesting because S.O. is frequently employed as a vehicle for hormones, and because other androgen-like activity of S.O. has recently been reported(11).

Summary and conclusions. 1. Some of our results confirm previous findings: hematocrits of rats castrated as adults are lower than those of intact animals; testosterone propionate returned hematocrits to normal after castration, but did not cause elevation in hematocrits of intact or castrated animals above normal. Estradiol benzoate did not significantly lower hematocrits of intact and castrate rats. 2. Other findings are new: hematocrits of neonatally castrated adult rats were not significantly different from those of intact adults of the same age, and thus differed from those of animals castrated as adults and examined 14 days later. Aqueous testosterone returned the hematocrits of castrates to normal. There was a trend in both intact and castrates for sesame oil to increase the hematocrit. Because sesame oil is widely used as a supposedly neutral vehicle for hormones, the possibility that it has an effect upon the hematocrit warrants further investigation.

1. Williamson, C. S., and Ets, H. N., *Am. J. Physiol.*, 1926, v77, 480.

2. Steinglass, P. A., Gordon, A. S., and Charipper,

H. A., PROC. SOC. EXP. BIOL. AND MED., 1941, v48, 169.

3. Crafts, R. C., *Endocrinology*, 1946, v39, 41.

4. Van Dyke, D. C., Contopoulos, A. N., Williams, B. S., Simpson, M. E., Lawrence, J. H., and Evans, H. M., *Acta Hemat.*, 1954, v2, 202.

5. Vollmer, E. P., and Gordon, A. S., *Endocrinology*, 1941, v29, 829.

6. Finkelstein, G., Gordon, A. S., and Charipper, H. A., *ibid.*, 1944, v35, 267.

7. Korenchevsky, V., and Hall, K., *J. Endocrinol.*, 1945, v4, 103.

8. Grant, W. C., and Root, W. S., *Physiol. Rev.*, 1952, v32, 449.

9. Strumia, M. M., Sample, A. B., and Hart, E. D., *Am. J. Clin. Path.*, 1954, v24, 1016.

10. Snedecor, G. W., *Statistical Methods*, Collegiate Press, Ames, Iowa, 1946.

11. Denison, M. E., Jasper, R. L., Hiestand, W. A., and Zarrow, M. X., *Am. J. Physiol.*, 1956, v186, 471.

Received December 21, 1956. P.S.E.B.M., 1957, v94.

Relation Between P-Aminohippuric Acid Synthesis and Amino Acid Incorporation into Protein.* (23041)

H. BURLINGTON† (Introduced by William D. Lotspeich)

Department of Physiology, State University of N. Y. Medical College, Syracuse, N. Y.

Systems carrying out synthesis of "peptidic" links, as in formation of hippuric acid, glutamine, glutathione, and pantothenic acid, are considered useful models for the study of peptide formation because of their accessibility and relative simplicity. These reactions have yielded useful information about possible steps leading to peptide bond formation and have provided a basis for a working hypothesis of protein biosynthesis(1). The 4 "model" systems have at least two things in common; namely, carboxyl activation and utilization of energy of one of the pyrophosphate linkages of ATP. It appeared worth while to determine whether a change in endocrine environment affecting peptide synthesis would influence protein formation in a qualitatively similar fashion. To answer this question it was decided to study the "PAH synthetase" system described by Cohen and McGilvery(2-4) while simultaneously studying the rate of protein synthesis. Response of each of these parameters to an evoked change in one of them provides the basis of this report. Measurement of rate of uptake of isotopically labelled amino acids into the protein fraction was chosen as the means of assessing rate of protein synthesis.

Methods. Studies were confined to liver slices from rats in the "hyperthyroid" and "hypothyroid" state. Males of the Sprague-Dawley strain were used. Maintenance on a diet containing 0.5% thyroid powder for 5 weeks was sufficient to produce hyperthyroidism in rats weighing 50-60 g at the outset. Similarly, thyroid function was suppressed significantly by a diet containing 0.05% propylthiouracil fed for 5 weeks. Degree of thyroid dysfunction was gauged by resting metabolic rate (determined as described in 5). From 60-75 mg wet weight of tissue slice was added to each incubation flask, containing 0.001 M PAB and 0.01 M glycine in phosphate-buffered Krebs-Ringer solution. Para-aminohippuric acid (PAH) was determined colorimetrically by the Bratton and Marshall procedure after differential extraction of PAB from PAH according to the method of Cohen and McGilvery(2). PAH synthetase activity is expressed as γ of PAH formed/mg dry weight of tissue/3 hours. In isotope experiments, uptake of glycine-1-C^{14} and lysine-2-C^{14} into the protein fraction of liver slices was determined as follows: Tissue slices (60-75 mg wet weight) were incubated at 38°C for 1 and 2 hours in phosphate-buffered Krebs-Ringer solution containing 0.001 M

* This investigation was supported in part by research grant from Nat. Cancer Inst. of Public Health Service.

† Present address: Department of Physiology, College of Medicine, University of Cincinnati.