to elevation of blood pressure. To examine this thesis saline infusion was administered to 16 mongrel dogs while individual organs were perfused with subpressor doses of metaraminol. The dose of metaraminol was that which the organ would receive during a systemic pressor infusion known to result in exaggerated natriuresis in the dog. The local pharmacologic effects of metaraminol on kidney, liver or brain did not result in an augmented natriuretic response to the infusion of saline. These data are therefore consistent with the view that hypertension itself is necessary for the appearance of exaggerated natriuresis in response to saline infusion in the dog.

1. Papper, S., Belsky, J. L., Bleifer, K. H., J. Clin. Invest., 1960, v39, 876.

2. Birchall, R., Tuthill, S. W., Jacobs, W. S., Trautman, W. J., Jr., Findley, T., Circulation, 1953, v7, 258.

3. Biglieri, E. G., Forsham, P. H., Am. J. Med., 1961, v30, 564.

4. Ortuzar, R., Crozatto, R., Thomsen, P., Gonzalez, J., J. Lab. & Clin. Med., 1959, v54, 712.

5. Hanenson, I. B., Taussky, H. H., Polasky, N., Ransohoff, W., Miller, B. F., Circulation, 1959, v20, 498.

6. Birchall R., Batson, H. M., Jr., Anderson, E. G., ibid., 1964, v29, 6.

7. Vaamonde, C. A., Sporn, I. N., Lancestremere, R. G., Belsky, J. L., Papper, S., J. Clin. Invest., 1964, v43, 496.

8. Eisinger, R. P., Proc. Soc. Exp. Biol. and Med., 1966, v122, 804.

9. Aviram, A., Czaczkes, W. J., Ullmann, T. D., Nephron, 1965, v2, 82.

10. Thompson, J. E., Silva, T. F., Kinsey, D., Smithwick, R. H., Circulation, 1954, v10, 912.

11. Hulet, W. H., Baldwin, D. S., Biggs, A. W., Gombos, E. A., Chasis, H., J. Clin. Invest., 1960, v39, 389.

12. Smith, H. W., Principles of Renal Physiology, Oxford Univ. Press, New York, 1956, p208.

13. Baldwin, D. S., Biggs, A. W., Goldring, W., Hulet, W. H., Chasis, H., Am. J. Med., 1958, v24, 893.

Received June 2, 1966. P.S.E.B.M., 1967, v124.

Effect of Advancing Age on Thyrotropin Content of the Pituitary and Blood Of the Rat.*† (31831)

J. N. PANDA AND C. W. TURNER
Department of Dairy Husbandry, University of Missouri, Columbia

The effect of advancing age from 25 to 115 days on the thyroid hormone secretion rate (TSR) of female rats was reported(1). It was observed that the TSR of the same rats at 25 days was 1.52 μg L-thyroxine (L-T_4)/100 g BW and gradually declined to a level of 0.88 μg/100 g BW at 115 days. An explanation of the mechanism involved in the decline in TSR during this period has been sought. Since the secretion and discharge of thyrotropin (TSH) is clearly involved in TSR, it seemed of interest to determine the change in the pituitary and blood content of TSH during advancing age up to maturity.

Levey(2) determined TSH concentration in the pituitary of rats by a bio-assay from birth to 56 days of age and noted a progressive increase from 13 mU/mg to over 100 mU/mg. However, he was unable to determine the level of TSH in the serum samples because they were all below the minimum sensitivity of the bio-assay method(3).

Since Levey showed a progressive increase in pituitary TSH in young rats with age, whereas our study showed a progressive decrease in TSR during the same period of growth, it seemed of interest to repeat the study of the changes in pituitary TSH but at the same time assay the plasma concentration of TSH. Instead of a bio-assay of TSH as used by Levey, the TSH of both pituitary and plasma was assayed by an immuno-

* Contribution from Missouri Agr. Exp. Sta. Journal Series No. 4033. Approved by Director. Supported in part by a grant from U.S. Atomic Energy Commission, Contract AT(11-1)-301-129.

† TSH was kindly supplied by NIH, Bethesda, Md.

chemical method which has been found to detect 0.1-0.2 mU/ml of USP Reference Standard of TSH, thus making possible the assay of blood plasma. Comparison of duplicate assays within the samples did not show greater than 2% variation. Experimental error involved in this technique of TSH assay averages 6.25% of the final reading due to dilution procedure.

This report presents data on the pituitary and plasma TSH concentration of rats from 24 to 110 days of age.

Materials and methods. Experimental animals. Weaning female rats of the Sprague-Dawley-Rolfsmeyer strain were maintained on Purina Lab Chow with tap water *ad libitum* in a constant environmental temperature of 78 ± 1°F. Groups of 10 rats were sacrificed at increasing ages from 24 to 110 days. Blood was collected from the dorsal aorta under ether anaesthesia using heparin as an anticoagulant. Plasma was collected and stored in sterile vials at —20°C until assayed. The pituitary of each rat was collected on ice immediately after decapitation. The glands were then weighed and homogenized in cold sterile saline so that the final concentration of the homogenate was 1 gland/ml. After centrifuging the supernatant was stored at —20°C until assayed.

Immuno-chemical assay. The hemagglutination-inhibition technique applied by Read and Bryan(4) for quantitative estimation of growth hormone and by Wide and Gemzell(5) for HCG was used for assay of thyrotropin in plasma and pituitary. This technique has been found to be sufficiently sensitive to detect 0.1-0.2 mU/ml of TSH in USP Reference Standard(6).

Both for antibody production and tanning of sheep erythrocytes, bovine TSH (NIH-TSH-B3) has been used as the antigen. This preparation has a potency of 2.73 units/mg USP Reference Standard. According to recent studies of Selenkow *et al*(7), although the bovine TSH preparations contain TSH and LH, yet the antibodies against each are quite distinct and separable. Young healthy albino rabbits of 4-5 lb BW were employed in antisera production. A healthy sheep was maintained as a constant donor of erythrocytes. All the chemical reagents, various plasma dilutions and the tanning and sensitizing of the erythrocytes with TSH was performed as recommended by Stavitsky(10). A 1:30,000 solution of tannic acid in saline was used for tanning the erythrocytes. The agglutination reactions were read after 4 and 24 hours of incubation at 25°C. The unknown plasma was used for assay undiluted and the pituitary homogenate was diluted 100× before assay. Results were compared and expressed in milliunits (mU) of TSH, USP Reference Standard. As a test of the validity of the assay method for blood plasma TSH, plasma from 15 hypophysectomized adult female rats was obtained and assayed in duplicate. No TSH was detected, indicating either an absence of TSH or an amount less than 0.1 mU/ml, the limit of sensitivity of the method.

Results. The mean weight of the pituitary glands increased gradually from 2.81 ± 0.16 mg at 24 days of age to 10.6 ± 0.37 mg at 110 days of age (Table I). The mean TSH content of the pituitaries increased from 46.35 ± 2.85 mU/mg at 24 days to a maximum of 93.34 ± 8.06 mU/mg at 80 days of age followed by a reduction to 69.19 ± 6.78 mU at 95 days and to 65.13 ± 5.82 mU/mg at 110 days when the rats weighed 245 g (Table I).

The mean plasma TSH increased from 0.316 ± 0.04 mU/ml at 24 days of age to 1.178 mU/ml at 95 days of age, then fell slightly to 1.046 ± 0.10 mU/mg at 110 days of age.

Discussion. In the previous study(1), it was shown that the TSR of this strain of rats was highest at 25 days of age and then gradually declined to 115 days. In the present study it was shown that the mean TSH content of the pituitary increased gradually until 80 days of age and the plasma content increased until 95 days of age. This indicates a close relation between the TSH content of the pituitary and the level of TSH in the plasma until 80 days of age (Fig. 1).

While the plasma level of TSH in young rats has not been reported previously, the pituitary levels of TSH reported by Levey(2) are similar in the age range of 20 to 40 days but at 56 days of age (spring) a value of 126

TABLE I. TSH Content of Pituitary and Blood of Rats of Increasing Age.

Group No.	Age (days)	No. of rats	Mean body wt (g)	TSH/ml plasma mean ± S.E. (mU)	Pituitary wt mean ± S.E. (mg)	TSH/pituitary gland mean ± S.E. (mU)
1	24	10	43	.316 ± .04	2.81 ± .16	127.2 ± 4.8
2	40	10	83	.463 ± .22	5.10 ± .08	300.9 ± 7.57
3	50	10	150	.573 ± .04	6.8 ± .22	478.2 ± 24.10
4	65	10	171	.797 ± .01	7.81 ± .12	693.4 ± 7.82
5	80	10	197	.912 ± .01	9.0 ± .003	834.0 ± 17.9
6	95	8	236	1.178 ± .01	9.75 ± .61	637.1 ± 53.92
7	110	9	245	1.046 ± .10	10.6 ± .37	673.4 ± 34.8

mU/mg was reported compared to our value of 90.23 mU/mg at 65 days of age. This difference may be accounted for by the fact that the animal room temperature was not controlled and groups were compared in fall, winter and spring. In the study of D'Angelo (8) of rats from 5 to 15 months of age, the mean TSH was 71 ± 17 mU/mg and 690 ± 40 mU/gland. This value is quite similar to the value reported in this paper for 110-day-old rats. The mean TSH value for the serum of his rats was 0.824 ± 9.4 mU/ml, slightly lower than the mean of our 110 day group.

On the basis of the present observations of an increase in pituitary and plasma TSH of rats up to 80 or 95 days of age, one might expect the TSR to increase correspondingly. However, the opposite is true.

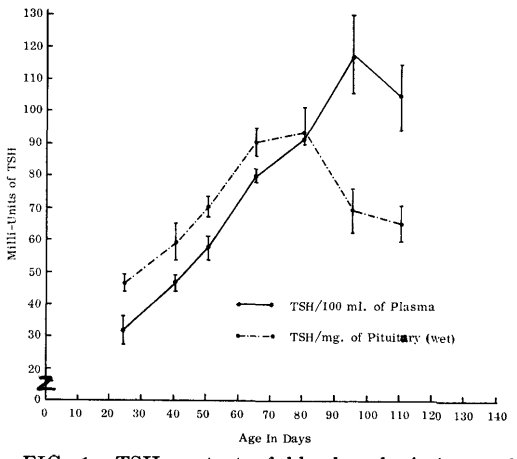

FIG. 1. TSH content of blood and pituitary of growing female rats from 24 to 110 days of age. There is a marked parallelism between the TSH/100 ml of plasma and TSH/mg of pituitary (wet) up to 65 days of age. Each point represents the mean of a group of 10 rats. Vertical lines are standard errors of mean.

A number of suggestions may be offered to explain these observations. Levey(2) suggested that his data supported the concept that "the thyroid of the immature rat is, if anything, *more sensitive* to TSH than that of the adult rat." If this were the only factor involved, the variation in sensitivity would necessarily be very great since there is a 3-fold concentration of TSH/ml of plasma between 24 and 80 days.

A second factor which might, in part, account for a decrease in TSR associated with an increase in plasma TSH would be a relation of age to the $t_{1/2}$ of TSH. Bakke and Lawrence(9) have determined the $t_{1/2}$ of TSH in the normal mature rat at 13.7 minutes, in the hypothyroid as 34 minutes, and of the thyrotoxic as 2.2 minutes. While the possible age effect on the $t_{1/2}$ of TSH was not studied, it is quite possible that the $t_{1/2}$ of TSH might be lengthened in the immature rat and decrease with advancing age. This would increase the time of the low level of TSH to act upon the thyroid gland and thus produce a higher TSR.

The relative rate of secretion of L-thyroxine (L-T_4) and L-triiodothyronine (L-T_3) by the immature rat might also be involved. Pitt-Rivers and Rall(11) reported in the serum of male Sprague-Dawley rats 92 to 94% of L-T_4 and 4.2 to 4.4% of L-T_3. Since it has been shown(12) that L-T_3 is 2.6 times as active as L-T_4 in the estimation of TSR in the rat, the secretion of a larger proportion of L-T_3 in comparison to L-T_4 in the immature rat would tend to give higher estimations of TSR.

Summary. The thyroid hormone secretion rate (TSR) of female Sprague-Dawley-Rolfsmeyer rats has previously been shown to de-

cline markedly from weaning time to about 4 months of age. In the present study it was shown that the pituitary and plasma levels of TSH were low at weaning time and gradually increased until 80 or 95 days of age, then showed a slight decline. In order to explain the observations of a declining TSR associated with an increase in plasma TSH, it has been suggested that (1) the sensitivity of the thyroid gland to TSH decreases with age, (2) the $t_{½}$ of TSH may decrease with increasing age, and (3) the secretion of L-T_3 in the immature rat may be high and decline with age.

1. Narang, G. D., Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1966, v121, 203.
2. Levey, H. A., Endocrinology, 1962, v71, 763.
3. Levey, H. A., Cheever, E., Roberts, S., ibid., 1956, v58, 420.
4. Read, C. H., Bryan, G. T., Recent Progr. Hormone Res., 1960, v16, 187.
5. Wide, L., Gemzell, C. A., Acta Endocrinol., 1960, v35, 261.
6. Pascasio, F. M., Selenkow, H. A., Endocrinology, 1962, v71, 254.
7. Selenkow, H. A., Saravis, C. A., Garcia, A. M., Acta Endocrinol., 1966, v51, 32.
8. D'Angelo, S. A., Endocrinology, 1961, v69, 834.
9. Bakke, J. L., Lawrence, N. L., ibid., 1962, v71, 43.
10. Stavitsky, A. B., J. Immunol., 1954, v72, 360.
11. Pitt-Rivers, R., Rall, J. E., Endocrinology, 1961, v68, 309.
12. Bauman, T. R., Turner, C. W., ibid., 1955, v76, 537.

Received June 16, 1966. P.S.E.B.M., 1967, v124.

Effect of Splenectomy on Renal Arteriovenous Reduction in Hemoglobin Concentration in the Anesthetized Dog.* (31832)

J. SANTOS-MARTINEZ AND ROGER M. REINECKE
School of Medicine, University of Puerto Rico, San Juan, P. R.

In a preceding study it was found in the anesthetized dog that the kidney apparently removed something measured as cyanmethemoglobin from the blood passing through it (1). In the investigation presented here the effect of splenectomy on this finding was studied.

Procedure. The apparent concentration of hemoglobin measured as carbon monoxide hemoglobin, acid hematin, and cyanmethemoglobin in the renal artery and vein was observed first in the nonsplenectomized and then in the splenectomized dog. In general the procedure with some exceptions was the same as that followed in the second group of experiments of the preceding study. Adult mongrel dogs of both sexes were anesthetized with sodium pentobarbital given intravenously. An initial dose of 20 mg/kg was supplemented with additional doses of 5 mg/kg as needed to maintain anesthesia throughout the experimental period of some hours. Polyethylene catheters were implanted in the left renal artery and the left renal vein with their open tips within the renal hilum through a ventral laparotomy. The left gonadal vein was ligated. The catheters were led out of the body through stab wounds and the laparotomy was closed with towel clamps. When the spleen was removed, this was done just before the catheters were implanted. The subjects were heparinized some minutes after the laparotomy was closed. Samples of about 1 ml of blood were drawn alternately in close succession instead of simultaneously from the arterial and venous catheters. In the nonsplenectomized animals the interval between samples was about 60 seconds and in the splenectomized animals about 30 seconds. Two to 5 ml of blood were drawn and discarded just before a sample was to be taken to clear the catheter of stagnant blood. Duplicate subsamples of each sample were analyzed for hemoglobin as carbon monoxide hemoglobin, as acid hematin, and as cyanmethemoglobin as in the second group of

* Supported in part by grant No. H-3053 (C_2) from Nat. Inst. Health, USPHS.