

Thyroid function in pyridoxine-deficient young rats

K. Dakshinamurti, C. S. Paulose, J. A. Thliveris* and J. Vriend*

Department of Biochemistry, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3

*Department of Anatomy, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3

REVISED MANUSCRIPT RECEIVED 15 August 1984

ABSTRACT

In pyridoxine-deficient young rats hypothalamic serotonin was decreased with no changes in the dopamine and noradrenaline content. Serum thyroxine and tri-iodothyronine concentrations were much lower in the deficient rats as compared to pyridoxine-supplemented controls. No significant difference between deficient and control groups in the

serum TSH concentration was detected. Highly significant decreases in the content of pituitary TSH and in the number of pituitary thyrotroph secretory granules were found. These results suggest that the hypothyroidism of pyridoxine-deficient young rats might be of hypothalamic origin.

J. Endocr. (1985) **104**, 339–344

INTRODUCTION

The central regulation of thyroid hormone secretion by monoamine neurotransmitters through the hypothalamic-pituitary pathway has been reported (DiRenzo, Quattrone, Schettini & Preziosi, 1978, 1979; Chen & Ramirez, 1981; Dupont, Dussault, Rouleau *et al.* 1981; Morley, Brammer, Sharp *et al.* 1981; Smythe, Bradshaw, Cai & Symons, 1982). Although much contradictory opinion has been reported on the effect of individual monoamines on the secretion of pituitary hormones, it is generally accepted that dopamine and serotonin have mutually antagonistic effects (Krush, 1979). Smythe *et al.* (1982) have demonstrated a direct relationship between serotonin turnover and thyroid-stimulating hormone (TSH) release.

Several experimental models have been devised to alter the hypothalamic serotonin content to examine the effect of such alterations on secretion of various anterior pituitary hormones. In the pyridoxine-deficient rat we have an animal model with a physiologically significant decrease in brain serotonin with no change in the concentrations of dopamine and noradrenaline (Dakshinamurti, LeBlancq, Herchl & Havlicek, 1976). The impaired myelination seen in the pyridoxine-deficient rats is similar to the defect in the hypothyroid rat (Stephens & Dakshinamurti, 1976). In both conditions the elongation of long-chain fatty acids is decreased. One would expect in the deficient rat a decrease in serotonergic stimulus on pituitary secretion. We have used this animal model to examine

the effect of pyridoxine deficiency on pituitary-thyroid hormones and on the morphology of pituitary and thyroid glands.

MATERIALS AND METHODS

Materials

S-[Methyl-³H]Adenosyl-L-methionine ([³H]SAM) was purchased from New England Nuclear, Boston, MA, U.S.A. *N*-Acetyl-5-hydroxytryptamine, 5-hydroxytryptamine-creatinine sulphate complex, 3-hydroxytyramine hydrochloride, *N*-acetyl-5-methoxytryptamine, metanephrine hydrochloride, methoxytyramine hydrochloride, noradrenaline hydrochloride and normetanephrine hydrochloride were purchased from Sigma Chemical Co., St Louis, MO, U.S.A. *N*-Benzyl-*N*-methyl propargylamine hydrochloride (pargyline) was purchased from Saber Laboratories Inc., Morton Grove, IL, U.S.A.

Animals

The objective was to compare pyridoxine-deficient pups with pups that had gone through an equivalent period of generalized malnutrition while receiving adequate pyridoxine (control) and with pups on a freely available normal diet (normal). Pyridoxine-deficient rats have, generally, a reduced food intake. Timed-pregnant Sprague-Dawley rats were housed individually (under a 12 h light : darkness cycle, lights

off 18.00 h) and fed a pyridoxine-supplemented diet (pyridoxine-deficient diet to which a supplement of pyridoxine, 50 mg/kg diet, was added) for the period of gestation. At the time of delivery the dams were divided into three groups. The number of pups left with the dam was manipulated soon after birth so that each dam in the deficient (fed the pyridoxine-deficient diet) and normal (fed the normal diet) groups had eight pups. In the control group (fed the pyridoxine-supplemented diet) each dam was left with 16 pups. The percentage composition of the pyridoxine-deficient diet (Stephens, Havlicek & Dakshinamurti, 1971) was as follows: vitamin-free casein, 30.0; dextrose, 59.85; corn oil, 5.0; salt mix, no. 446, 4.0; vitamin mix without pyridoxine, 1.0; choline chloride, 0.15. All dams had free access to the respective diets. However, because of the large number per litter, the pups in the control group were receiving less milk and thus were subjected continually to a generalized malnutrition so that their body weights were close to those of deficient pups. There was, generally, no mortality in the litters of any of the three groups during the experimental period. There were six dams in each of the three experimental groups.

Pups from each group were killed at 21 days of age between 16.00 and 18.00 h. Blood was collected after decapitation. Serum was used for assay of thyroxine (T_4), tri-iodothyronine (T_3) and TSH. Brain regions were dissected according to Glowinski & Iversen (1966). Tissues were frozen immediately on dry ice and stored at -70°C . Pituitary glands were sonicated in phosphate-buffered saline (pH 7.6). The homogenate was centrifuged at 2000g for 20 min and the supernatant fraction used for TSH assay. Protein content in the pituitary homogenate was measured according to Lowry, Rosebrough, Farr & Randall (1951).

Assay of monoamines in hypothalamus

Dopamine and noradrenaline were determined radioenzymatically using the method of Sole & Hussain (1977). Serotonin was assayed according to the method of Hammel, Naot, Ben-David & Ginsburg (1978) with modifications. Hydroxyindole-*o*-methyltransferase (HM) used for the assay was partially purified from pineal glands (Pel-Freez Biologicals Inc., Rogers, AR, U.S.A.) and assayed as described by Saavedra, Brownstein & Axelrod (1973).

Tissues were homogenized in 9 volumes of ice-cold 0.05 M-sodium phosphate buffer, pH 7.2, containing 1 mM-pargyline, using a Polytron homogenizer (Brinkman Instruments, New York, NY, U.S.A.). The homogenate was left on ice for 20 min and then centrifuged at 4°C for 20 min at 5000g. The resulting supernatant fraction was used for the assay of serotonin.

Tissue homogenate (40 μl) and serotonin standard (0.001–0.1 nmol) respectively were acetylated by adding 20 μl 1% acetic anhydride in acetone to each tube. Blanks were run using buffer instead of acetic anhydride. The tubes were incubated at room temperature for 10 min and then placed in a water bath (60 – 70°C) for 10 min to evaporate the acetone, complete acetylation, and decompose the remaining acetic anhydride. S-[Methyl- ^3H]adenosyl methionine (0.3 nmol) in 0.5 M-sodium phosphate buffer, pH 7.95 and 25 μl HM (4 units) were added to each tube and incubated at 37°C for 30 min. The reaction was stopped by the addition of 1 ml 0.5 M-borate buffer, pH 10. The reaction mixture was transferred to scintillation vials, 10 ml organic counting scintillant (OCS; Amersham Corporation, Arlington Heights, IL, U.S.A.) were added and radioactivity was determined in a Beckman LS-2800 liquid scintillation spectrometer. Serotonin was assayed as a measure of the amount of [^3H]melatonin formed in the reaction. The serotonin content in the tissue was expressed in nmol/g wet wt.

When radioactivity is determined using OCS, only the counts in the organic phase are registered. Less than 0.25% of the counts in the aqueous phase appeared in the total counts. We used [^3H]SAM and [^3H]melatonin to determine the cross-over and found that there was no interference in determining the radioactivity associated with melatonin from [^3H]SAM which was present in the aqueous phase. Using the OCS, the radioactivity associated with melatonin, the product of the reaction, can be determined in the presence of the excess radioactive substrate, [^3H]SAM in the aqueous phase. In view of this, we compared this method with that where the melatonin formed was extracted using chloroform before determining the radioactivity. There was no significant difference in the melatonin-associated radioactivity determined using either procedure. Hence we have used the simpler procedure of determining melatonin formed using the OCS.

Assay of hormones

Thyroxine and T_3 were determined using the T_4 and T_3 solid-phase radioimmunoassay kit (^{125}I) purchased from Becton-Dickinson & Co., Orangeburg, NY, U.S.A. Serum T_4 and T_3 concentrations were expressed in nmol/l. Pituitary and serum TSH were assayed using the reagents and protocol of the NIADDK, Bethesda, MD, U.S.A. The rat TSH reference preparation (NIADDK-rTSH-RP-2) now supplied by NIADDK is 176 times more potent than the NIADDK-rTSH-RP-1 previously supplied. Accordingly, the TSH values expressed in terms of NIADDK-rTSH-RP-2 are about 200 times lower than

values expressed in terms of NIADDK-rTSH-RP-1, the previously supplied standard.

Morphological study

For morphological study, the pituitary and thyroid glands from control and pyridoxine-deficient animals were removed, cut into small pieces and fixed in 2% glutaraldehyde in 0.1 M-phosphate buffer (pH 7.4) for 2 h at 4 °C. After an overnight rinse in 0.1 M-phosphate buffer (pH 7.4) containing 0.2 M-sucrose, the tissues were post-fixed in 1% osmium tetroxide in 0.1 M-phosphate buffer (pH 7.4) for 2 h at 4 °C, dehydrated in ascending concentrations of ethanol and embedded in Epon 812. Thick (1 µm) sections were cut and stained with toluidine blue and examined for routine orientation. Thin sections were stained with uranyl acetate and lead citrate, viewed and photographed in a Philips EM 201 electron microscope. In order to eliminate observer bias, tissues were examined blind using coded grids. Since thyrotrophs contain the smallest granules (140–200 nm) of the various cell types in the pituitary gland they were readily identified using the criteria established by Farquhar, Skutelsky & Hopkins (1975). Morphometric analysis of the number of secretory granules was done on thyrotrophs from 400 electron micrographs: 200 from control and 200 from pyridoxine-deficient pituitaries.

RESULTS

Body weights of rat pups in the three experimental groups were as follows (mean weight in g ± S.D.): normal, 49.2 ± 3.6; pyridoxine-deficient, 22.2 ± 3.6; control, 32.1 ± 2.9. There was a significant ($P < 0.01$) difference between any two of the three groups as analysed by Duncan's multiple range test. Pups in the control group were subjected to generalized malnutrition. However, they had adequate pyridoxine intake as the pyridoxine supplement of the diet of the

dams was ten times their requirement. This was reflected in the significantly ($P < 0.001$) higher hypothalamic content of pyridoxal phosphate of the pups in this group (Table 1).

Effect of pyridoxine deficiency on brain monoamines

There was no significant difference in the dopamine and noradrenaline contents in the hypothalamus of the control and deficient rats (Table 1), whereas a significant ($P < 0.01$) decrease in serotonin content was observed.

Effect of pyridoxine deficiency on T_4 , T_3 and TSH concentrations in serum and on the TSH content of the pituitary

Comparison of the control with the normal group indicates the effect of generalized malnutrition on the hormonal status of the pups. Comparison of the pyridoxine-deficient group with the control group would then indicate the specific effects of pyridoxine deficiency, apart from that of the generalized malnutrition experienced by both groups. The results are given in Table 2. Pituitary content of TSH was expressed both on a per pituitary and on a per mg protein basis. The only significant ($P < 0.05$) difference between the normal and control groups was in the pituitary TSH expressed on a protein basis. There was a tendency in the control group toward lower levels of serum T_4 and T_3 as compared to normals.

The concentrations of serum T_4 and T_3 of the pyridoxine-deficient pups were significantly ($P < 0.01$) lower in comparison with both the pyridoxine-supplemented groups, normal and control. There was no significant change in the concentration of TSH in the serum of the deficient rats. However, pups in the deficient group had a significantly ($P < 0.01$) lower content of pituitary TSH regardless of the way this was expressed. No significant difference was noted in either the pituitary weight or its protein content between deficient and control groups.

TABLE 1. Pyridoxal phosphate, serotonin, dopamine and noradrenaline contents in control and pyridoxine-deficient rat hypothalamus. Values are means ± S.E.M. of eight separate determinations in each group

Animal status	Pyridoxal phosphate (nmol/g)	Serotonin (nmol/g)	Dopamine (nmol/g)	Noradrenaline (nmol/g)
Pyridoxine-supplemented (control)	2.71 ± 0.19	1.70 ± 0.20	1.18 ± 0.07	2.17 ± 0.09
Pyridoxine-deficient	1.17 ± 0.07**	1.00 ± 0.27*	1.25 ± 0.10	2.01 ± 0.10

* $P < 0.01$, ** $P < 0.001$ compared with controls (Student's unpaired *t*-test).

TABLE 2. Serum thyroxine (T_4), tri-iodothyronine (T_3), TSH and pituitary TSH in normal, control and pyridoxine-deficient 3-week-old rats. Values are means \pm S.E.M.; numbers of experiments are shown in parentheses

	Normal (group 1)	Control (group 2)	Pyridoxine- deficient (group 3)
T_4 (nmol/l)	87.52 \pm 3.76 (19)	82.98 \pm 1.88 (40)	58.39 \pm 2.66** (41)
T_3 (nmol/l)	1.54 \pm 0.07 (20)	1.40 \pm 0.06 (42)	0.98 \pm 0.03** (41)
Serum TSH (μ g/l)	2.63 \pm 0.15 (16)	2.75 \pm 0.16 (22)	2.45 \pm 0.18 (15)
Pituitary TSH (μ g/mg protein)	6.00 \pm 0.38 (17)	8.21 \pm 0.39* (41)	4.68 \pm 0.32** (38)
Pituitary TSH (μ g/pituitary)	2.12 \pm 0.15 (17)	1.80 \pm 0.12 (41)	1.05 \pm 0.05** (38)

* $P < 0.05$ compared with group 1; ** $P < 0.01$ compared with group 1 and group 2 respectively (Duncan's multiple range test).

Morphological study

Thyroid

Thyroid follicular cells from control animals (Plate, fig. 1) were characterized primarily by numerous profiles of rough-surfaced endoplasmic reticulum often with markedly dilated cisternae. Golgi membranes and associated vesicles were also present as were elongated mitochondria with plate-like cristae. Also seen were small, slightly electron-dense and larger, notably electron-dense bodies that were located mainly in the apical portion of the cell. Infrequently detected were large membrane-bounded droplets which were morphologically similar to luminal colloid. The apical surface of the cells exhibited numerous relatively short microvilli. Rarely observed were microvilli in the form of long pseudopods that extended into and surrounded a portion of luminal colloid.

The fine structural features of the thyroid follicular cells from pyridoxine-deficient animals (Plate, fig. 2) were similar to those observed in the control animals with one notable exception. The cisternae of the rough-surfaced endoplasmic reticulum were far less frequently observed in the markedly dilated form.

Thyrotrophs

Thyrotroph cells in pituitary glands from control (Plate, fig. 3) and pyridoxine-deficient (Plate, fig. 4) animals were similar in their fine structural appearance characterized by well-developed organelles and the presence of numerous secretory granules. However, morphometric analysis of the numbers of secretory granules revealed a significant ($P < 0.05$) reduction in pyridoxine-deficient animals compared with control animals. The number of secretory granules per micrograph of thyrotroph examined were as follows

(mean \pm S.E.M.): control, 176 \pm 11; pyridoxine-deficient, 142 \pm 12.

DISCUSSION

In the normally growing rat the thyroid becomes fully developed during weaning (Dussault & Labrie, 1975). The highest serum concentrations occur during weeks 3 and 4 of life and subsequently decrease to adult levels. Our results indicate that the thyroid function of rats fed a pyridoxine-deficient diet from birth was decreased at 21 days of age. Morphological studies of the thyroid glands of deficient rats having decreased serum levels of T_4 and T_3 indicate decreased secretion of T_4 . We compared pyridoxine-deficient rats with pyridoxine-supplemented control as well as normal rats. The results on thyroid status presented here are thus specific to pyridoxine deficiency. In contrast, in protein malnutrition (Tulp, Krupp, Danforth & Horton, 1979) and vitamin A deficiency in the rat (Morley, Damassa, Gordon *et al.* 1978), the concentrations of both T_4 and T_3 are actually increased. In acute starvation there is a decrease in circulating T_3 with no change in serum T_4 concentration (Jung, Shetty & James, 1980).

In determining the location of the biochemical lesion leading to the hypothyroid state in pyridoxine deficiency, various possibilities were considered. The lesion could be primary, at the site of the thyroid itself, secondary, with a defective pituitary gland, or tertiary, with a defect at the level of the hypothalamus (Fisher & Klein, 1981).

If the defect were only at the level of the thyroid tissue, low serum T_4 and T_3 values would be coupled with a compensatory high level of serum TSH. A defect

at the level of the pituitary thyrotrophs may also be considered. The classical formulation of the hypothalamic-pituitary-thyroid axis would suggest pituitary (secondary) hypothyroidism to be associated with a decrease in serum T_4 and T_3 coupled with a sharp decrease in serum TSH and unresponsiveness to thyrotrophin-releasing hormone (TRH) (Jackson, 1982). Infants with hypothalamic hypothyroidism (tertiary) due to TRH deficiency are seen with persistently low serum T_4 and T_3 and a low range of slightly increased serum TSH (Stanbury, Aiginger & Harrison, 1979; Fisher & Klein, 1981). Transient hypothyroxinaemia with low serum T_4 , with normal levels of serum TSH associated with a normal TSH response to TRH, seen in infants, has been ascribed to hypothalamic immaturity. In a preliminary experiment in which six pyridoxine-deficient and six control pups respectively received daily intraperitoneal injections of TRH (15 µg/100 g body wt) for 1 week, there was no significant difference in pituitary TSH values between the two groups (11.14 ± 0.65 and 10.70 ± 0.76 µg/mg pituitary protein for control and deficient groups respectively).

The present results are interpreted as showing that in the pyridoxine-deficient rat the site of the defect is the hypothalamus rather than the pituitary or the thyroid gland. The decrease in pituitary TSH in the deficient rat and its response to injected TRH support this view. The data showing no significant effect on serum TSH, however, are not conclusive. The results, while clearly showing that there was no increase, as would be expected in primary hypothyroidism, did not show a decrease, as would be expected from the pituitary TSH and serum T_4 and T_3 results. Clinical cases showing similar data have been reported (Stanbury *et al.* 1979) and have been interpreted as tertiary hypothyroidism (Jackson, 1982). Explanations for such results might include the following: (1) limited time-point data which might have excluded the effects of circadian rhythm (Jordan, Pigeon, McRae-DeGuerce *et al.* 1979; Jordan, Rousset, Perrin *et al.* 1980); (2) changes in the sensitivity of the pituitary gland to feedback suppression by thyroid hormones; TRH functions as the principal determinant of the 'set point' of this interaction (Reichlin, Martin & Jackson, 1978); (3) the short half-life of TSH might have made it difficult to obtain values reflecting long-term TSH secretion.

The synthesis and release of TRH is under the control of various monoamines. Pharmacological studies using inhibitors of noradrenaline synthesis or α -adrenergic blockers like phentolamine indicate a stimulatory role for noradrenaline in the control of TRH-mediated TSH secretion. However, the primary effect of the noradrenergic system is on the cold-stimulated secretion (Krulich, 1979). There was, of course, no difference in the noradrenaline content of

the hypothalamus between the deficient and control rats. The dopaminergic system exerts an inhibitory effect on the secretion of TSH but this effect is at the level of the pituitary gland itself as bromocriptine blunts the stimulatory effect of TRH in euthyroid subjects (Brown, Bacchus, Sachs *et al.* 1979). The inhibitory effect of dopamine is abolished by dopamine receptor antagonists like domperidone (Delitala, Deville & Lotti, 1980).

A stimulatory role for serotonergic neurones on growth hormone and prolactin secretion and the inhibition of such secretion by serotonin receptor antagonists have been reported (Arnold & Fernstrom, 1978). The postulate that serotonergic neurones stimulate TSH secretion in rats is supported by the observation that injection of serotonin into the third ventricle causes a rapid increase in serum TSH. This effect is completely reversed by pretreatment of rats with cyproheptadine, a serotonin receptor antagonist (Jordan, Poncet, Mornex & Ponsin, 1978). Smythe *et al.* (1982) have provided very convincing evidence to support the conclusion of Chen & Ramirez (1981) that serotonin stimulates the release of TRH from the hypothalamus. We have shown that serotonin in various brain areas is significantly decreased in the pyridoxine-deficient rat, with no change in the contents of dopamine and noradrenaline. Although this decrease in serotonin results in supersensitivity of the post-synaptic membrane to this neurotransmitter (Dakshinamurti & Paulose, 1984), there is a functional deficiency of serotonin in the pyridoxine-deficient rat brain (Dakshinamurti, 1982). Our results indicate that one of the consequences of this is hypothyroidism of hypothalamic origin.

ACKNOWLEDGEMENTS

This work was supported by grants from the Medical Research Council of Canada.

REFERENCES

- Arnold, M. A. & Fernstrom, J. D. (1978). Serotonin receptor antagonists block a natural, short term surge in serum growth hormone levels. *Endocrinology* **103**, 1159-1163.
- Brown, P. M., Bacchus, R., Sachs, L., Sonksen, P. H. & Wheeler, M. (1979). Bromocriptine suppression of TRH stimulated prolactin and thyrotrophin release and accompanying inhibition of bromocriptine induced growth hormone release by TRH in normal man. *Clinical Endocrinology* **10**, 481-488.
- Chen, Y. F. & Ramirez, V. D. (1981). Serotonin stimulates thyrotrophin-releasing hormone release from superfused rat hypothalamus. *Endocrinology* **108**, 2359-2366.
- Dakshinamurti, K. (1982). Neurobiology of pyridoxine. In *Advances in nutritional research*, vol. 4, pp. 143-179. Ed. H. H. Draper. New York: Plenum Press.

- Dakshinamurti, K., LeBlancq, W. D., Herchl, R. & Havlicek, V. (1976). Nonparallel changes in brain monoamines of pyridoxine-deficient growing rats. *Experimental Brain Research* **26**, 355–366.
- Dakshinamurti, K. & Paulose, C. S. (1984). Consequences of decreased brain serotonin in the pyridoxine-deficient young rat. *Progress in Neuropsychopharmacology and Biological Psychiatry* **7**, 743–746.
- Delitala, G., Deville, L. & Lotti, G. (1980). Domperidone, an extracerebral inhibitor of dopamine receptors, stimulates thyrotropin and prolactin release in man. *Journal of Clinical Endocrinology and Metabolism* **50**, 1127–1130.
- DiRenzo, G. F., Quattrone, G., Schettini, P. & Preziosi, P. (1978). Effect of quipazine and D-fenfluramine, two serotonin-like drugs, on TSH secretion in basal and cold stimulated conditions in the rat. *Life Sciences* **22**, 1879–1886.
- DiRenzo, G. F., Quattrone, A., Schettini, G. & Preziosi, P. (1979). Effect of selective lesioning of serotonin-containing neurons on the TSH-inhibiting action of D-fenfluramine in male rats. *Life Sciences* **24**, 489–494.
- Dupont, A., Dussault, J. H., Rouleau, D., DiPaolo, T., Coulombe, P., Gagne, B., Merand, Y., Moore, S. & Barden, N. (1981). Effect of neonatal thyroid deficiency on the catecholamine, substance P, and thyrotropin-releasing hormone contents of discrete rat brain nuclei. *Endocrinology* **108**, 2039–2045.
- Dussault, J. H. & Labrie, F. (1975). Development of the hypothalamic-pituitary-thyroid axis in the neonatal rat. *Endocrinology* **97**, 1321–1324.
- Farquhar, M. G., Skutelsky, E. H. & Hopkins, C. R. (1975). Structure and function of the anterior pituitary and dispersed pituitary cells. In *The anterior pituitary*, pp. 83–135. Eds A. Tixier-Vidal & M. G. Farquhar. New York: Academic Press.
- Fisher, D. A. & Klein, A. H. (1981). Thyroid development and disorders of thyroid function in the newborn. *New England Journal of Medicine* **304**, 702–712.
- Gilowski, J. & Iversen, L. L. (1966). Regional studies of catecholamines in the rat brain - I. The disposition of [³H]norepinephrine, [³H]dopamine and [³H]dopa in various regions of the brain. *Journal of Neurochemistry* **13**, 665–669.
- Hammel, I., Naot, Y., Ben-David, E. & Ginsburg, H. (1978). A simplified microassay for serotonin: modification of the enzymatic isotopic assay. *Analytical Biochemistry* **90**, 840–843.
- Jackson, I. M. D. (1982). Thyrotropin-releasing hormone. *New England Journal of Medicine* **306**, 145–155.
- Jordan, D., Pigeon, P., McRae-DeGuerce, A., Pujol, I. F. & Mornex, R. (1979). Participation of serotonin in thyrotropin release. II. Evidence for the action of serotonin on the phasic release of thyrotropin. *Endocrinology* **105**, 975–979.
- Jordan, D., Poncet, C., Mornex, R. & Ponsin, G. (1978). Participation of serotonin in thyrotropin release. I. Evidence for the action of serotonin on thyrotropin releasing hormone release. *Endocrinology* **103**, 414–419.
- Jordan, D., Rousset, B., Perrin, F., Fournier, M. & Orgiazzi, J. (1980). Evidence for circadian variation in serum TSH, 3,5,3'-triiodothyronine and thyroxine in the rat. *Endocrinology* **107**, 1245–1248.
- Jung, R. T., Shetty, P. S. & James, W. P. T. (1980). Nutritional effects on thyroid and catecholamine metabolism. *Clinical Science* **58**, 183–191.
- Krulich, L. (1979). Central neurotransmitters and the secretion of prolactin, GH, LH and TSH. *Annual Review of Physiology* **41**, 603–615.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.
- Morley, J. E., Brammer, G. L., Sharp, B., Yamada, T., Tuwiler, A. & Hershman, J. M. (1981). Neurotransmitter control of hypothalamic-pituitary-thyroid function in rats. *European Journal of Pharmacology* **70**, 263–271.
- Morley, J. E., Damassa, D. A., Gordon, J., Pckary, A. E. & Hershman, J. M. (1978). Thyroid function and vitamin A deficiency. *Life Sciences* **22**, 1901–1906.
- Reichlin, S., Martin, J. B. & Jackson, I. M. D. (1978). Regulation of thyroid-stimulating hormone (TSH) secretion. In *The endocrine hypothalamus*, pp. 229–269. Eds S. L. Jeffcoate & J. S. M. Hutchinson. New York: Academic Press.
- Saavedra, J. M., Brownstein, M. & Axelrod, J. (1973). A specific and sensitive enzymatic-isotopic microassay for serotonin in tissues. *Journal of Pharmacology and Experimental Therapeutics* **186**, 508–515.
- Smythe, G. A., Bradshaw, J. E., Cai, W. Y. & Symons, R. G. (1982). Hypothalamic serotonergic stimulation of thyrotropin secretion and related brain-hormone and drug interactions in the rat. *Endocrinology* **111**, 1181–1191.
- Sole, M. J. & Hussain, M. N. (1977). A simple specific radio-enzymatic assay for the simultaneous measurement of picogram quantities of norepinephrine, epinephrine and dopamine in plasma and tissues. *Biochemical Medicine* **18**, 301–307.
- Stanbury, J. B., Aiginger, P. & Harrison, M. D. (1979). Familial goiter and related disorders. In *Endocrinology*, vol. 1, pp. 523–539. Eds L. J. DeGroot, G. F. Cahill, Jr, W. D. Odell *et al.* New York: Grune & Stratton.
- Stephens, M. C. & Dakshinamurti, K. (1976). Galactolipid fatty acids in brain of pyridoxine-deficient young rats. *Experimental Brain Research* **25**, 465–468.
- Stephens, M. C., Havlicek, V. & Dakshinamurti, K. (1971). Pyridoxine deficiency and development of the central nervous system in the rat. *Journal of Neurochemistry* **18**, 2407–2416.
- Tulp, O. L., Krupp, P. P., Danforth, E., Jr & Horton, E. S. (1979). Characteristics of thyroid function in experimental protein malnutrition. *Journal of Nutrition* **109**, 1321–1332.

DESCRIPTION OF PLATE

FIGURE 1. Photomicrograph of thyroid follicular cells from a control animal. Note the abundance of endoplasmic reticulum primarily in a dilated appearance (arrows) ($\times 3500$).

FIGURE 2. Photomicrograph of thyroid follicular cells from a pyridoxine-deficient rat. Whereas numerous profiles of endoplasmic reticulum are present, they are primarily in a non-dilated form (arrows) ($\times 3500$).

FIGURE 3. Electron micrograph of a thyrotroph cell (TSH) from a control animal. Note moderate numbers of secretory granules (arrows) ($\times 6000$).

FIGURE 4. Electron micrograph depicting reduction of secretory granules (arrows) in a thyrotroph cell (TSH) from a pyridoxine-deficient animal ($\times 6000$).

