THE EFFECT OF THYROID DEFICIENCY INDUCED BY METHYL THIOURACIL ON THE MATURATION OF THE CENTRAL NERVOUS SYSTEM

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Thyroid hormone has a beneficial effect on body growth in the congenital cretin, but unless treatment is begun very early, it has little influence on mental development (Lewis, 1937; Brown, Bronstein & Kraines, 1939). In hypothyroidism of later life (myxoedema), it improves both the mental and bodily states (Means, 1948). The clinical evidence thus suggests that thyroid deficiency is associated with changes in the maturation of the central nervous system which cannot later be reversed.

This important inference has not been adequately studied by experimental methods. Benda (1947) examined the tissues of the central nervous system in 'a few experimental animals' about which he gives no details, and observed that the most constantly occurring effects of thyroid deficiency are vascular stasis, oedema, an impairment of myelination and loss of cortical cells. These effects are said to resemble those associated with chronic anoxia, and have consequently been attributed to a lowered tissue metabolism. Similar changes have been reported in the brains of young rats born of thiouracil-treated mothers (Barrnett, 1948).

The present experiment on rats was undertaken to study the problem in greater detail. Observations were restricted to the sensory and motor areas of the cerebral cortex (Krieg, 1946), but were correlated with associated changes in the growth and metabolic rate of the experimental animals and in the time of appearance of stereotyped behaviour.

MATERIALS AND METHODS

Experimental procedure

Twenty-eight female albino rats were mated, and on the day of birth half of the young of each sex was interchanged with half of the same sex from another litter born on the same day, any odd rat being discarded. In this way, two reconstituted litters of equal size and similar composition were formed. One of these reconstituted litters received subcutaneous injections of a 4 % suspension of methyl thiouracil in a 2 % solution of sodium alginate. 0·05 ml. of this suspension was injected daily for the first 10 days of life, 0·1 ml. for the next 10 days and 0·2 ml. for the remainder of the experiment. The other litter, which acted as control, was given a similar amount of the sodium alginate solution only. In order to test whether the experimental treatment was effective, the oxygen consumption of the experimental and control litters was compared at intervals by the method used by Krohn & White (1950).

Tests of behaviour

The air-righting reaction and the placing reflex were used as tests of the maturation of automatic behaviour. Each animal was held back downwards, and was then dropped on to a cotton-wool pad from a height of 1 ft. The air-righting response was recorded as present in those rats which landed on all fours.

The presence of the placing reflex was tested by touching the chin against a metal bar. The mature animal responds by immediately placing its fore-paws on the bar on either side of its chin. If this response did not occur within 3 sec. of applying the stimulus it was judged to be absent.

Preparation of tissues

The rats were weighed at 15 and 24 days of age and were then killed by chloroform. Their tissues were fixed in formalin by the perfusion method (Eayrs, 1950) using the perfusing and fixing fluids of Koenig, Groat & Windle (1945). The brains were

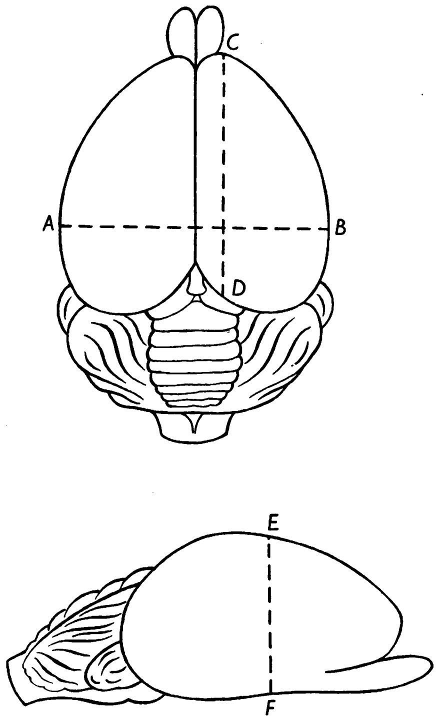

Fig. 1. Linear dimensions used to estimate growth of rat brain (see Table 2). The plane *EF* passes vertically through the optic chiasma.

removed, weighed, and placed in fixative for a week. They were then measured with callipers along the three axes shown in Fig. 1, cut in a standard vertical plane passing through the optic chiasma and the sensorimotor cortical areas and subsequently embedded in paraffin wax. Coronal sections at 7 μ were taken from the cut surface and were progressively stained for Nissl substance in gallocyanin chromalum for 24 hr. at 37° C. (Einarson, 1932).

The trachea with the thyroid gland attached was also removed, embedded in paraffin, cut at 7 μ and stained with Ehrlich's haematoxylin and eosin.

Quantitative estimations

Two fine lines were drawn in Indian ink on the coronal sections of the cerebral cortex, one passing horizontally through the most central of a constantly occurring group of blood vessels lying at the base of the longitudinal fissure and the other vertically through the apex of the centrum ovale (Fig. 2). These lines mark off the approximate extent of the sensorimotor cortex, the lamina ganglionaris forming a broad arc between them. In four adjoining microsopic fields taken from the middle of this arc, the outline of the cell body of each neurone in which a nucleolus was visible was drawn under the projection microscope. The exact magnification of these drawings (approx. 2000 ×) varied slightly between sections, but was accurately determined for each with a stage micrometer. The cross-sectional areas of the cell bodies were measured on the drawings with a planimeter, and after correction for magnification the mean area of each cell body was recorded in square microns. In addition, the method described by Chalkley (1943) was used to estimate the pro-

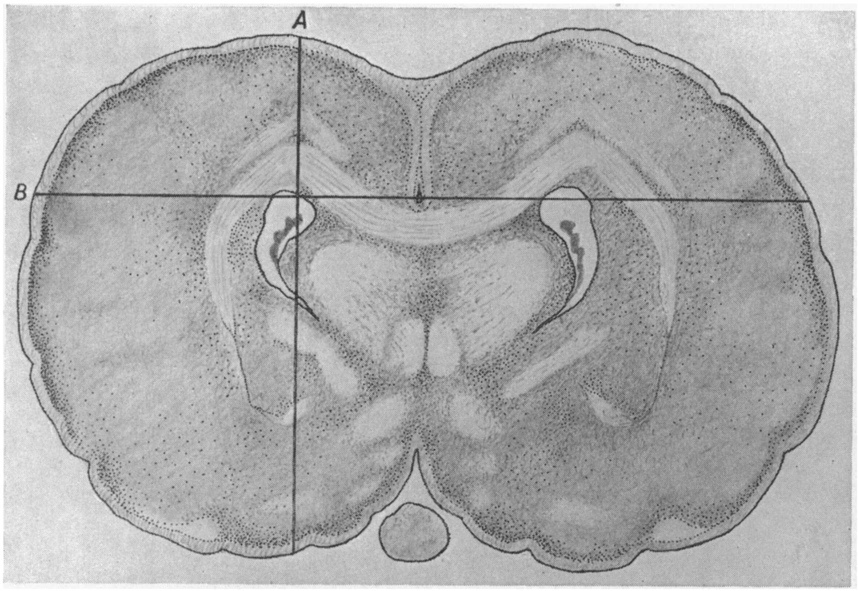

Fig. 2. Coronal section through rat brain passing through optic chiasma. The region of cortex measured extended between the guide lines from *A* to *B*.

portion of the lamina ganglionaris occupied by the pyramidal cell bodies in relation to that occupied by other tissue components. The whole of that part of layer 5 which was contained between the guide lines was measured in this way at a magnification of about 650 ×. Layer 5 has no sharp boundaries, and it is therefore possible that small parts of the adjacent layers 4 and 6 may have been included in some of the measurements. Since, however, this was as likely to occur in the experimental as in the control tissues, there is no reason to believe that any systematic error was involved which would invalidate the results.

These estimations were made on the tissues of six pairs of male litter-mates at both 15 and 24 days of age, and the data were statistically analysed for differences between the experimental and control animals.

RESULTS

Depression of thyroid function

The administration of methyl thiouracil resulted in an early depression in the amount of oxygen consumed by the experimental litters. Fig. 3 shows that at 4 days of age the mean oxygen consumption of the experimental litters had fallen by 18 % compared with that of control litters of the same size. This depression had

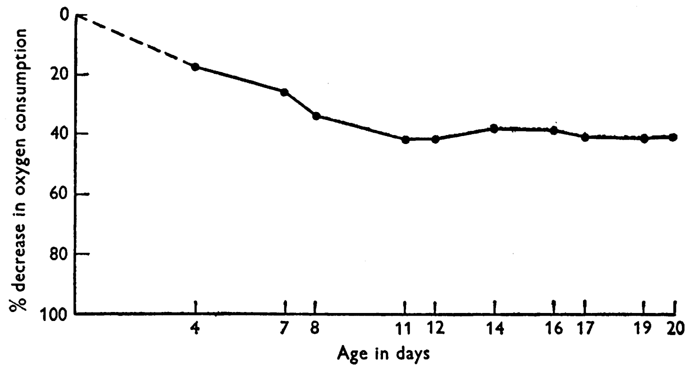

Fig. 3. Depression of oxygen consumption of litters treated with methyl thiouracil from the day of birth. Percentage decrease when compared with litter-mate controls plotted against age.

increased to about 40 % by the 11th day and remained at this level for the rest of the experiment.

The thyroid glands of the experimental animals showed the histological changes which normally follow treatment with thyroid depressant substances (MacKenzie & MacKenzie, 1943).

Growth

The experimental treatment retarded the growth of both body and brain (Table 1). At 24 days old the mean weight of the animals given thiouracil was 11·0 g. less, and that of their brains 0·25 g. less than those of normal litter-mates. Both differences were statistically significant. There was no significant difference in body weight between the two groups at 15 days old, but the bodily proportions of the experimental rats were characteristic of cretinism. The agreement in body weight seemed to be partly due to a disproportionate enlargement of the gut in the thyroid-deficient animals (previously reported by Scow & Simpson (1945) as a consequence of surgical thyro-parathyroidectomy). The brains of the 15-day-old experimental rats weighed significantly less (0·18 g.) than those of the controls.

Comparison of the ratio of brain weight to body weight (Table 1) showed that at 15 days old the bodies of the experimental animals were larger in relation to the size of their brains than were those of the controls. This ratio changed very little in experimental rats between 15 and 24 days of age, but decreased significantly in the the controls. It may therefore be inferred that between 15 and 24 days of age the growth of the body was more severely disturbed by the experimental treatment than was that of the brain.

All the linear dimensions measured on the brains of the experimental animals were about 8 % smaller than those of the controls both at 15 and 24 days old. (Table 2). Furthermore, the brains of the thyroid-deficient rats differed from those of the controls in being wider in relation to their length (ratio AB/CD in Table 2). There also was a difference in the trend of growth, for between 15 and 24 days of age the brains of the control animals grew longer in relation to their width while those of the experimental animals grew wider in relation to their length.

Table 1. *Comparison of body weight and brain weight of rats treated from birth with methyl thiouracil and their litter-mate controls*

Measurement	Age (days)	No. of observations	Control	Experimental	Difference	t	P
Mean weight of body (g.) ± standard error of mean	15	32	18·1±0·98	17·1±1·21	1·0±1·67	0·599	0·6–0·5
	24	30	31·8±2·23	20·8±1·43	11·0±2·65	4·155	<0·001
Mean weight of brain (g.) ± standard error of mean	15	32	0·92±0·025	0·74±0·028	0·18±0·037	4·884	<0·001
	24	30	1·08±0·024	0·83±0·020	0·25±0·031	8·004	<0·001
Mean ratio: brain weight ×100/body weight ± standard error of mean	15	32	5·21±0·17	4·46±0·20	0·75±0·265	2·832	0·01–0·001
	24	30	3·60±0·21	4·22±0·25	0·62±0·336	1·880	0·1–0·05

Table 2. *Comparison of linear dimensions of the brains of hypothyroid rats and normal controls at 15 and 24 days old*

Measurement (cm.) ± standard error of mean (see Fig. 1)	Age (days)	No. of observations (litter means)	Control	Experimental	Difference	Percentage difference from control measurement	t	P
Mean width (AB)	15	16	1·28 ±0·020	1·19 ±0·019	0·09 ±0·028	7·0	3·290	0·01–0·001
Mean length (CD)		16	1·14 ±0·027	1·03 ±0·023	0·11 ±0·035	8·8	2·963	0·02–0·01
Mean height (EF)		16	0·83 ±0·013	0·78 ±0·010	0·05 ±0·016	6·0	2·813	0·02–0·01
Ratio: AB/CD		16	1·130±0·0121	1·155±0·0073	0·025±0·1042	2·2	1·765	0·1 –0·05
Mean width (AB)	24	15	1·34 ±0·014	1·23 ±0·017	0·11 ±0·021	8·2	4·993	<0·001
Mean length (CD)		15	1·19 ±0·016	1·06 ±0·015	0·13 ±0·022	10·9	6·105	<0·001
Mean height (EF)		15	0·85 ±0·007	0·80 ±0·007	0·05 ±0·010	5·9	5·204	<0·001
Ratio: AB/CD		15	1·123±0·0067	1·161±0·0060	0·038±0·0090	3·4	4·238	<0·001

Tests of behaviour

The development of both the air-righting response and the placing reflex was significantly retarded in the experimental animals. The ability to land on their feet was present in more than 10 % of control rats at 15 days old, and in 90 % at 19 days. The corresponding percentages in the experimental animals were 0 and 20 % ($\chi^2 = 43{\cdot}8$ with one degree of freedom; $P < 0{\cdot}001$). Similarly, the placing reflex was elicited in 70 % of the control but in none of the experimental animals at 19 days old.

Development of the cerebral cortex

The mean cross-sectional area of the pyramidal cell bodies in layer 5 of the sensorimotor cortex of thiouracil-treated rats was only two-thirds of that of the controls (Table 3). This difference was present at both 15 and 24 days old, and was statistically significant at both ages. The pyramidal cells of the control animals did not increase in cross-sectional area between 15 and 24 days of age. A slight increase in the mean size of those of the experimental rats was not statistically significant ($P = 0{\cdot}7$–$0{\cdot}6$).

There were more pyramidal cell bodies per unit area in the experimental tissues than in the control. Statistical analysis (Table 3) shows that this difference was significant at the 5 % level in 15-day-old rats, and at the 1 % level at 24 days of age. Between these two ages, the number of cells per unit area decreased significantly in the control animals ($t = 2{\cdot}582$; $P = 0{\cdot}02$–$0{\cdot}01$), but a relatively small decrease in the thiouracil-treated rats was not statistically significant ($P = 0{\cdot}6$–$0{\cdot}5$).

Table 3. *Comparison of (i) the mean cross-sectional area and (ii) the mean number in a microscopic field of standard size of pyramidal cell bodies in the lamina ganglionaris of the cortex of rats treated with methyl thiouracil and of litter-mate controls*

Measurement	Age (days)	No. of standard fields	Control	Experimental	Difference	t	P
Mean cross-sectional area	15	12	260·4 ± 11·20	166·4 ± 11·64	94·0 ± 16·15	5·819	<0·001
(μ^2) ± standard error	24	12	259·3 ± 4·11	176·3 ± 11·97	83·0 ± 12·58	6·559	<0·001
Mean no. of cell bodies in	15	12	24·5 ± 1·72	34·2 ± 3·22	9·7 ± 3·66	2·648	0·05–0·02
an area of standard size ± standard error	24	12	18·3 ± 1·31	32·0 ± 2·05	13·7 ± 2·44	5·621	<0·001

Table 4. *Comparison of the space occupied by the bodies of the pyramidal neurones of the lamina ganglionaris in relation to that of other constituents; and of the rate of change of this ratio with age*

Measurement	Age (days)	No. of observations	Control	Experimental	Difference	t	P
Ratio: $\frac{\text{intercellular substance}}{\text{pyramidal cells}}$	15	12	4·25 ± 0·115	3·17 ± 0·088	1·08 ± 0·145	7·439	<0·001
± standard error	24	12	6·57 ± 0·212	3·71 ± 0·116	2·86 ± 0·242	11·82	<0·001
Regression coefficient: change of ratio $\frac{\text{intercellular substance}}{\text{pyramidal cells}}$ with age	15–24	24	0·257 ± 0·0268	0·060 ± 0·0162	0·197 ± 0·0313	6·311	<0·001

These results show that during the normal growth of the lamina ganglionaris there is a reduction in the number of neurones in a unit area of tissue, while at the same time the size of the cell bodies remains unchanged. The wider spacing of the cell bodies can be attributed to an increase in one or more of the components of the intervening tissue ('intercellular substance'). The inference that this change was much less marked in the tissues of the experimental animals was tested by estimating, in all groups of rats, the volume occupied by pyramidal cell bodies relative to that taken up by the intercellular substance (Chalkley, 1943). Comparison of these measurements (Table 4) shows that, at both 15 and 24 days old, the amount of the intercellular substance relative to the volume occupied by the cell bodies of pyramidal neurones was much (and significantly) less in the experimental animals than in the controls. A plot of this measure against age showed that a significant increase in the relative amount of intercellular substance occurred in both experimental and control animals between 15 and 24 days of age. Regression coefficients were calculated and compared. The rate of increase proved to be significantly greater in the control tissues than in the experimental (Table 4).

DISCUSSION

Changes in growth

While the extent to which body growth was depressed in the present experiments was less than that reported after thyroid deficiency by other workers (Hughes, 1944; Salmon, 1938; Scow & Simpson, 1945), the evidence is clear that the animals given thiouracil were suffering from chronic thyroid deficiency. It is also plain that the treatment depressed the growth of the brain. The two changes do not appear to be interdependent, for while the growth of the body is restored in the thyro-para-thyroidectomized rat by treatment with growth hormone, that of the brain is not (Scow & Marx, 1945). It seems therefore that a deficiency of thyroid hormone specifically impairs brain growth.

The brains of the experimental animals were also different in shape from those of the controls. During the first days of life the brain of the normal rat grows more rapidly in length than in width (Sugita, 1918*a*), but this tendency, while present in the controls, was significantly less marked in the experimental animals whose brains were consequently more infantile in general appearance. It is not possible to say whether this change was a direct effect or a secondary result of an impairment to the growth of the skull. Similar, but more severe, changes in the cranio-neural relationship which follow hypophysectomy performed at 6 days old have been attributed to disparity in the rates of growth of brain and skull (Walker, Simpson, Asling & Evans, 1950). The present results suggest that these effects may be largely due to the withdrawal of the thyrotrophic hormone of the pituitary.

Development of automatic behaviour

The appearance of the air-righting response and of the placing reflex were delayed in the thyroid-deficient rats. It is possible that in these animals the stimulus was inadequate to elicit the response, for the reduced excitability of peripheral nerve in hypothyroidism (Horsten & Boeles, 1949) would be expected to be associated with a rise in the threshold of sensory receptors. The fact that the air-righting reflex eventually appeared in the experimental rats without a corresponding increase in the stimulus suggests, on the other hand, that the retarded development of this reaction was due to an impairment to central nervous maturation. It is therefore significant that morphological changes were found in that part of the cerebral cortex whose integrity is essential for the performance of the placing reflex (Brooks, 1933).

Changes in the cerebral cortex

The two major changes which were observed in the maturation of the lamina ganglionaris were in the size and spacing of the cell bodies. Sugita (1918*b*) found that the pyramidal cells grow very slowly in the rat after about the 12th day of age, and this is borne out by the measurements made in the present experiment. The pyramidal cell bodies in the experimental tissues, however, were significantly smaller than in the control. Qualitative observations suggested that this arrested growth involved a reduction in the size of both nucleus and cytoplasm. Since the outline of the nuclear membrane was frequently obscured by the Nissl substance it proved impracticable to test the inference quantitatively.

The cell body itself, however, is but a small part of the whole neurone, a considerable proportion of which consists of processes, contained in the intercellular substance, which are not visible in Nissl-stained preparations. The reduction in amount and the slower rate of increase of the intercellular substance in the brains of the thyroid-deficient rats probably have a greater functional significance than the smaller size of the cell bodies; for while it is likely that these differences were due in part to a shortage of myelin (Benda, 1947), another possibility is that some change occurred in the development of the axon-dendritic network which is so prominent a feature of cortical maturation (Conel, 1941, 1947). This would imply that a disturbance to the formation of the proper number and pattern of inter-neuronal connexions may occur in hypothyroidism which must undoubtedly be reflected in behaviour and might well account for the retarded mental development of the human cretin. The application of more specific histological techniques to demonstrate the structure of the intercellular substance should enable a quantitative assessment to be made of the nature of the disturbance to this aspect of cerebral maturation.

SUMMARY

1. The changes in the growth of the body and of the brain which follow the administration of methyl thiouracil from the day of birth have been studied in the albino rat.
2. Thiouracil-injected rats grew less well than their litter-mate controls, and were cretinous in their appearance.
3. The brains of thiouracil-injected rats weighed significantly less than those of the controls at both 15 and 24 days old.
4. The experimental animals were retarded in the time of acquisition of the air-righting response and of the placing reflex.
5. The pyramidal cells of layer 5 of the sensorimotor cortex of the experimental animals were smaller than those of the controls. There were more of these cells per unit area of cortex in the experimental rats than in the controls. This was due not only to the smaller size of the cell bodies but also to a reduced amount of intervening substance.
6. The size of the pyramidal cell bodies did not increase appreciably between 15 and 24 days of age, but the amount of the intervening substance did. The rate of increase of this substance was less in the thiouracil-injected animals than in the controls.
7. The significance of these findings in relation to the mode of influence of thyroid deficiency on the development of the central nervous system is discussed.

REFERENCES

Barrnett, R. J. (1948). Quoted in Pincus, G. and Thimann, K. V., *The Hormones*. New York: Academic Press.

Benda, C. E. (1947). *Mongolism and Cretinism*. London: W. Heinemann.

Brooks, C. M. (1933). Studies on the cerebral cortex. II. Localised representation of hopping and placing reactions in the rat. *Amer. J. Physiol.* **105**, 162–171.

Brown, A. W., Bronstein, I. P. & Kraines, R. (1939). Hypothyroidism and cretinism in childhood. VI. Influence of thyroid therapy on mental growth. *Amer. J. Dis. Child.* **57**, 517–523.

Chalkley, H. W. (1943). Method for the quantitative morphologic analysis of tissues. *J. Nat. Cancer Inst.* **4**, 47–53.

Conel, J. L. (1941). *The Post-natal Development of the Human Cerebral Cortex.* Vol. 2. *The Cortex of the One-month Infant.* Cambridge, Mass.: Harvard University Press.

Conel, J. L. (1947). *The Post-natal Development of the Human Cerebral Cortex.* Vol. 3. *The Cortex of the Three-month Infant.* Cambridge, Mass.: Harvard University Press.

Eayrs, J. T. (1950). An apparatus for fixation and supravital staining of tissues by the perfusion method. *Stain Tech.* **25**, 137–142.

Einarson, L. (1932). Method for progressive selective staining of Nissl and nuclear substance in nerve cells. *Amer. J. Path.* **8**, 295–308.

Horsten, G. P. M. & Boeles, J. Th. F. (1949). The influence of hypothyroidism on the excitability of peripheral nerves. *Arch. int. Pharmacodyn.* **78**, 93–99.

Hughes, A. M. (1944). Cretinism in rats induced by thiouracil. *Endocrinology,* **34**, 69–76.

Koenig, H., Groat, R. A. & Windle, W. F. (1945). A physiological approach to perfusion-fixation of tissues with formalin. *Stain Tech.* **20**, 13–22.

Krieg, W. J. S. (1946). Connections of the cerebral cortex. I. The albino rat. A. Topography of the cortical areas. *J. comp. Neurol.* **84**, 221–275.

Krohn, P. L. & White, H. C. (1950). The effect of hypothyroidism on reproduction in the female albino rat. *J. Endocrinol.* **6**, 375–385.

Lewis A. (1937). A study of cretinism in London. *Lancet,* **1**, 1505–1509; **2**, 5–9.

MacKenzie, C. G. & MacKenzie, J. B. (1943). Effect of sulfonamides and thioureas on the thyroid gland and basal metabolism. *Endocrinology,* **32**, 185–209.

Means, J. H. (1948). *The Thyroid and its Diseases,* 2nd ed. Philadelphia: Lippincott.

Salmon, I. N. (1938). The effect on the growth rate of thyro-parathyroidectomy in new born rats and of the subsequent administration of thyroid, parathyroid and anterior hypophysis. *Endocrinology,* **23**, 446–457.

Scow, R. W. & Marx, W. (1945). Response to pituitary growth hormone of rats thyroidectomised on the day of birth. *Anat. Rec.* **91**, 227–236.

Scow, R. W. & Simpson, M. E. (1945). Thyroidectomy in the newborn rat. *Anat. Rec.* **91**, 209–226.

Sugita, N. (1918*a*). Comparative studies on the growth of the cerebral cortex. III. On the size and shape of the cerebrum in the Norway rat and a comparison of these with the corresponding characters in the albino rat. *J. comp. Neurol.* **29**, 1–9.

Sugita, N. (1918*b*). Comparative studies on the growth of the cerebral cortex. VI. Part I. On the increase in size and on the developmental changes of some nerve cells in the cerebral cortex of the albino rat during the growth of the brain. *J. comp. Neurol.* **29**, 119–151.

Walker, D. G., Simpson, M. E., Asling, C. W. & Evans, H. W. (1950). Growth and differentiation in the rat following hypophysectomy at 6 days of age. *Anat. Rec.* **106**, 539–554.