A CEPHALOMETRIC STUDY OF VERVET MONKEYS WITH INDUCED HYPOTHYROIDISM

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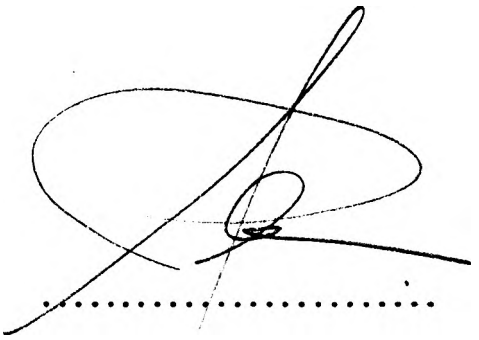
I hereby declare that this dissertation is my own work, and has not been submitted or incorporated in any other dissertation or thesis for any other degree.



C.B. Preston

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PROLOGUE

'Growth was conceived by an anatomist, born to a biologist, delivered by a physician, left on a chemist's doorstep, and adopted by a physiologist. At an early age she eloped with a statistician, divorced him for a psychologist, and is now being ardently wooed, alternately and concurrently, by an endocrinologist, a pediatrician, a physical anthropologist, and educationalist, a biochemist, a physicist, a mathematician, an orthodontist, a eugenist and the Children's Bureau ! '

Krogman, 1943.

ABSTRACT

Growth and development of the skull is influenced by metabolic factors such as the endocrinopathies. The purpose of this study is to examine the effects of induced hypothyroidism on growth and development of the skull of the Vervet monkey. Seven infant monkeys of the subspecies *Cercopithecus aethiops cloetei* were selected as the experimental animals in this study. Two of the seven monkeys were used as the control group, the remaining five monkeys were given radio-active iodine to depress their thyroid activity. Cephalometric records were taken of the animals for a period of approximately one year, at the end of which time they were sacrificed. At the age of 15 months the radiographs of the hypothyroidic monkeys, when compared to those of the control group, exhibit marked differences in cranial form. However, the most marked changes are seen in the cranial vaults of the experimental animals. Some of the findings are examined in the light of a number of theories which have been proposed for the control of the growth and development of the skull.

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INTRODUCTION

1.1 Aims of the investigation

Enlargement of the cranial vault and facial skeleton is due to intramembranous ossification, while growth at synchondroses, followed by endochondral ossification, is responsible for lengthening of the cranial base (van Limborgh, 1970). There are thus two types of bone growth responsible for enlargement of the skull.

Growth of the cranial vault occurs in harmony with that of the brain which reaches adult proportions early in the second decade of life (Brodie, 1955; Ford, 1958). Growth of the facial bones, being closely linked to somatic development (Hunter, 1966), is more gradual and continues till adulthood has been reached (Björk, 1964, 1968). From the fifth year onwards the size of the facial skeleton thus increases in relation to that of the neurocranium. At the same time the facial part of the skull gradually occupies a position more inferiorly situated, relative to the neurocranium (van Limborgh, 1970). The cranial base, situated between the neural and facial components of the skull, is concerned with the growth of both and may be regarded as a 'hafting zone' between them (Björk, 1955).

Growth and development of the skull is influenced by function as well as, amongst others, metabolic and hormonal factors (Salzman, 1954). Although certain effects of hypothyroidism on the growth of the skull have been described (Thoma, 1934; Ziskin and Applebaum, 1941; Korkhaus, 1957; Spiegel, Sather and Hayles, 1971) the relationship between reduced thyroid activity and craniofacial abnormality has yet to be explained (Graber, 1969).

The purpose of this study is to examine some of the effects of induced hypothyroidism on growth and development of the skull of the Vervet monkey.

1.2 Historical review

As early as the sixteenth century B.C., the ancient Chinese were aware that seaweed had a beneficial effect in some patients presenting with swelling of the neck. An understanding of this relationship was however, only gained in the nineteenth century, after Courtois in 1812, isolated the element which is now known as iodine (Nocenti, 1968). Prout in 1816 and Coindet in 1820 demonstrated that iodine administration brought about a regression of goiters; they believed that this element was the active ingredient in seaweed (Nocenti, 1968).

Fagge in 1871, cited by Nocenti in 1968, noted the association of thyroid atrophy with cretinism which was characterized by a dry, puffy skin and mental as well as physical lassitude. The syndrome was also termed myxoedema because it was believed that the integumental puffiness was due to an accumulation of mucus in the subcutaneous tissues. In the late nineteenth century Horseley (Tepperman, 1968) noted the development of the myxoedema-like syndrome in monkeys which had been thyroidectomized.

Among the first authors to record the effects of thyroid hormone deficiency on growth of the skull were Taylor and Appleton, who in 1929 reported a case of cretinism in which there was mandibular retrusion. Engel *et al.* (1941) presented radiographic findings in the crania of thirteen selected cases suffering from hypothyroidism. It was Engel's view that thyroid deficiency affected craniofacial growth by retarding its velocity rather than by modifying its growth pattern. Korkhaus (1957)

studied developmental abnormalities in the faces of individuals suffering from thyroid hormone deficiency. He concluded that an increase in tongue size was responsible for the mandibular over-development which he observed in a number of instances. Spiegel *et al.*, in 1971, studied children with various endocrine diseases and his results indicate that children with infantile myxoedema have retarded vertical facial growth.

1.3 Physical effects of hypothyroidism

Thyroid hormone deficiency produces growth changes which are dependent on the degree of the deficiency as well as upon the age at which it occurs (Harper, 1973; Hinrichs, 1966). Cretinism is a chronic disease beginning in foetal life or infancy and is caused by a congenital deficiency of thyroid substance (Collins and Crane, 1960). It is characterized by a retardation of mental and physical development; weak musculature, accompanied by obesity; dry coarse skin and hair; and a characteristic facial expression marked by a protruding tongue and open mouth (Harper, 1973). Childhood hypothyroidism, which is also known as juvenile myxoedema, has its origin later in life than does cretinism. Its effects on growth are generally less severe, and some of the typical cretinoid symptoms may be absent.

In childhood hypothyroidism there is a generalized retardation of growth of the cranium; but the different parts are not all equally affected by the condition (Thoma, 1934). The latter finding is in keeping with the concept of 'chronologic specificity' as published by Engel *et al.*(1941), which states that : 'The greater the activity of a tissue the greater its vulnerability'. Infantile myxoedema thus affects the frontal area, which is the first to mature (Björk, 1955),

the least, the parietal area more and the occipital region, which grows the longest (Björk, 1955) the most (Engel *et al.*, 1941). Despite the variation in degree to which the component bones of the cranial vault are affected in hypothyroidism, generalized abnormally wide sutures are a consistent finding which may be readily demonstrated radiographically (Weinmann and Sicher, 1955).

Individuals with juvenile myxoedema are reported to have retarded growth of the facial skeleton in general (Engel *et al.*, 1941; Gold, Engel and Bronstein, 1948). Korkhaus (1957), however, attributes most of the developmental disturbances seen in the human face, in juvenile myxoedema, to insufficient activity of the intersphenoidal and sphenoethmoidal synchondroses during the last month of foetal life, and immediately after birth. He noted a widening of the sphenoidal angle associated with a shortening of the anterior cranial base, as well as an increase in tongue size which, according to Korkhaus, may cause the mandivular mandibular enlargement. Spiegel *et al.* (1971), however, found the anterior cranial base to be advanced, the posterior cranial base to be retarded, and the cranial base angle to be more acute in 38 children suffering from juvenile myxoedema. In the same paper, Spiegel *et al.* reported a tendency towards maxillary protrusion, which he attributed to an advancement of the maxillo relative to the cranial base.

The adult form of myxoedema results in marked physiological abnormalities, but because of its late onset, it has much less effect on growth and development.

1.4 The effects of thyroxine on bone and cartilage

The combined and separate effects of thyroid - and growth hormone

on cartilage and bone growth, were studied by Smith, Graebler and Long, (1955), Asling and Nelson (1962), Kaplan and Shimizu (1963), Rigal (1964, Asling, Tse and Rosenberg (1968), Turbin (1971) and Toole (1973). The action of growth hormone is ubiquitous and all tissues except nervous- and eye-tissues are overtly stimulated to growth (Turban, 1971). This may be caused by the anabolic effects of growth hormone on protein metabolism (Smith *et al.*, 1955), while its action also enhances the transportation of certain amino acids across the cell membrane (Harper, 1973). Growth hormone induces epiphyseal cartilage proliferation resulting in a widening of the epiphyseal plates (Asling and Nelson, 1962; Asling, Tse and Rosenberg, 1968). On the other hand, when growth hormone was added to cultures containing explants of normal rabbit epiphyses, the hormone produced no detectable effects on the epiphyses (Rigal, 1964). The author attributes this finding to the absence of thyroid as well as other hormones from the culture medium.

Thyroid hormone also affects bone growth, however, thyroxine does not produce a widening of the epiphyseal plate, rather, it promotes erosion of the epiphyses, resulting in its narrowing, and eventual disappearance (Asling, Tse and Rosenberg, 1968). Asling *et al.*(1968) proposed that thyroid hormonal support was required for endochondral osteogenesis, and that thyroid augmentation of growth hormone, is manifested by increased maturational changes in the chondrocyte sequences.

In bones, hyaluronate synthesis is increased during the morphogenetic phase of 'cell migration and proliferation', while hyaluronate removal by the action of hyaluronidase accompanies subsequent differentiation, such as is seen during cartilage deposition (Toole, 1973). It was suggested by Toole that hyaluronate inhibits pre-cartilage cell aggregation, thereby

delaying further cellular differentiation. The control of hyaluronate turnover and of its interaction with mesenchymal cells, may be an important factor in the morphogenesis of the skeleton. Thyroxine prevents the inhibition of chondrogenesis by hyaluronate and in this manner is able to promote cartilage growth (Toole, 1973).

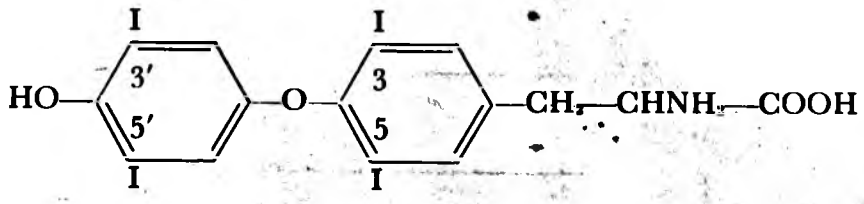
Some of the other actions attributed to thyroid hormone may also affect the growth of bone. Thus according to Harper (1973) thyroid hormone acts primarily via the protein synthesizing mechanisms of the target cells, or via its catalytic effect on the oxidative reactions of these cells (Nocenti, 1968).

Moderate concentrations of thyroid hormone have an anabolic effect, causing an increase in protein synthesis by stimulating growth hormone formation (Harper, 1973). In high concentrations, thyroid hormone may cause underdevelopment of the skeletal tissues. This could, in part, be due to a failure of the bone to compete for the protein required by the general increase in the basal metabolic rate (Albright and Reifenstein, 1948) resulting from the hyperthyroidism. These additional, non-specific, effects of thyroid hormone on the growth of bone; reflect indirect efforts of the thyroid secretions on metabolism in general (McClean and Urist, 1968).

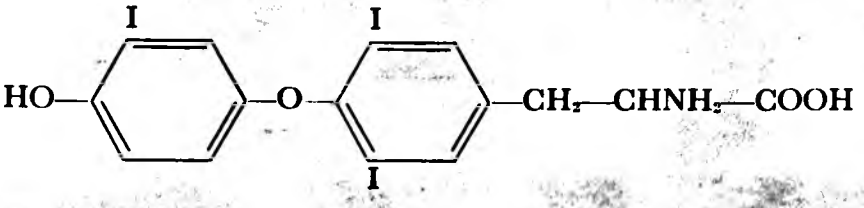
Histologic studies of long bones of thyroidectomized rats, reveal the absence of secondary centres of ossification; and greatly delayed epiphyseal closure due to the inhibition of cartilage removal (Weimann and Sicher, 1955). Clinically, delayed epiphyseal maturation is also a feature of hypothyroidism. This finding may be utilized in assessing whether thyroid hormone could be used to advantage, in the treatment of patients presenting with lack of growth (Fourman *et al.*, 1968).

1.5 Chemistry of the thyroid hormones

The thyroid gland liberates iodated amino acids of which L-Thyroxine (L-3,5,3',5', tetra-iodo thyronine) is the major circulating thyroid hormone, (Fig. 1) others are, L-Triiodothyronine (L-3,5,3', -triiodothyronine), L-3-monoiodotyrosine, L-2 and L-4 -monoiodohistidine. The latter three compounds are essentially inactive, and their biologic activity is not completely understood (Nocenti, 1968). The thyroid hormone with the highest biologic activity, amounting to five to ten times that of thyroxine, is 3,5,3' -triiodothyronine (Fig.1); it represents 5-7 per cent of the total iodine in the gland (McLean and Urist, 1968).



L-3,5,3′,5′-tetraiodothyronine (thyroxine)



L-3,5,3′-triiodothyronine

Fig.1 Structural formulas of some thyroid hormones

Thyroid tissue is able to concentrate iodine (Fig.2) which is required for the synthesis of thyroid hormones (Fig.3). Other tissues, such as gastric mucosa and salivary glands, also concentrate iodine, but they are unable to produce the thyroid hormones (Nocenti, 1968). Pharmacologically large doses of iodide do not affect the ability of the thyroid gland to concentrate it, but its organic binding is inhibited (Wolff and Chaikoff, 1948).

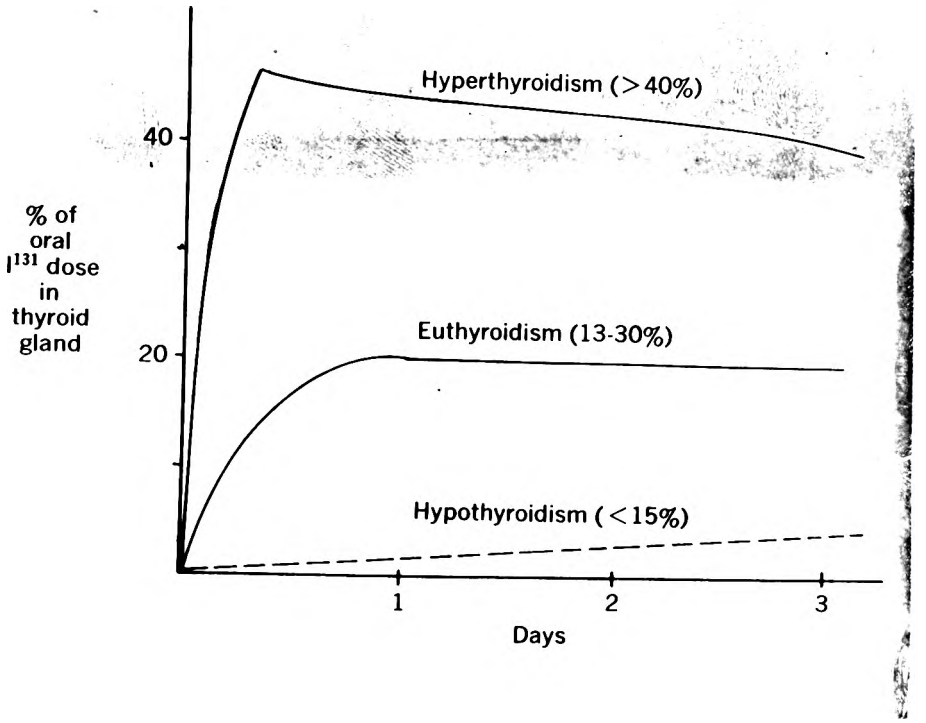


Fig.2 Idealized graph depicting thyroidal uptake of ^{131}I. (from Nocenti, 1968).

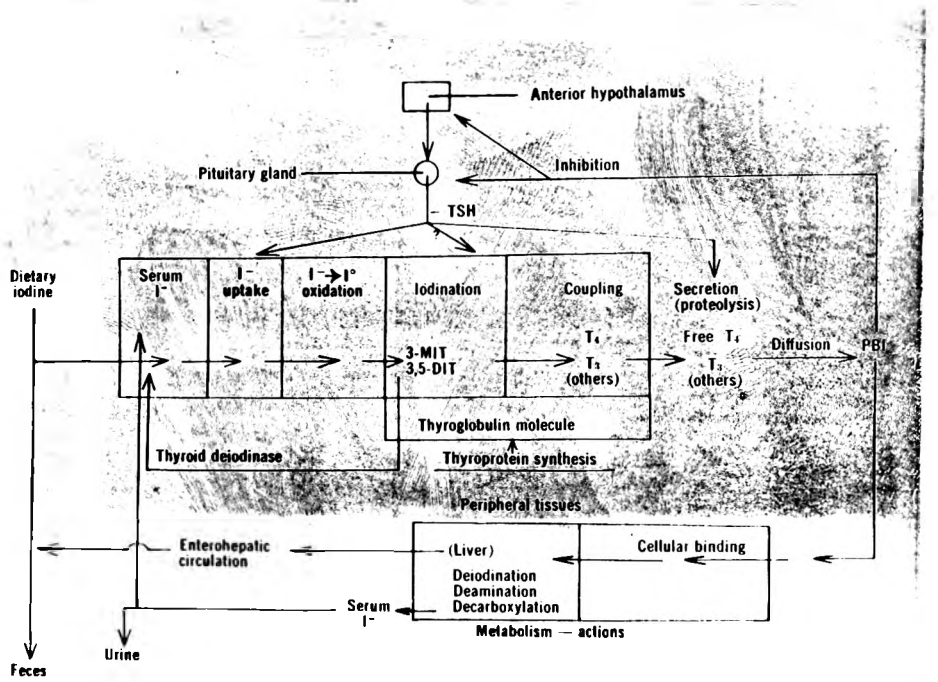


Fig.3 Diagrammatic illustration of iodine metabolism relative to thyroid function (From Nocenti,1968).

CHAPTER 2

MATERIALS AND METHODS

2.1 The experimental animals

Monkeys have been used in many studies of cranial growth and development (Moore, 1949; Gans and Sarnat, 1951; Craven, 1956; Baume, 1961 and Melsen, 1971). The results obtained indicate that there are many similarities between skull growth in monkeys and in humans (Melsen, 1971). For example, the areas of growth described by Moore (1949) in a *Macaque* monkey (Figs. 4 and 5) are similar to those described for human growth (Enlow, 1966). For this reason Vervet monkeys of the subspecies

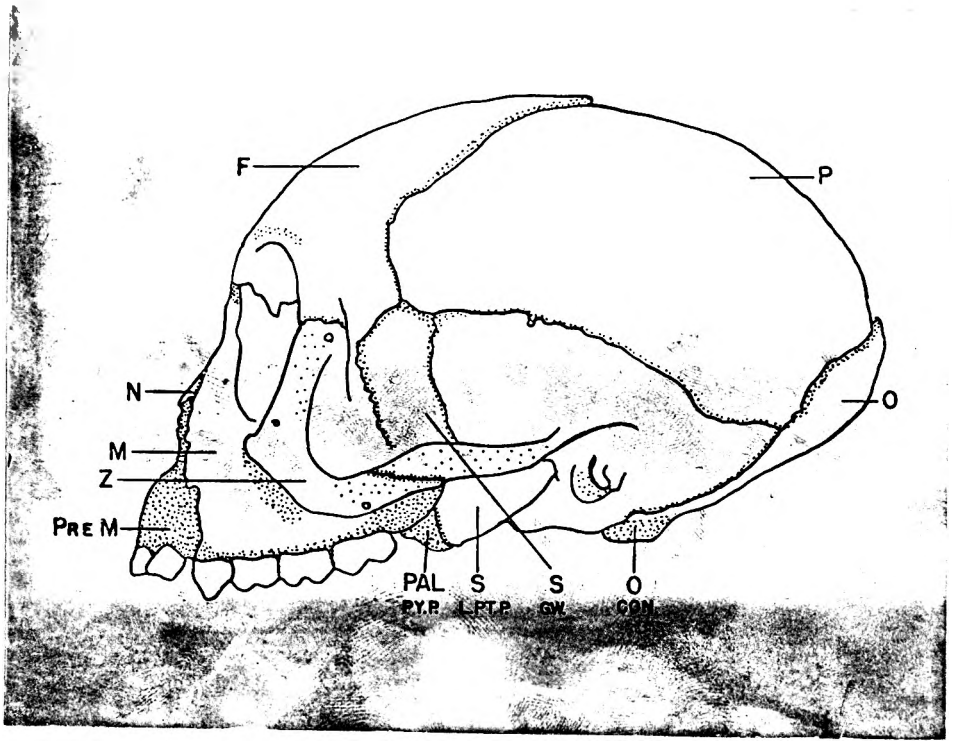


Fig.4 (From Moore, 1949.) For legend see Fig. 5.

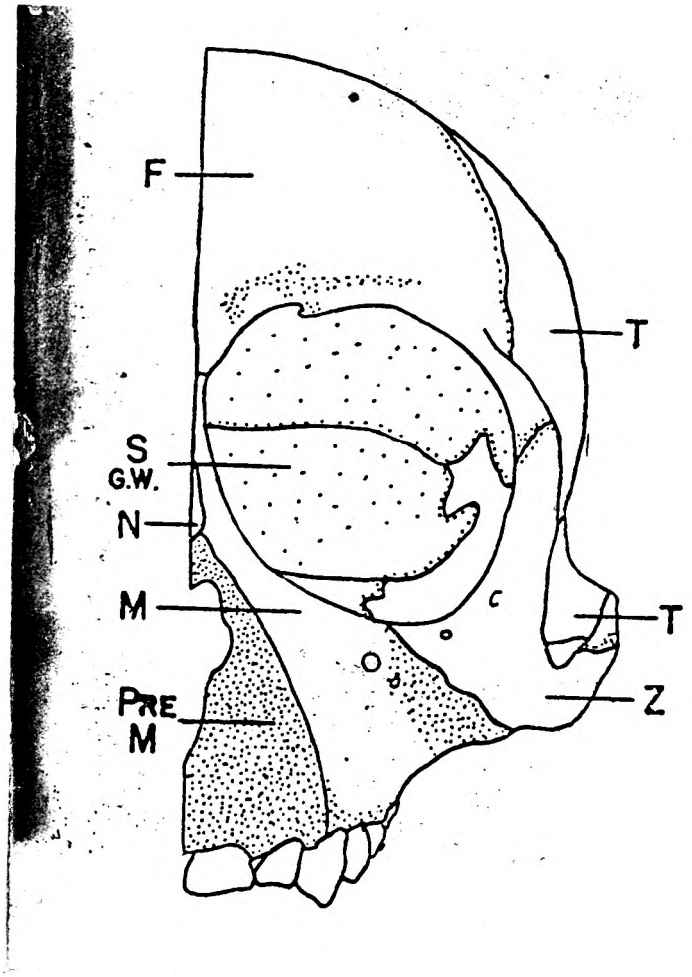


Fig.5 (From Moore, 1949)

F, frontal bone; M, maxilla; M F.P., frontal process of maxilla; N, nasal bone; O, occipital bone; O CON., condyle of occipital bone; O J.P., jugular process of occipital bone; P, parietal bone; PAL, palatine bone; PAL PY.P., pyramidal process of palatine bone; PRE M, premaxilla; PRE S, presphenoid; S, sphenoid bone; S B., body of sphenoid bone; S G.W., great wing of sphenoid bone; S L.PT.P., lateral pterygoid plate of sphenoid bone; S M.PT.P., medial pterygoid plate of sphenoid bone; T, temporal bone; T PET., Petrous portion of temporal bone; T SQ., squamous portion of temporal bone; Z zygomatic bone.

Figures 4 and 5 are lateral and anterior views, respectively, of the skull of a young *Macaque* monkey. Shaded areas are growth sites and the density of the shading is proportional to the degree of growth activity.

Cercopithecus aethiops cloetei (Fig.6) were selected as the experimental animals in this study.

Fig.6 Facial profile of male *Cercopithecus aethiops cloetei*.

Preston and Evans (1973) reported the radiographic cephalometric findings in normal adult Vervet monkeys and Ockerse (1959) described their teeth in detail. *Cercopithecus aethiops* has a dentition which is both heterodont and diphyodont with a dental formula similar to that of the human (Fig.7).

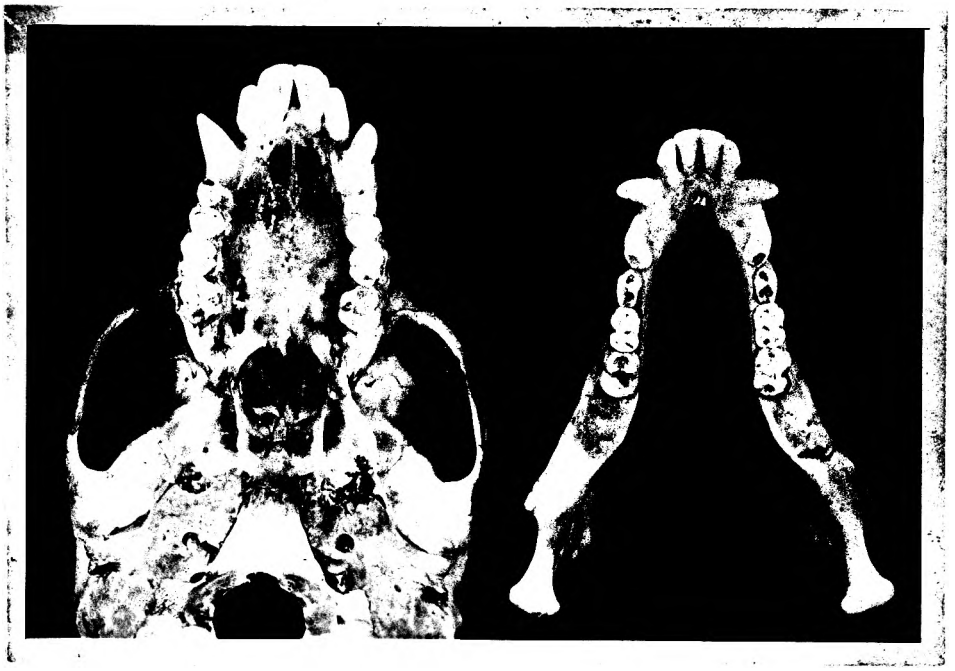


Fig. 7 Permanent dentition of *Cercopithecus aethiops*.

2.2 Source of the animals

Seven infant monkeys born at the South African Institute for Poliomyelitis Research, were used in the experiment. These babies were born to pregnant mothers, captured in the northern Transvaal area of the Republic of South Africa. The baby monkeys were not weighed at birth but they appeared to be reasonably uniform in size and were outwardly healthy. Six were males and one, the smallest, was a female.

2.3 Design of the experiment

The numbers one to seven were allocated to the experimental animals on a random basis and these were tatooed on the skin of the medial surface of the left thigh of each animal. Animals which had been allocated the numbers one to five became the experimental group, while those with the numbers six and seven were used as controls.

2.4 Rearing the animals

The animals were reared in the animal facilities of the South African Institute for Poliomyelitis Research. The intention was to house one mother plus her infant per cage. The female baby, number two in the series was, however, rejected by her mother and an attempt was made to use the mother of monkey number five as a foster mother for the rejected infant. Although weakened and obviously underdeveloped,this monkey survived for eight months, at which stage it died following an injection of radio-active iodine.

The above instance apart, each mother and her infant monkey was housed in a separate cage constructed from galvanized iron and approximately 1 x 1½ x 2 meter in size. The individual cages were kept in a single air-conditioned room with natural light, but no sun, from an east facing window. The process of weaning was allowed to take its natural course, food and water being freely available to the monkeys at all times.

2.5 Feeding

The monkeys were fed cubes, the composition of which is set out in Fig.8. The cubes were placed in a hopper which was regularly topped up. In addition the diet was supplemented with vegetables, fruits, ground nuts and stale whole wheat bread.

2.6 Induction of hypothyroidism

2.6.1 Chemical means

Einhorn and Säterborg (1962) investigated various antithyroid drugs and concluded that the agent of choice, for use prior to radiation therapy,

Composition of Monkey Cubes

Analysis

Protein	24.3%
Fibre	4.6%
Ca	1.2%
P	0.98%
Fat	5.4%
Metabolisable energy 2434 K cals/Kg	
Total digestive nutrients	69%

Constituents (Main)

Components of grain and wheat

Groundnut oil cake

Carcassemeal

Bonemeal

Salt

Sugar, Synthetic lysine and methionine

Binders e.g. molasses

Vitamine additions per 2½ tons

Vit. A	18 Mill. i.u.
Vit. D.	600,00 i.u.
Riboflavin	3½ gram
Pyridoxine	2¼ gram
B_{12}	46 mg
E	100,000 i.u.
Choline	1.6 gram

Mineral additions per 2½ tons

Iodine	18 gram

Fig.8 Composition of the monkey cubes.

was 'Neo-Mercazole'. It contains no sulphydryl group to protect the thyroid tissue against ionizing radiation, and does not reduce the thyroid uptake of radio-iodine to the same degree as does thiouracil. Neo-Mercazole (Fig.9) is the trade name for carbimazole, a creamy-white almost tasteless powder, which is slightly soluble in water and which is believed to exert its antithyroid effect, by 'blocking' the organic binding of iodine. Severe cases of thyrotoxicosis in humans are treated with 60 mgs. of Neo-Mercazole per day.

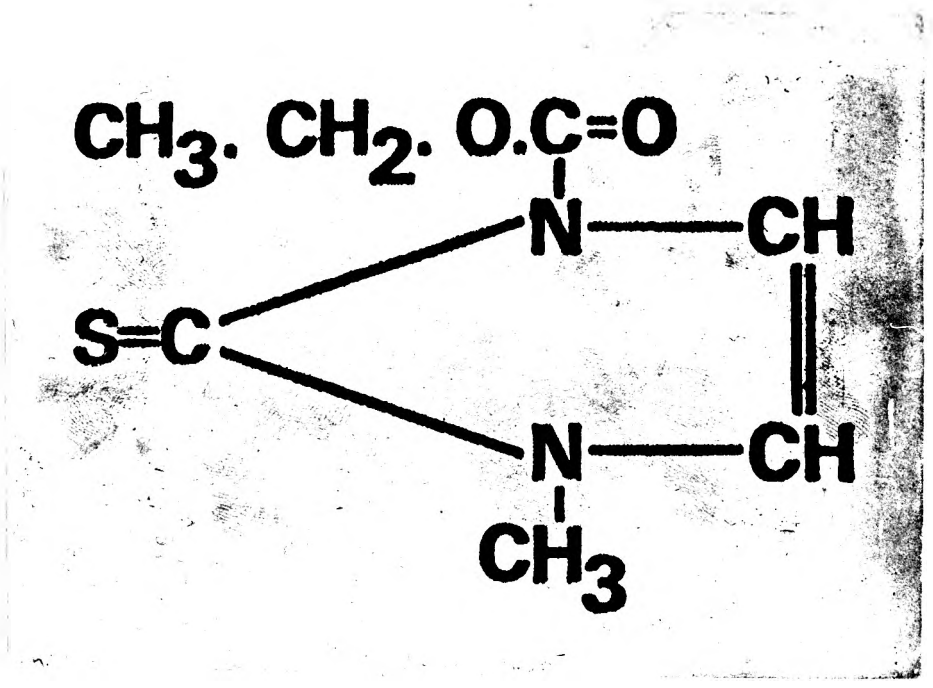


Fig.9 Structural formula of carbimazole

2.6.2 Thyroidectomy

Surgical removal of the thyroid gland may be complicated by injury to the recurrent laryngeal nerve as well as by damage to the parathyroid glands. This method of thyroid destruction requires highly specialized surgical technique to obviate the above complications.

2.6.3. Radio-isotopes

^{131}I is the most extensively used isotope for biochemical, diagnostic and therapeutic purposes (Wolff, 1970). Most of the effective radiation of ^{131}I results from its beta emisions, with the gamma radiation passing through the thyroid tissue, without causing many ionizations (Nocenti, 1968).

In cases of hyperthyroidism a single dose of ^{131}I causes remission in 60 to 80 per cent of cases (Wolff, 1970), however, repeated treatments will raise these figures. In treated cases there is a continually rising incidence of hypothyroidism at a rate of 2-3% per year, which may be due to failure of thyroid cells to multiply after radiation (Nocenti, 1968). Parathyroid function is generally believed to remain unimpaired in these patients (Wolff, 1970).

The greatest difficulty in determining ^{131}I dosage is the problem of thyroid weight estimation. Wolff (1970) quotes a formula which was published by Rall (1953) and which is based on the administration of a pre-treatment tracer dose of ^{131}I.

$$D = \frac{100\ (110 + 27B)\ x\ G}{U}$$

D = the dose to be given in millicuries

G = the gland weight in grams

U = the 48 hour tracer uptake (%) and B is the whole blood ^{131}I at 48 hours (in per cent per litre).

2.6.4 The experimental animals

When the youngest of the experimental animals had reached the age three months they were fed the thyroid depressant drug Neo-Mercazole.

Calculated on the human dose each monkey in the experimental group was given 1 mg. of Neo-Mercazole daily. Monkey number two weighed 600 mgs. while all the others weighed within 100 grams of 1 kg. A 5 mg. tablet of Neo-Mercazole was dissolved in 10 ml. of sterile water. Each animal was given 2 ml. of this solution by means of an endogastric tube attached to a 5 ml. syringe. The endogastric tube was fashioned from a soft, latex catheter with an outer dimension of 4 mms. The monkeys were handled with extreme care by well trained staff but it was impossible to avoid injuries to them, either due to the catching; or from the passing of the tube. After two weeks of this treatment it was decided to use a less traumatic means of lowering the level of circulating thyroid hormones and radiation by ^{131}I was chosen.

The ^{131}I isotope which was used was supplied by Pelindaba and has a half life ($t_{\frac{1}{2}}$) 8,04 days,a primary energy of 360 Kev with 79 per cent gamma radiation. The biological half life ($t_{\frac{1}{2}(biol)}$) was not determined. Calculated on the average human ^{131}I ablative dose of 80 millicurie (Malcomb and Fletcher, 1967), it was decided to give each monkey 2 millicurie, of the drug, per Kg. of its body weight.

Two weeks after the administration of Neo-Mercazole had been stopped, each monkey was weighed (Table 1) and those in the experimental group, were then given an intravenous injection of ^{131}I (Fig.10). The ^{131}I was supplied as an injectable solution with a concentration of 1 millicure per ml

Table 1

Monkey No.	Weight in Grammes
1	1150
2	620
3	1120
4	1280
5	1040
6	1260
7	960

Fig.10 Administration of ^{131}I

This dose was repeated four weeks later, and again two days before the animals were sacrificed, at an average age of fifteen months. The final monkey died twenty-four hours after the first injection of the radio-active isotope.

2.7 Assessment of thyroid activity after administration of radio-active iodine

Twenty four hours after its ^{131}I injection, each monkey in the experimental series, was subjected to a total body scan. It was thus possible to determine the anatomical distribution of the radio-active material. The scan was performed by means of a 25 mm. sodium iodide detector, colmated by a 6 mm. aperture and connected to a Canberra linear ratemeter (Figs. 11, 12 and 13). The range was set at 1,000 counts per minute.

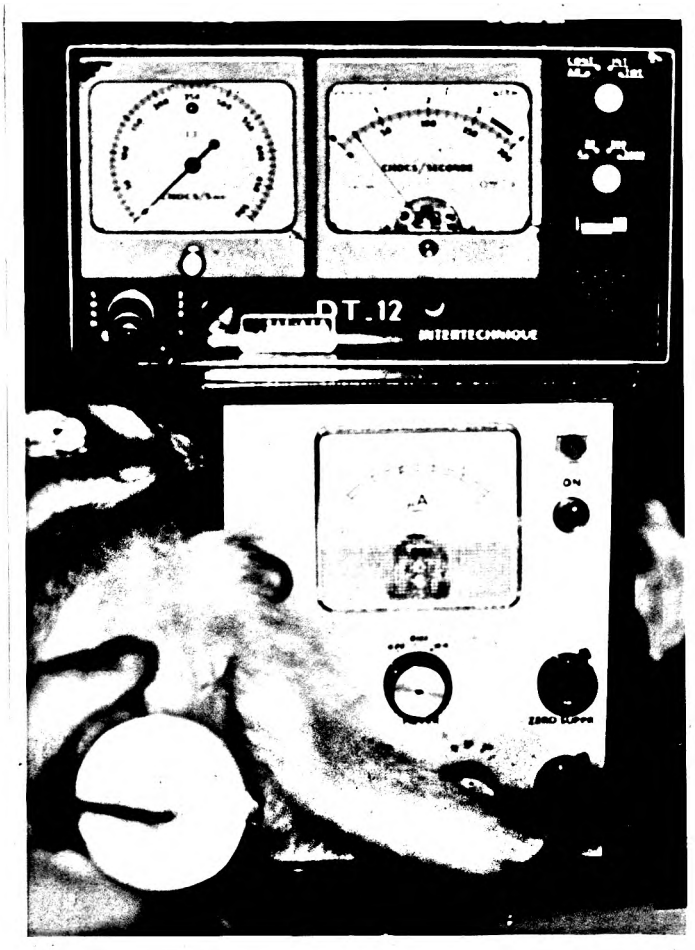


Fig.11 Detector system being used to assess ^{131}I levels. Hepatic region giving a reading of 20 on the scale.

Fig.12 Detector being used to assess ^{131}I level in the thyroid gland.

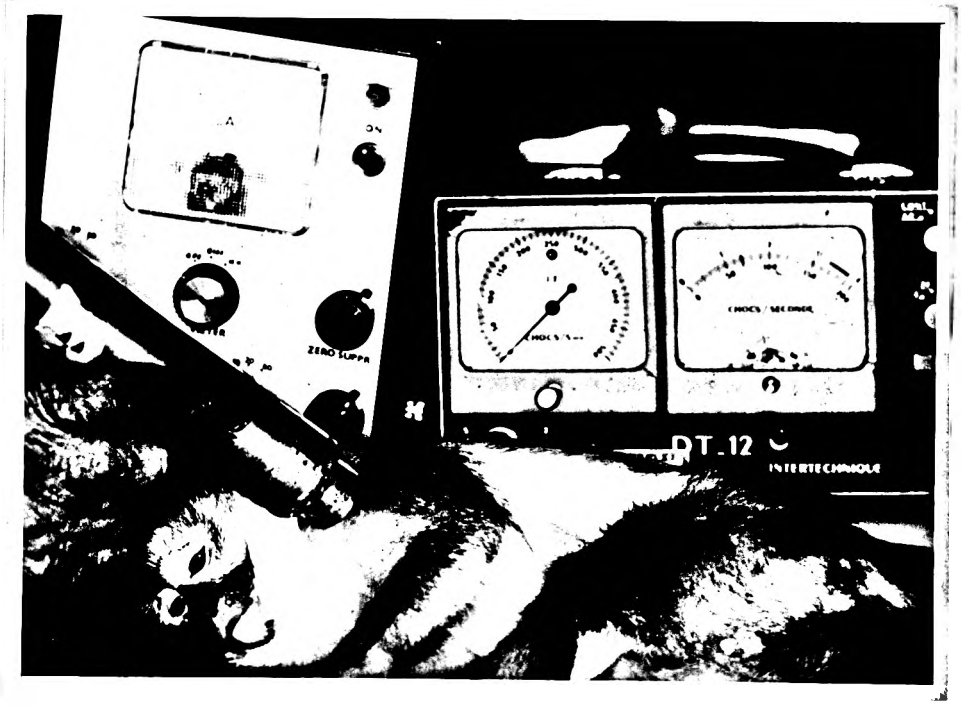


Fig.13 Reading in neck region indicating 15 on the scale.

2.8 Cephalometric radiographic records

Cephalometric radiographs were taken at three stages during the experiment. The first plates were taken at the start of the experiment when the monkeys were two and a half months of age. The second X-rays were taken when the first dose of ^{131}I was given at five months of age. The final records were taken at fifteen months of age, at the end of the experiment. The radiographs were taken using a standardised technique. The Wehmer cephalostat used was modified by extending the ear-posts of the unit by means of round perspex rods. These were 15 mms. long and five mms. in diameter, and were joined to the normal rods by means of perspex collars.

The central rays of the tube were directed perpendicular to the films and at the ear-posts. The film to midsagittal plane was kept constant at 75 mm. During the radiographic procedures the monkeys were sedated with intramuscular injections of Sernylan (Phencyclidine hydrochloride). They were supported on a stool, of suitable height, by packing sandbags around

them. For the exposures a Siemen's (Ergophos 4) X-ray machine was used and this was set at 50 Kvp. and 100 mA, while the exposure was for .1 seconds. Kodak Blue Brand film was used in a cassette fitted with Kodak high speed screens, and were developed for 3 minutes at 68°F in D.X.80 developer.

2.9 Cephalometric data

The landmarks defined in the glossary were traced from the radiographs onto acetate paper of 4/1000" thickness (Figs. 14 and 15). The relevant points were joined as shown in the figures and the distances and angles required were measured. All the linear measurements were taken to the nearest 0.5 mm. and the angular measurements to the nearest 0.5 of a degree. It was possible to correct linear measurements by means of a millimeter scale which forms part of the cephalostat, and which is orientated on the midsagittal plane. Due to poor alignment, lateral landmarks, sometimes presented with double images. In those instances both images were traced, and the point midway between them used for measurement purposes.

2.10 Glossary of points and measurements

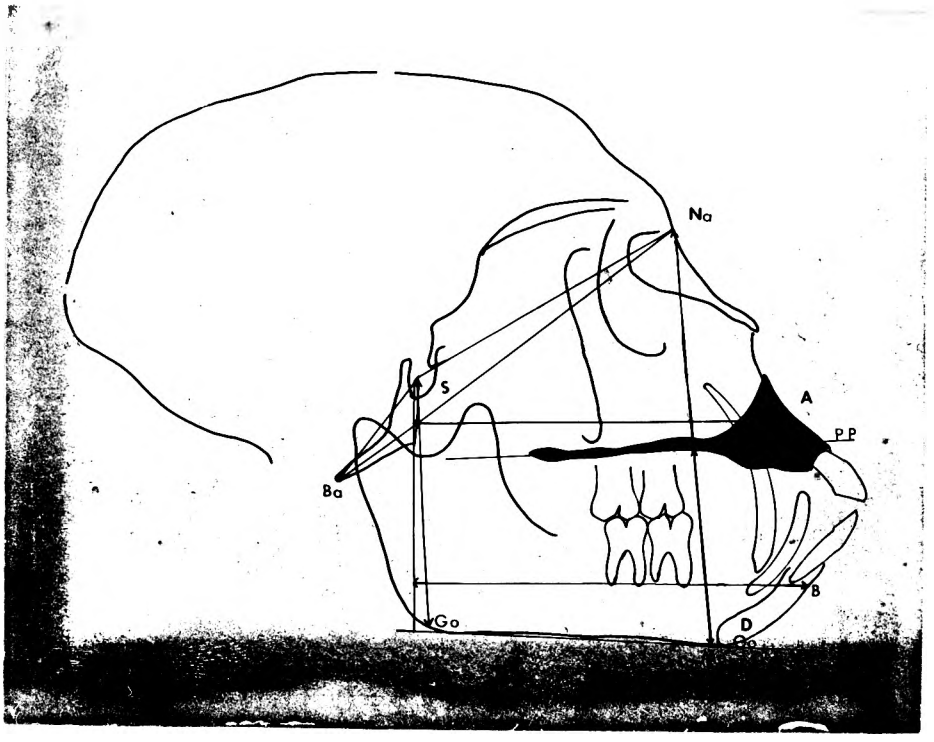


Fig. 14 Cephalometric landmarks, planes and dimensions.

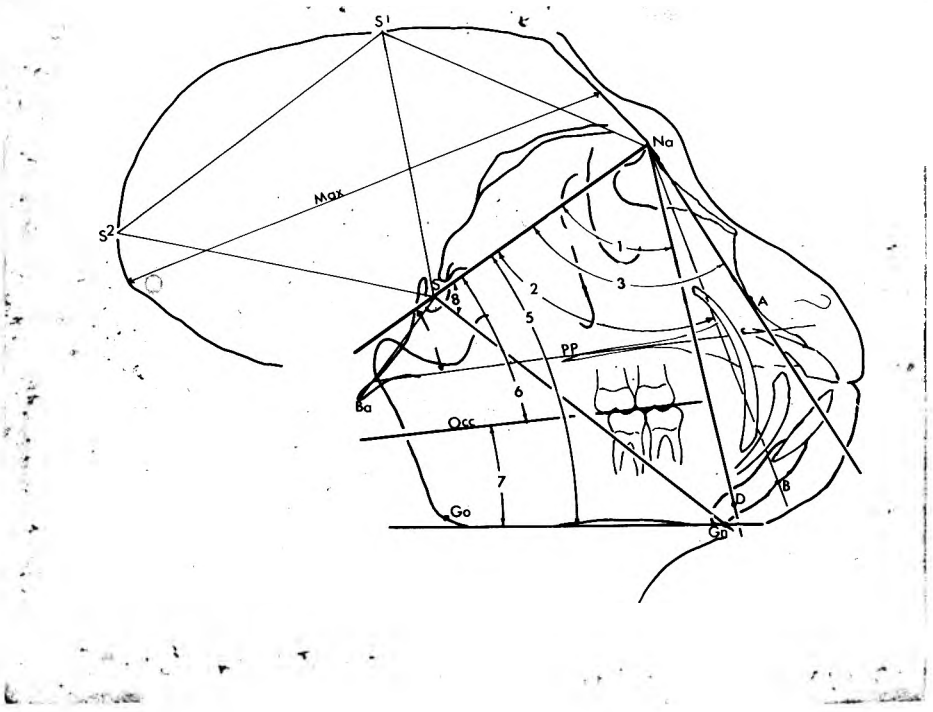


Fig.15 Cephalometric landmarks, planes, angles and dimensions.

Lateral cephalometric analysis points (Figs. 14 and 15)

Na - Nasion	A midline point at the anterior limit of the nasofrontal suture.
S - Sella	The centre of the sella turcica, selected by inspection.
Ba - Basion	The lowermost point on the anterior margin of the foramen magnum in the midsagittal plane.
S^1	The centre of the fronto-parietal suture (coronal).
S^2	The centre of Parieto-occipital suture lambdoid).
A - Subspinale	The deepest point on the maxilla between the anterior nasal spine and prosthion. (Anterior nasal spine and the dental alveolus).
B - Supramentale	The deepest point on the anterior curve of the midsagittal symphysis.

G - Gonion	The most posterior - inferior point on the posterior angle of the mandible.
Gn - Gnathion	The most anterior - inferior point on the mid-sagittal symphysis.
D - Symphysis	A point selected in the centre of the symphysis by inspection.
M - Menton	A point located at the lowest point on the midline curve of the symphysis.
AP max	The maximum anterio - posterior dimension of the cranial vault taken on the outer surface of the bone.

Lateral cephalometric analysis planes (Figs. 14 and 15)

Mandibular plane	The plane tangent to the lower border of the symphysis and the lower border of the angle of the mandible.
Palatal plane	A plane through the maxilla which best seemed to define the midsagittal axis of the bony palate.
Occlusal plane	A plane selected from bisection of the overbite of upper and lower incisor teeth and of the second deciduous molars.
Y-axis	A line joining sella and gnathion. In this series expressed as the angle Na.S.Gn.

Dimensions taken from the lateral cephalometric radiographs (Figs. 14 and 15)

Posterior facial height (PFH)	sella to gonion
Anterior facial height (TAFH)	nasion to menton
Upper facial height (UAFH)	nasion to palatal plane
Lower facial height (LAFH)	palatal plane to menton
Anterior cranial base length	nasion to sella
Posterior cranial base length	sella to basion
Total cranial base length	nasion to basion
Maxilla to cranial base	perpendicular distance from subspinale to a line from sella and perpendicular to the mandibular plane.
Mandible to cranial base	perpendicular distance from supramentale to a line from sella and perpendicular to the mandibular plane.
Orbital Height	maximum vertical dimension of orbit (Fig. 17).

Angular measurements in the lateral cephalometric radiographs (Fig.15)

All angles are defined by three points and are self explanatory. To determine the general facial patterns, a vertical and an antero-posterior (AP) index was used (Spiegel *et al.*, 1971).

$$\text{Vertical index} = \frac{S - Go}{Na - Me} + \frac{Na - PP}{pp - Me}$$

AP index = (Maxilla to cranial base) - (Mandible to cranial base).

2.11 Dental measurements

Dental observations were limited to an intra-oral examination during which the teeth present in the mouth were noted. Any tooth which had partly or completely erupted through the mucosa was taken as being present.

CHAPTER 3

FINDINGS

3.1 Clinical findings

At the end of the experiment the hypothyroidic monkeys, suffered from a generalized loss of hair and they also appeared to be less aggressive than those in the control group. There were, however, no other obvious physical differences between the two groups of animals (Fig. 16).

Fig.16 Facial features of a hypothyroidic monkey at 15 months of age. Note the loss of pigmentation on the eyelids, which was a finding in both groups of animals.

3.2 Radiographic findings

Figures 17, 18, 19 and 20 show examples of the radiographic appearances, seen in the two groups of monkeys, and the cephalometric findings are summarized in the Tables 2 to 9. Values which are of doubtful accuracy due to difficulty in determining anatomical landmarks, are printed between brackets in the tables. Contrary to the clinical findings there are marked differences in the radiographic appearances of the skulls of these two groups. The lateral radiographs of the experimental monkeys taken at three and six months of age show no obvious deviations of growth. The parietal bones are closely aligned with the occipital and frontal bones, and the sutures between them only slightly separated.

The radiographs of the four experimental monkeys taken at 15 months have many features in common. The parietal bones are depressed below the distal and mesial margins of the frontal and occipital bones respectively. The effect of this is to give the skulls of the hypothyroid monkeys, a flattened appearance, with pointed ends anteriorly and posteriorly (Figs. 18 and 19). The lambdoid and coronal sutures in the crania of the experimental animals are widely separated, while those in the control group appear to be virtually clossed. The separation is greater at the coronal suture and the depression of the parietal bone is more marked at the junction of this bone with the occipital bone. In the cranial vaults of the hypothyroidic monkeys, the bones have well developed external and internal tables. They do, however, appear to be thinner than those of the control group of monkeys. The only monkey with well developed cranial superstructures, such as superciliary arches, is the monkey number seven which had normal thyroid activity.

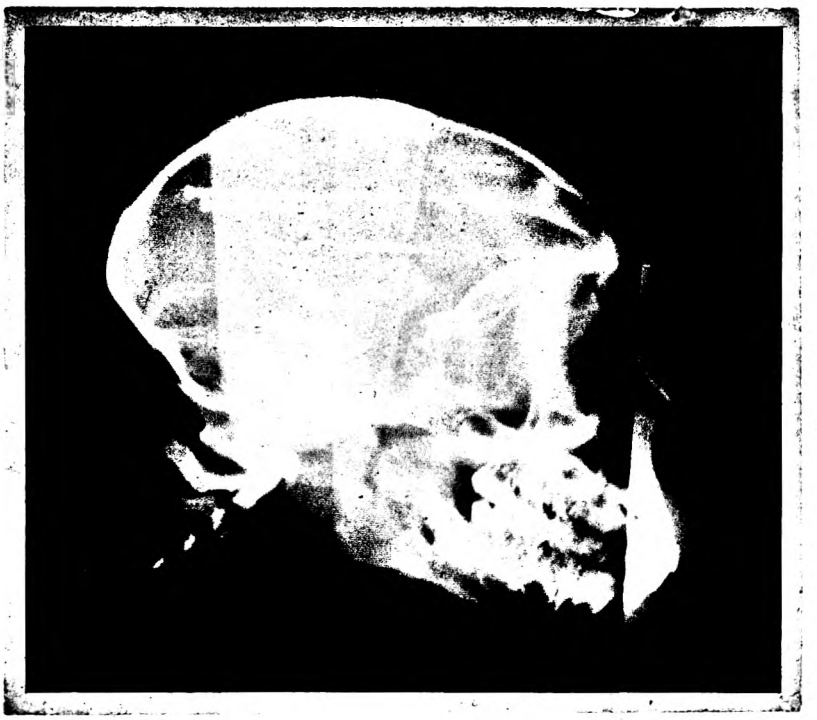


Fig.17 Post-mortem lateral radiograph of 15 month old monkey in the control group.

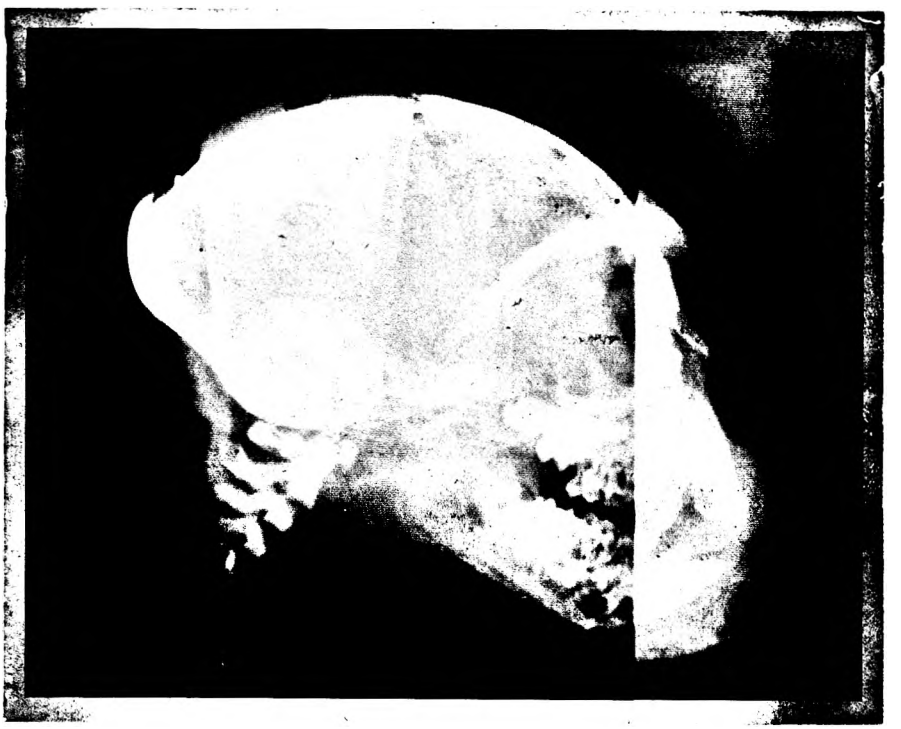


Fig. 18 Post-mortem lateral radiograph of hypothyroidic monkey at age of 15 months which shows the sutural widening and depression of the parietal base.

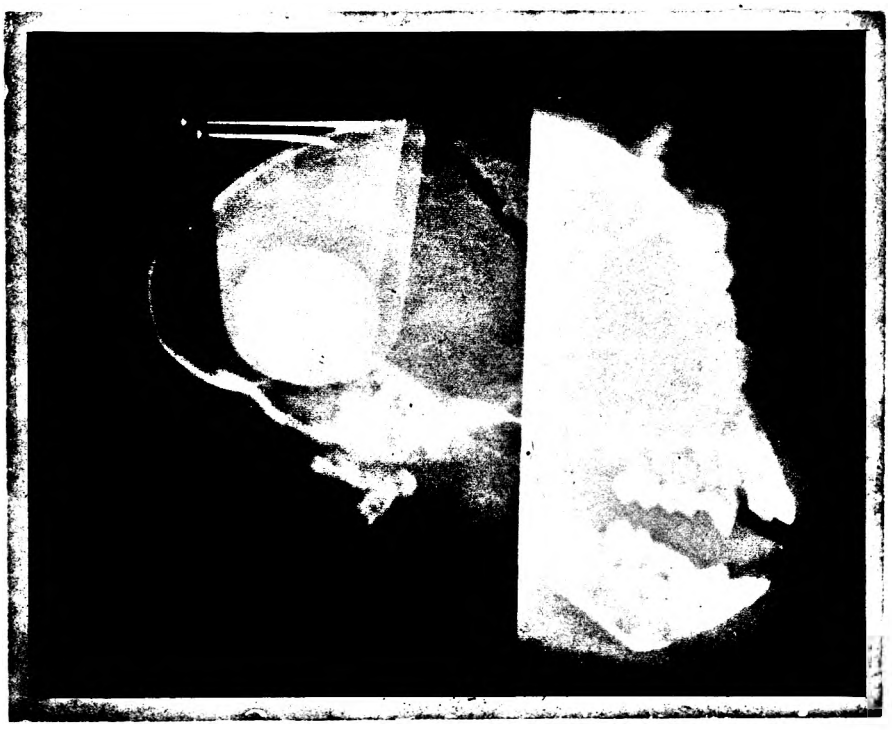


Fig.19 Post-mortem lateral radiograph of hypothyroidic monkey at age 15 months showing similar features to those in Fig.18. In addition this monkey appears to be developing superciliary arches.

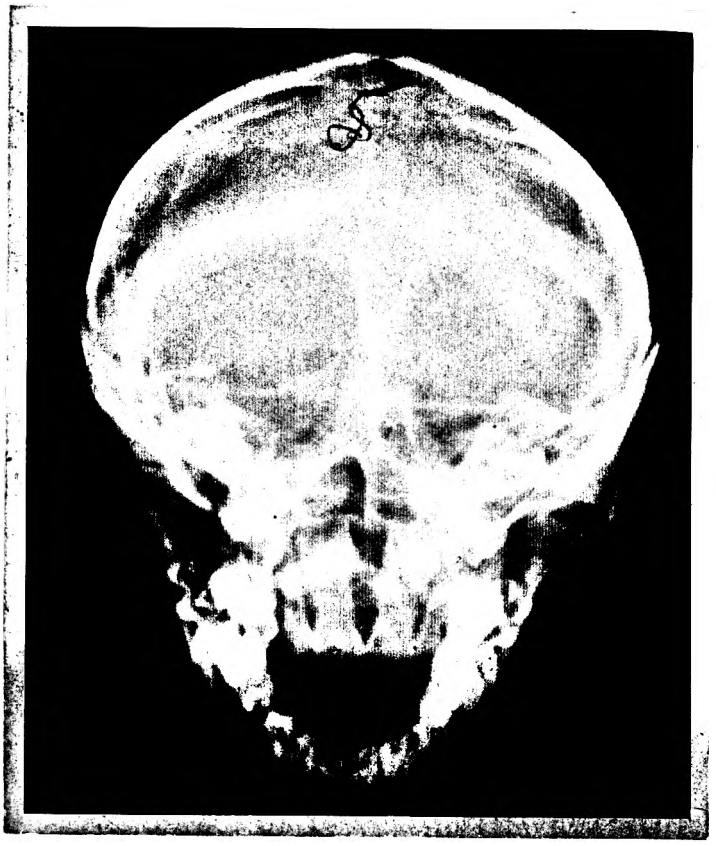


Fig.20 Post-mortem antero-posterior, radiograph of 15 month old hypothyroidic monkey, showing sutural separation and poor alignment of bones both in the neural and facial skeletons.

Table 2

Monkey Number		3 Months	5 Months	15 months
1	Vertical index	2,5	3,0	2,0
	AP index	1,48	1,45	1,36
2.	Vertical index	1,32	1,32	
	AP index	4,0	4,0	
3.	Vertical index	1,28	1,27	1,18
	AP index	4,0	5,0	4,5
4.	Vertical index	1,36	1,37	1,25
	AP index	7,5	7,5	6,0
5.	Vertical index	1,27	1,27	1,45
	AP index	2,5	3,5	3,5
6.	Vertical index	1,28	1,57	1,60
	AP index	5,5	6,0	2,5
7.	Vertical index	1,27	1,22	1,40
	AP index	2,0	4,0	0,5

Table 3

Monkey Number 1

	3 months	5 months	15months
A.P. Vault			
A.P. Max.	71,0	71,0	71,5
S - S^1	35,0	36,0	36,5
S - S^2	43,5	44,0	53,0/46,0
S^1 - S^2	37,01	37,0	35,0
Na - S^1	40,0	43,5	43,5
S - Na	32,0	32,0	33,0
Ba - S	15,5	18,5	20,0
NA - Ba	47,0	48,5	52,0
Na.S.Ba^o	$158^o0'$	$151^o0'$	$170^o0'$
Face			
Post.H.(S.Go.)	21,5	23,0	24,0
UAFH	20,5	20,5	20,5
LAFH	21,0	22,5	24,5
TAFH	41,5	42,5	44,5
Orbital Height	21,0	21,0	23,0
A - S.Go.	32,5	33,0	37,0
B - S.Go.	30,0	30,0	35,0
Y - Axis	$72^o0'$	$74^o0'$	$78^o0'$
SNA	74o0'	$81^o0'$	$78^o0'$
SNB	($63^o0'$)	$69^o0'$	($65^o0'$)
SND	60,0	$60^o0'$	($56,^o0'$)
O. SN.	$42^o0'$	$38^o0'$	($42^o0'$)
M. SN.	$45^o0'$	$48^o0'$	$42^o0'$
PP. SN.	$29^o0'$	$29^o0'$	$21^o0'$

Table 4

Monkey No. 2	3 months	5 months	15 months
A.P. Vault			
A.P. Max.	63,5	65,0	
$S - S^1$	31,0	33,5	
$S - S^2$	41,5	41,5	
$S^1 - S^2$	37,5	40,0	
$Na - S^1$	35,0	37,5	
S - Na	25,5	27,0	
Ba - S	18,5	18,5	
Na - Ba	43,5	44,0	
Na. S. Ba^0	$152^0 0'$	$150^0 0'$	
Face			
Post. H. (S.Go.)	20,0	20,0	
UAFH	17,0	17,0	
LAFH	21,0	21,0	
TAFH	38,0	38,0	
Orbital Height	20,0	20,0	
A - S.Go.	28,0	29,0	
B - S.Go.	24,0	25,0	
Y - Axis	$80^0 0'$	$79^0 0'$	
SNA.	$72^0 0'$	$79^0 0'$	
SNB.	$57^0 30'$	$61^0 0'$	
SND.	$52^0 30'$	$54^0 0'$	
O. SN.	$(38^0 0')$	$34^0 0'$	
M. SN.	$47^0 30'$	$42^0 0'$	
PP. SN.	$24^0 0'$	$20^0 0'$	

Table 5

Monkey No. 3	3 months	5 months	15 months
A.P. Vault			
A.P. Max.	68,5	72,0	73,0
S - S^1	36,0	38,5	37,5
S - S^2	41,5	42,5	41,5 - 44
S^1 - S^2	38,5	41,0	41,0
Na - S^1	44,0	45,5	46,0
S - Na	(31,0)	(34,5)	(36,0)
Ba - S	(15,0	15,0	17,0
Na - Ba	(44,5	48,0	52,0
Na. S. Ba^o	$155^o0'$	$140^o0'$	$155^o0'$
Face			
Post. H. (S.Go)	19,0	22,0	24,5
UAFH	16,5	17,5	20,0
LAFH	21,0	23,5	29,0
TAFH	37,5	41,0	49,0
Orbital Height	21,5	23,0	23,0
A - S.Go.	32,0	34,0	40,5
B - S.Go.	28,0	29,0	36,0
Y - Axis	$75^o0'$	$63,5^o$	$68^o0'$
SNA.	$84^o0'$	$82^o0'$	$89^o0'$
SNB.	$68^o0'$	$68^o0'$	$75^o0'$
SND.	$62^o0'$	$62^o30'$	$62^o0'$
O.SN.	$29^o0'$	$27^o0'$	$25^o0'$
M.SN.	$34^o30'$	$34^o0'$	$36^o0'$
PP. SN.	$25^o0'$	$24^o0'$	$19^o0'$

Table 6

Monkey No.4	3 months	5 months	15 months
A.P. Vault			
A.P. Max.	71,0	73,0	74,0
S - S^1	36,0	37,0	36,0
S - S^2	48,0	48,0	45,5(48,0)
S^1 - S^2	42,0	42,0	42,0
Na - S^1	42,5	42,5	43,0
Na - S	30,0	32,0	33,5
Ba - S	18,0	18,0	19,0
Na - Ba	47,5	48,5	52,5
Na. S. Ba^o	$169^o0'$	$161^o0'$	$166^o0'$
Face			
Post. H. (S.Go.)	22,0	23,0	23,0
UAFH	16,5	20,0	20,5
LAFH	21,0	23,5	26,5
TAFH	37,5	43,5	47,0
Orbital Height	21,0	22,0	24,0
A - S.Go.	34,0	36,0	42,0
B - S.Go.	26,5	28,5	36,0
Y - Axis	$74^o0'$	$76^o0'$	$72^o0'$
SNA.	$83^o0'$	$82^o0'$	$86^o0'$
SNB.	$72^o0'$	$67^o0'$	$68^o0'$
SND.	$67^o0'$	$61^o0'$	$62^o0'$
O.'SN.	$41^o0'$	$42^o0'$	$39^o0'$
M. SN.	$40^o0'$	$47^o0'$	$42^o0'$
PP. SN.	$25^o0'$	$26^o0'$	$23^o0'$

Table 7

Monkey No.5	3 months	5 months	15 months
A.P. Vault			
A.P. Max.	74,0	74,0	71,0
S - S^1	38,0	38,0	33,0
S - S^2	46,5	47,0	43,5/46,0
S^1 - S^2	41,5	45,0	41,5
Na - S^1	43,5	43,5	45,0
S - Na	32,5	32,0	34,0
Ba - S	17,0	17,0	18,5
Na - Ba	47,0	47,0	50,5
Na. S. Ba^o	$148^o0'$	$149^o0'$	$149^o0'$
Face			
Post. H.(S.Go.)	18,0	18,5	26,0
UAFH	20,0	20,0	20,5
LAFH	23,0	23,5	23,5
TAFH	43,0	43,5	44,0
Orbital Height	21,5	21,5	24,0
A - S.Go.	34,5	35,0	36,0
B - S.Go.	32,0	31,5	32,5
Y - Axis	$75^o0'$	$74^o0'$	$(70^o0')$
SNA.	$79^o0'$	$79^o0'$	$83^o0'$
SNB.	$65^o0'$	$65^o0'$	$75^o0'$
SND.	$59^o0'$	$58^o0'$	$68^o0'$
O. SN.	$43^o0'$	$43^o0'$	$24^o0'$
M. SN.	$45^o0'$	$45^o0'$	$35^o0'$
PP. SN.	$35^o0'$	$35^o0'$	$22^o0'$

Table 8

Monkey No. 6	3 months	5 months	15 months
A.P. Vault			
A.P. Max.	68,5	75,0	79,0
S - S^1	34,0	38,0	40,0
S - S^2	43,5	47,0	48,0
S^1 - S^2	39,0	43,0	43,0
Na - S^1	42,0	42,0	46,0
Na - S	31,5	34,5	38,5
Ba - S	17,0	18,0	22,5
Na - Ba	47,0	51,0	60,0
Na. S. Ba^o	$162^o0'$	$160^o0'$	$161^o0'$
Face			
Post. H. (S.Go.)	19,5	26,0	32,0
UAFH	17,5	25,0	28,5
LAFH	22,0	24,0	29,0
TAFH	39,5	49,0	51,0
Orbital Height	22,0	24,0	27,0
A - S.Go.	32,5	36,0	43,0
B - S.Go.	27,0	30,0	41,5
Y - Axis	$81^o0'$	$79^o0'$	$81^o0'$
SNA.	$79^o0'$	$71^o0'$	$73^o0'$
SNB.	$67^o0'$	$60^o0'$	$64^o0'$
SND.	$56^o0'$	$52^o30'$	$58^o0'$
O. SN.	$30^o0'$	$40^o0'$	$37^o0'$
M. SN.	$36^o0'$	$44^o0'$	$43^o0'$
PP. SN.	22 0'	34 0	31 0'

Table 9

Monkey No. 7	3 months	5 months	15 months
A.P. Vault			
A.P. Max.	67,0	70,0	74,0
S - S^1	36,0	36,0	38,5
S - S^2	43,5	43,5	47,5
S^1 - S^2	38,5	38,5	38,5
Na - S^1	42,5	43,5	46,0
S - Na	28,0	32,0	32,5
Ba - S	14,0	17,5	21,0
Na - Ba	41,0	47,0	53,0
Na. S. Ba^o	$155^o0'$	$145^o0'$	$156^o0'$
Face			
Post. H. (S.Go.)	18,0	18,5	29,0
UAFH	17,0	17,0	22,0
LAFH	21,0	22,5	27,0
TAFH	38,0	39,5	49,0
Orbital Height	20,5	21,0	24,0
A - S.Go.	29,5	34,5	39,0
B - S.Go.	27,5	30,5	38,5
Y - Axis	$75^o0'$	$74^o0'$	$78^o0'$
SNA.	$76^o0'$	$79^o0'$	$80^o0'$
SNB.	$63^o0'$	$64^o0'$	$68^o0'$
SND.	$55^o0'$	$58^o0'$	$60^o0'$
O. SN.	$36^o30'$	$36^o0'$	$35^o0'$
M. SN.	$45^o0'$	$45^o0'$	$48^o0'$
PP. SN.	$(28^o30')$	$32^o0'$	$26^o30'$

The individual measurements show that the antero-posterior dimensions of the cranial vaults increased less in the experimental animals than they did in the control group. In the case of monkey number five this dimension actually decreased. The vertical heights of the cranial vaults ($S\text{-}S^1$) and ($S\text{-}S^2$) (Fig.15) tended to decrease in the hypothyroidic monkeys rather than show the growth increments seen in the control group. The sagittal dimensions of the frontal and parietal bones ($Na\text{-}S^1$ and $S^1\text{-}S^2$) manifest small comparable growth increases in both groups of animals.

Cranial base length (Na-Ba) shows less increase in the experimental than in the control monkeys. The growth in both groups occurred with more or less even distribution between the anterior (Na-S) and the posterior (S-Ba) components of the cranial base. The one exception, monkey number one, had much more growth of the posterior cranial base, than of the anterior part. The angle between the anterior and posterior segments of the cranial base (Na.S.Ba.) remained remarkably constant, not only between the different age groups but also between the two groups of monkeys. Again monkey number one was the exception, and in this instance the angle increased from 150^{o} to 170^{o}.

The Tables 2 to 9 reflect the general growth changes which took place in the facial skeletons of the monkeys. The facial indices (Table 2) correlate a number of related findings and express them in a single value. An increase in the numerical value for the vertical index indicates a change in the facial pattern, towards a deep-bite type, which is characterized by a large PFH, a small TAFH and LAFH (Spiegel *et al.*, 1971). The reverse holds true when the value for the vertical index decreases *viz.* a change towards the open bite facial pattern is indicated. All monkeys in

the experimental group, excepting number four show changes in their facial patterns towards the open-bite type while the reverse holds for the control group. Changes in the AP index for monkey number one demonstrates a decrease in maxillary prognathism similar to those seen in the control group of animals. The remainder of the monkeys in the experimental group have a tendency towards maxillary protrusion as expressed by the AP index. The angle ANB, which may also be used to measure maxillary protrusion is calculated by subtracting the angle SNB from the angle SNA.

3.3 Dental findings

At fifteen months of age the control monkeys had all their first permanent molars fully erupted, in addition to having complete deciduous dentitions. The hypothyroidic monkeys of the same age all had complete sets of deciduous teeth, but none of them had permanent teeth present in their oral cavities.

CHAPTER 4

DISCUSSION

Three major theories have been proposed to explain the process of cranial growth. Firstly, Sicher (1952) considers growth of the skull to be largely controlled by intrinsic genetic factors located in the bones themselves. According to this theory periosteal, sutural and endochondral osteogenesis all play an equally important part in the growth of the skull (van Limborgh, 1970).

Secondly, Scott (1953, 1956, 1958), concludes that the growth controlling factors are mainly present in the cartilage precursors of the bone. Accordingly sutural growth is secondary and entirely dependent on the sutural separation resulting from cartilage growth. The cartilagenous parts of the skull, such as the nasal septum and synchondroses, are thus seen as the primary growth centres of the head (Koski, 1968).

More recently Moss and his co-workers (1960, 1962, 1964) have postulated that growth of the skull is secondary to growth and functional demands of the soft tissues. This view is based on the theory developed by van der Klauw (1952) according to which the skull is essentially composed of bony units, the size,shape and position of which is determined by their functions. Moss accordingly formulated his concept of a 'functional matrix' and he attached little importance to the growth regulatory mechanisms intrinsic to the skull bones. The sites at which bone grows during the enlargement of the skull may be studied in order to give a better understanding of the mechanisms involved in regulating skull growth.

Moore (1949) gives a detailed account of growth sites in the skull

of a female *Macaca rhesus* monkey, with a dental age comparable to that of a five year old human (Figs. 4 and 5). At the end of the present study the six Vervet monkeys had reached the mixed dentition stage and the areas of active growth in their skulls, should be similar to those described by Moore. These regions of active growth are, according to Engel (1941), particularly vulnerable to metabolic abnormalities. They should therefore show the most obvious deviations in growth, after induced hypothyroidism. The present study shows that while minor deviations could be detected in the facial regions of all of the experimental animals, the most obvious changes occurred in their cranial vaults. Three of them showed marked changes in the sutural pattern and shapes of their cranial vaults, while the fourth also showed similar but less dramatic changes. Contrary to this finding in the monkeys, Korkhaus (1957) concluded that the neural component of the human skull is much less affected than the facial, by hypo-function of the thyroid gland. Hypothyroidism according to Harper (1973) decreases production of growth hormone. Since growth of neural tissues is less dependent on growth hormone than are the bony tissues (Turbin, 1971), growth of the cranial vault will be reduced to a greater degree than that of the brain, in hypothyroidic states. This explanation may lend support to the theory of Moss (1960, 1962, 1964) according to which bone growth is secondary, to soft tissue development. It does not , however, explain why the parietal bones become depressed, or even more important, why the dimensions of the cranial vaults in these animals show little increase, or in some instances even decrease in size. Depression of the parietal bones and possibly the widening of the sutures may in part be due to the functional contractions of the temporal and the extensor muscles which are inserted into the bones of the vault.

Growth of the cranial base as a whole, and of its component parts

individually, appears to be reduced in the hypothyroidic monkeys. Despite the reduction in growth of the cranial base, changes in cranial base angle (Ba.S.N.), similar to those described by Korkhaus (1957) in humans, were anticipated. However, these were absent not only in the hypothyroidic, but also in the control group. This finding makes it unlikely that changes in the cranial base are primarily responsible for those observed in the vaults.

The human open-bite facial type is characterized by a small posterior facial height, a large anterior facial height, and a large lower anterior facial height. Conversely, a deep bite facial type is characterized by a large posterior facial height, a small anterior facial height, and a small lower anterior facial height (Spiegel *et al.*, 1971; Sassouni and Nanda, 1964; Sassouni, 1969; Sassouni, 1972). The vertical index derived from these three facial dimensions is used to classify human facial types. Applying this index to the experimental monkeys, shows that three of the experimental animals have a tendency towards an open bite.

Although the vertical facial measurements used did not make it possible to determine, with certainty, the areas of growth most responsible for these findings, the posterior facial height appears to be the dimension most affected. It has been suggested that the growth centres of the mandible show a greater response to thyroid hormone, than do those of the other parts of the skull (Spiegel *et al.*, 1971). A suggestion which may account for the finding, that the posterior facial heights of three of the experimental monkeys, is affected to a greater degree than are the other vertical dimensions of their facial skeletons.

The two methods used for measuring maxillary protrusion in relation

to the mandible have their shortcomings but when applied to the monkeys they indicate that the experimental animals are more retrograthic (mandibular) at the termination of this experiment than they were at the start. The control monkeys, however, are more prognathic (mandibular) at the end of the study. In the experimental monkeys, vertical as well as antero-posterior development of the upper face is retarded by the hypothyroidic state. These findings, however, appear to be largely overshadowed by the reduced mandibular growth in these same animals. It is thus difficult to assess the contribution of the nasal septum, to the changes seen in the faces of the experimental animals.

Other factors may play a role in affecting the growth of the captive monkeys. Experiments have shown that monkeys experience a marked drop in serum ascorbic acid levels, when kept in captivity on diets which should be sufficient to prevent scurvy (de Klerk *et al.*, 1973). Factors such as these affected both groups of monkeys but may augment the effects of the endocrinopathy.+

+ The delayed eruption of teeth seen in the 15 month old hypothyroidic monkeys, may be due to lack of growth, and thus space, in the jaws of these animals.

CONCLUSIONS

1. The administration of radio-active iodine proved to be a convenient and reliable method of depressing thyroid activity in Vervet monkeys.

2. At the age of 15 months the hypothyroidic monkeys when compared to the control group, exhibited marked cranial differences:

   (a) The most marked changes occurred in the cranial vaults.

   (b) The cranial base regions appeared to be the least affected by the hormonal deficiency.

   (c) The cranial base angle remained relatively constant in the experimental, as well as the control group of monkeys.

   (d) There was an increased tendency towards an open bite facial pattern in the experimental animals, at the age of fifteen months, as compared to the patterns seen at three months of age.

   (e) The experimental monkeys were more retrograthic (mandibular) at the end of the experiment than they were at the start.

3. The eruption of the first permanent molar teeth was delayed in the 15 month old hypothyroidic monkeys, as compared to the control monkeys of the same age.

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