DENTAL CARIES

CLINICAL AND EXPERIMENTAL INVESTIGATIONS

by

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TABLE OF CONTENTS.

I. INTRODUCTION

II. MATERIALS AND METHODS

III. FINDINGS

IV. DISCUSSION

Page

V. SUMMARY AND RECOMMENDATIONS

I. INTRODUCTION

DENTAL caries is described by Noyes (1939) as a bacterial disease of the calcified dental tissues, producing typical lesions that originate in characteristic locations.

PREVALENCE OF DENTAL CARIES

Dental caries is the most prevalent of all diseases among civilized people. Rarely do we find a person of middle age to-day with a full complement of thirty-two perfectly healthy teeth. From various statistics obtained from practically every country in the world, the incidence is estimated to be over 95 per cent. By this is meant that more than 95 out of every 100 persons suffer or have suffered at some time from one or more carious teeth. Statistics are based mostly on dental examinations of school children, because of the obvious difficulty of examining large groups of adults for dental defects. There is a lamentable lack of reliable and accurate statistics concerning the incidence of dental caries in most civilized countries.

Klein and Palmer (1938) reported that the incidence of dental caries (as defined above) among elementary school children in the United States is 95 per cent. Day and Sedwick (1935) found the incidence among Rochester (N.Y.) schoolchildren to be 99 per cent. The final report of the Mixed Committee of the League of Nations of 1937 shows that in Norway, of 25,000 school children examined, only 160 possessed perfect sets of teeth, or 99 per cent. affected by dental caries. Day and Sedwick (1935) state that, in the county of Shropshire in England, 97 per cent. of the children at the age of 12 had dental caries. The Director-General of Health of New Zealand, in his annual report of 1941, states that of 52,500 children examined, 95 per cent. were affected by caries. In India, Day and Tandan (1940) reported that the incidence of dental caries among urban children in Lahore was 94 per cent. In South Africa, Friel and Shaw (1931) found 93 per cent. of urban children suffering from dental caries. Staz (1938) reported that of 300 European adults examined in Johannesburg none showed caries-free mouths.

The serious problem of dental caries is to-day occupying the minds of many eminent research workers and health authorities in the fields of dentistry, medicine and nutrition throughout the civilized world.

In order to investigate the contributory causal factors from as many angles as possible, and also the control and prevention of dental caries, it is necessary initially to consider:—

1. TOOTH DEVELOPMENT

In man, teeth consist of the calcified propriodontal tissues (enamel and dentine) and the pulp. The periodontal or supporting tissues consist of the cementum, periodontal membrane, alveolar bone and gingivae. Embryologically, the enamel is of ectodermal origin and the other tissues of mesodermal origin.

The bulk of the tooth is made up of dentine, which is covered by enamel on the crown and cementum on root. The enamel is the hardest tissue in the body. The central cavity of the dentine is filled with its formative organ, the pulp. The periodontal membrane connects the cementum of the tooth to the alveolar bone. The tooth arises from the oral mucosa and passes through the following developmental stages:—

(*a*) Growth—

(1) Proliferation;

(2) Histo-differentiation;

(3) Apposition.

(*b*) Calcification.

(*c*) Eruption.

(*a*) Growth

(1) *Proliferation*

The first of the three stages in the growth of the tooth is the very rapid growth of cells from the ectodermal layer of the oral mucosa. The tooth germ, which consists of the enamel organ, the dental papilla (dentine organ) and the dental sac (supporting tissue organ), is formed during this stage. The enamel organ plays the dominant role in the proliferative growth of the tooth germ. The epithelium beneath the free margin of the jaws proliferates and forms an arch-like dental lamina. It then undergoes three stages of transformation—

(*a*) Bud stage; (*b*) Cap stage; (*c*) Bell stage.

The cells of the inner enamel epithelium at this stage are high columnar and show many mitotic figures. The dental papilla is the mesenchyme which is enclosed by the invaginating enamel organ. It will later form dentine and remain in the adult tooth as the pulp.

Dental Sac.—As the dental papilla and enamel organ take on their full form, a modification of the surrounding mesoderm occurs and fibrous tissue begins to encircle the outer surface of the enamel organ and the base of the dental papilla. This is the dental sac, which will later form the cementum, periodontal membrane, alveolar bone and gingivae.

(2) *Histo-differentiation*

The second stage in the growth of the tooth germ is characterized by marked histologic changes in the cells and by the assumption of the final morphologic form plan of the tooth. At the end of the bell stage of the enamel organ the tooth germ is composed of a group of cells which are fully equipped for appositional growth.

The Enamel Organ.—The enamel organ at this stage consists of four layers—

(i) the outer enamel epithelium, which has now changed from columnar-shaped cells to flattened cells just prior to enamel apposition;

(ii) the stellate reticulum, which is composed of squamous epithelium;

(iii) the stratum intermedium, which lies between the stellate reticulum and the inner enamel epithelium, and consists of several layers of flat cells. These cells are thought to be concerned with amelogenesis and the ameloblasts do not form enamel, except when lying next to the stratum intermedium;

(iv) inner enamel epithelium, composed of tall columnar enamel-forming cells or ameloblasts. At this stage their polarity changes, so that, unlike other epithelium columnar cells, their nuclei are now situated at what was formerly the distal end of the cells. No mitotic figures are seen.

The Dental Papilla.—During the bell stage of the enamel organ the peripheral cells of the dental papilla differentiate themselves opposite the ameloblastic layer. Between these cells will be the future dentino-enamel junction.

The Dental Sac.—The cells of the dental sac will give rise to the supporting tissues, i.e., the cementum, the periodontal membrane, the alveolar bone and gingivae. The differentiation of the dental sac does not occur until amelogenesis is complete and root formation and eruption have begun. The connective tissue cells of the dental sac then differentiate into cementoblasts and osteoblasts. Before appositional growth a number of changes occur in the enamel organ:—

(*a*) The enamel organ separates from the oral epithelium and the dental lamina is resorbed.

(*b*) The stellate reticulum becomes reduced adjacent to the site of apposition.

(*c*) The result is that the outer epithelium approaches the stratum intermedium and the inner enamel epithelium. They fuse and become the united enamel epithelium.

(*d*) The outer enamel epithelium becomes irregular and connective tissue papillae containing capillary loops project into it.

(3) *Apposition*

The third and last phase of growth is one of a quantitative addition of extracellular material to the morphologic outline, the dentino-enamel junction which was established in the histo-differentiation phase.

Amelogenesis: Enamel Prisms.— Although the presence and the differentiation of the inner enamel epithelium precede and are essential to dentine formation, the ameloblasts do not show any secretory activity until a microscopic cuspal portion of dentine has been formed. Amelogenesis begins with the formation of minute drop-like inclusions within the cytoplasm situated between the nucleus and the enamel end of the cell. These globules react like young, newly-formed enamel and have been called pre-enamel globules. They coalesce and become larger as they approach the enamel end of the cell. Tomes described a truncated cone-shaped process which projects beyond the enamel end of the ameloblasts. The pre-enamel globules are probably secreted through the Tomes processes. The coalesced globules are deposited in the form of a large calcospherite, the enamel globule, approximately 4μ in diameter, one upon the other, until a column-like enamel prism is produced. The pressure of succeeding deposition of the globules causes compression, so that they appear somewhat flattened in the formed enamel. The globules do not completely fuse and are demarcated by the intervening cross-stratum seen in the completed enamel rod. Each enamel prism is thus the extracellular product of a single ameloblast. A cross-section of completely formed enamel presents a picture that is practically identical in form and arrangement with that of a cross-section of the ameloblasts and their intercellular spaces: the prisms correspond to the ameloblasts and the interprismatic substance to the intercellular space.

Interprismatic Substance.—The intercellular spaces between the ameloblasts are closed off first on the basal and later on the peripheral surfaces by a condensed solid cement substance. This cement substance first appears immediately before amelogenesis begins and later becomes the interprismatic substance.

Final Product and Fate of the Enamel Organ.—When the deposition of the enamel matrix is completed, the ameloblasts secrete a final cuticular product called the primary enamel cuticle or Nasmyth's membrane. It is 1μ thick and may become calcified. After the formation of the enamel cuticle, the united enamel epithelium regresses to a stratified squamous epithelium. In the process of eruption the epithelial remains of the enamel organ and the enamel cuticle are carried into the oral cavity and worn away during mastication. The enamel thus has lost its formative organ and presumably is incapable of further growth or regeneration.

Dentinogenesis.—Dentinogenesis begins at the dentino-enamel and dentino-cemental junction and proceeds from without inward at the expense of its formative organ, the pulp. The cells of the inner enamel epithelium in the crown are essential to the formation of the dentine. They organize the dentine-forming cells or odontoblasts and initiate dentinogenesis. Their outlines determine the outer surface of the dentine, so that the pattern of the crown is determined prior to dentine apposition. As each odontoblast recedes, it leaves behind it a long cytoplasmic process called Tomes' fibre. The deposition of the ground substance and the subsequent calcification of the dentine proceed around and between the Tomes' fibres, and thus enclose them within the dentinal tubules. The Tomes' fibres completely fill the tubules and normally do not calcify. They are present throughout the period of dentine deposition. They arise from the deeper cell layers of the pulp and unite at the base of the odontoblastic row. They then extend spirally in the intercellular spaces, pass between the odontoblasts and unravel in the layer of ground substance surrounding the dentinal tubules.

The dentine-forming cells, unlike the enamel-forming cells, retain their vitality and continuity with their formed tissues (by means of the Tomes' fibres), and under the stimulating influence of abrasion or caries, can redifferentiate, acquire a secondary growth potential and produce an atypical secondary dentine.

(*b*) Calcification

Very little is known about the mechanism of calcification. Rainey (1857) and Harting (1872) found that the precipitation of a calcium salt in the presence of albumen resulted in the formation of calcospherites and not of ordinary crystals of calcium salts, as would occur in water. The calcospherites were formed through the precipitation of a more or less distinct gelatinous nuclear mass, about which the calcium salts were deposited in the form of large round lamellae which were at once radiate and concentric. Individual calcospherites tend to aggregate and coalesce so that a homogeneous layer is formed as they enter the sphere of influence of one another. This results in the formation of larger rhythmic layers of calcified material. D'Arcy Thompson (1917), in reviewing the work of Rainey (1857) and Harting (1872), is of the opinion that probably the actual precipitation of the calcium salts is not due to the direct action of carbonic acid, etc., on a more soluble salt (as was at one time

believed), but to catabolic changes in the proteids of the organism, which tend to throw down the salts already formed, which had remained hitherto in albuminous solution. Robison (1923) is of the opinion that the enzyme phosphatase liberates inorganic phosphate from phosphoric esters and raises locally the concentration of the $PO_4^{\equiv}$ ion. The product of the concentrations of the Ca^{++} and $PO_4^{\equiv}$ ions then exceeds the solubility product of calcium phosphate, which is, in consequence, deposited in the cartilagenous matrix.

Calcification of Enamel.—During amelogenesis minute globules are found within the cytoplasm of the ameloblasts. As these progress from the distal toward the enamel end of the cell they increase in size and fuse. The enamel globule is then deposited as a large calcospherite. Fully calcified enamel contains about 1 per cent. of organic material, the organic enamel matrix deposited by the ameloblasts being almost completely replaced by inorganic salts. After decalcification, during histologic preparation of teeth, no vestige of the original structure of enamel remains.

Calcification of Dentine.—During dentinogenesis the dentine-forming cells recede pulpally and deposit new dentine matrix, while the previously deposited matrix is calcifying. The most recently formed and still soft and uncalcified dentine matrix is called predentine. and corresponds to young osteoid bone. The calcification process begins at the border of the predentine which is farthest from the pulp and is called the intermediate dentine. The intermediate dentine represents an early stage of the calcification process. The soft organic matrix of the predentine layer begins to calcify at this level. The first evidence of calcification is seen in the formation of tiny individual calcospherites. These grow by the radiate concentric deposition of the inorganic calcium salts. As the calcospherites approximate each other a fusion occurs and there results a homogeneously calcified incremental layer of dentine. When fully calcified dentine is decalcified during histologic preparation an acid-resistant calcoglobulin matrix remains in the form of the original framework. The adult dentine appears to increase calcification with age and this results in the reduction in the size of the dentinal tubules. It is generally believed that the calcium carbonate and phosphate of enamel and dentine are not present as separate compounds simply mixed together with smaller amounts of other mineral salts, but as a complex compound closely resembling the apatite series with the general formula of $3Ca_3(PO_4)_2\ Ca\ X_2$; the X_2 ordinarily may represent CO_3, Cl_2, $(OH)_2$, F_2, O or SO_4. Some investigators are of the opinion that the solid phase of enamel and dentine consist of hydroxyapatite.

CHRONOLOGY OF CALCIFICATION OF HUMAN DENTITION

Teeth.		In Utero.	After Birth.	
		First Evidence Calcification.	Crown Completed.	Root Completed.
Deciduous dentition: upper and lower	Central incisors	5 months	4 months	1½–2 years.
	Lateral incisors	5 months	5 months	1½–2 years.
	Canines	6 months	9 months	2½–3 years
	First molars	5 months	6 months	2–2½ years.
	Second molars	6 months	10–12 months	3 years.
			After Birth.	
Permanent dentition: upper	Central incisors	3–4 months	4–5 years	10 years.
	Lateral incisors	1 year	4–5 years	11 years.
	Canines	4–5 months	6–7 years	13–15 years.
	First premolars	1½–1¾ years	5–6 years	12–13 years.
	Second premolars	2–2¼ years	6–7 years	12–14 years.
	First molars	At birth	2½–3 years	9–10 years.
	Second molars	2½–3 years	7–8 years	14–16 years.
	Third molars	7–9 years	12–16 years	18–25 years.
Permanent dentition: lower	Central incisors	3–4 months	4–5 years	9 years.
	Lateral incisors	3–4 months	4–5 years	10 years.
	Canines	4–5 months	6–7 years	12–14 years.
	First premolars	1¾–2 years	5–6 years	12–13 years.
	Second premolars	2¼–2½ years	6–7 years	13–14 years.
	First molars	At birth	2½–3 years	9–10 years.
	Second molars	2½–3 years	7–8 years	14–15 years
	Third molars	8–10 years	12–16 years	18–25 years.

Calcification of Cementum and Alveolar Bone.—The calcification of cementum and bone presents essentially the same picture as observed in the dentine. An organic matrix is deposited (precementum and osteoid border), which is calcified through the same calcospherite mechanism. The degree of calcification, as is the case in the dentine, is much less than that of the enamel, so that after decalcification in acid, the organic framework remains. That portion of the fibres of the periodontal membrane that is embedded in the cementum and alveolar bone is also calcified by a process that is analogous to the one already described.

CHRONOLOGY OF CALCIFICATION OF RAT MOLARS

Upper Molars.	*Beginning of Calcification.*	*Crown Completed.*
1st molar	1st to 2nd day post-natal	11th to 12th day post-natal.
2nd molar	3rd to 4th day post-natal	13th to 14th day post-natal.
3rd molar	16th day post-natal	20th to 22nd day post-natal.
Lower Molars.	*Beginning of Calcification.*	*Crown Completed.*
1st molar	Birth to 1st day post-natal	10th to 11th day post-natal.
2nd molar	2nd to 3rd day post-natal	12th to 13th day post-natal.
3rd molar	15th day post-natal	19th to 21st day post-natal.

(*c*) Eruption

Eruption is the process whereby the crown of the tooth moves from its intra-osseous location to its full clinical position within the mouth. During the movement toward its final occlusal plane, the tooth undergoes passive as well as active changes in position. Not only does the tooth itself actually change position in respect of its bony crypt or the oral epithelium but also the growth of the surrounding jaw changes the relative position of the tooth even if itself be stationary. The tooth exhibits an inherent active potentiality to erupt. Passive changes in position also occur during the active eruption of the tooth. Eruption begins at completion of the anatomic crown and when enamel formation is finished. The bony crypt then opens to allow for the passage of the crown. The tooth moves towards the oral epithelium, followed by the emergence of the crown tip into the oral cavity. The opposing teeth then make occlusal contact but are not yet fully erupted. The final stage is full clinical occlusion. No one theory at present can explain all the factors involved in the eruptive process. (Schour, 1938.)

CHRONOLOGY OF ERUPTION OF HUMAN TEETH AND RAT MOLARS

	Human Teeth.	*Rat Molars.*
Upper and Lower Deciduous:		
Central incisors	6–8 months	—
Lateral incisors	8–10 months	—
Cuspids	16–20 months	—
First molars	12–16 months	—
Second molars	20–30 months	—

	Human Teeth.	*Rat Molars.*
Permanent Upper Dentition:		
Central incisors	7–8 years	—
Lateral incisors	8–9 years	—
Cuspids	11–12 years	—
First bicuspids	10–11 years	—
Second bicuspids	10–12 years.	—
First molars	6–7 years	20th day.
Second molars	12–14 years	22nd to 23rd day.
Third molars	17–21 years	36th day.

	Human Teeth.	*Rat Molars.*
Permanent Lower Dentition:		
Central incisors	6–7 years	—
Lateral incisors	7–8 years	—
Cuspids	10–11 years	—
First bicuspids	10–12 years	—
Second bicuspids	11–12 years.	—
First molars	6–7 years	19th day.
Second molars	12–13 years	21st to 22nd day.
Third molars	17–21 years	35th day.

2. FACTORS INFLUENCING DEVELOPMENTAL STAGES

(*a*) Heredity

Very little is known about what role heredity plays in growth, calcification and eruption of teeth. The question as to whether certain characteristics, such as shallow fissures and low cusps, usually associated with a low caries rate, and deep fissures and high cusps, usually associated with a high caries rate, may be inherited cannot be answered. Bossert (1933, 1937) found that teeth with acute cusp angulation are more often carious than those with obtuse angles. Fisher (1934) has also studied the relation of tooth form to caries. Fish (1932) consider

that hypoplastic areas or lamellae which may appear to predispose to caries, may be inheritable. Hunt *et al* (1944) and Hunt and Hoppert (1944) have produced a caries-susceptible and a caries-resistant strain of rats by selection, progeny testing and close inbreeding.

(*b*) Minerals

Whole sound teeth contain approximately 35 per cent. calcium, 17 per cent. phosphorus and 0·49 per cent. magnesium. Rats placed on a diet very low in all inorganic salts show severe disturbances in the calcification of the growing teeth. Arnim *et al* (1936) found that no enamel hypoplasias occurred in rats, but the predentine becomes wider than normal and the amount of dentine formed becomes decreased until the incisors are merely shells and fracture easily. In the molars, secondary dentine-formation becomes irregular and pathologic cuspal fractures occur, followed in turn by carious lesions, pulpal necrosis and periapical abscesses.

(1) *Calcium and Phosphorus Deficiency*

Enamel.—Gaunt and Irving (1940) found no changes in the enamel organ, or in enamel formation or calcification in rats placed on a low calcium and phosphorus diet. Boyle and Wesson (1943) however, employing a low calcium diet, observed premature atrophy of the enamel organ and in severe chronic cases disturbed calcification and hypoplasia.

Dentine.—Gaunt and Irving (1940) found that rats placed on a diet deficient in calcium and phosphorus show disturbances in the calcification of the growing dentine. Gaunt *et al.* (1939) and Wesson and Boyle (1943) found that a calcium and phosphorus deficiency in rats results in a progressive increase in the width of the predentine and irregular border between the predentine and dentine, interglobular dentine and, in severe deficiencies, vascular inclusions. Boyle and Wesson (1943) also showed that the pre-experimental dentine of molars of rats placed on a diet deficient in calcium is unaffected, but the secondary dentine, forming during the experimental period, is poorly formed and calcified. Schour (1938*a*) showed that dentine is not subject to calcium withdrawal by resorption as in bone. Massler *et al.* (1941) and Follis *et al.* (1943) have demonstrated that in calcium deficiency in man the changes in growing dentine and bone are similar to those observed in experimental animals. Schour (1938a) found that the fully formed enamel and dentine in man are not affected by calcium deficiency. Dean (1943), Malan and Ockerse (1941) and Day (1944) observed no relationship between post-eruptive calcium intake and caries. Shelling and Asher (1933) conclude that in the rat there is no correlation between Ca and P intakes, caries-immunity and caries-susceptibility. Armstrong and Brekhus (1937) have shown that caries is not dependent upon the calcium content of the enamel and dentine.

(2) *Magnesium Deficiency*

Enamel.—Becks and Furuta (1939, 1941) found that the enamel epithelium of rats fed a magnesium-deficient diet undergoes early atrophy and degeneration, and in advanced stages of deficiency the enamel is severely hypoplastic. Irving (1940) describes distortion and atrophy of the enamel organ and calcareous granules which become embedded in the basal end. Radiographic findings by Gagnon (1940) showed a disturbed contour of the enamel surface.

Dentine.—Gagnon *et al.* (1942) and Irving (1940) found disturbances in the calcification of the dentine characterized by prominent delineated stratifications. These striations are thought to be associated with the intermittent interference with calcification and the convulsive attacks of magnesium deficiency. Watchorn and McCance (1937) also found these striations in subacute magnesium deficiency in which no convulsions occurred. The rate of dentine apposition is progressively decelerated and a temporary local cessation of dentine growth may occur.

Eruption.—Duckworth and Godden (1940) and Gagnon *et al.* (1942) found that a magnesium deficiency retards eruption to one-third the normal rate.

(*c*) VITAMINS

Vitamin A

Vitamin A deficiency plays a very important role in the growth, calcification and eruption of teeth. Wolbach and Howe (1933) were the first to observe early and characteristic changes in the enamel organ of rats fed a vitamin A deficient diet. Wolbach and Bessey (1942) have established that the function of vitamin A is concerned primarily with epithelium cells and, in these cells, with the process of differentiation. Schour *et al.* (1941) found that the primary effect of vitamin A deficiency causes failure of the young cells of the odontogenic epithelium to undergo normal histo-differentiation and morpho-differentiation. This highly specialized odontogenic epithelium is responsible for enamel formation and guiding the formation of dentine. Experiments by Mellanby (1939*a*, 1941) have shown that young rats whose mothers were on a vitamin A deficient diet for five months preceding their birth, had distortion in the shapes of both the incisors and molars. The mature ameloblasts become small in size and disturbed in function, so that the enamel matrix is arrested in its formation and calcification, resulting in enamel hypoplasia.

Irving and Richards (1939), and Smith and Lantz (1933), found that in vitamin A deficiency the normal orange pigment of the enamel in rats is lacking and the tooth has a paper-white, unglazed appearance.

Wolbach and Howe (1933) observed early atrophy and depolarization in the odontoblasts on the lingual portion of the incisor of the rat in vitamin A deficiency. The growth of the dentine is thereby seriously affected.

Smith and Lantz (1933), King (1937), Schour *et al.* (1941) and Wolbach and Howe (1933) have demonstrated deficient calcification in both the enamel and dentine in vitamin A deficiency. The enamel becomes hypoplastic and the dentine shows an interglobular texture.

Irving (1943), Mellanby (1941, 1930) and Wolbach and Howe (1933) found that the super-position of a vitamin D deficiency upon a basic diet deficient in vitamin A, increases the disturbance in dentine calcification.

Smith and Lantz (1933) found that eruption of the teeth of rats is retarded in vitamin A deficiency, while Schour *et al* (1941) found that eruption ceases entirely in prolonged deficiencies.

Vitamin A Deficiency in Man.—Vitamin A deficiency may cause a disturbance in the developing tooth germ only in children under six years of age. Bloch (1930, 1931) and Sarnat and Schour (1941, 1942), however, found no association between dental changes and a vitamin A deficiency.

Caries and Vitamin A Deficiency.—Day (1944) is of the opinion that there is no evidence to show that caries is increased because of a deficiency in vitamin A. Day and Sedwick (1934) observed no significant effect on caries progress in children who received supplements of Vitamin A and D over a period of a year. Wolbach and Bessey (1942) could not find significant dental changes in experimental hypervitaminosis A.

From experimental studies with vitamin A, Marshall (1927, 1928) concluded that a vitamin A deficiency in the diet results in caries in the rat.

Vitamin B

Vitamin B deficiencies apparently do not affect growth, calcification and eruption of teeth, but are primarily concerned with the oral soft tissues.

Vitamin C

Vitamin C deficiency affects the growth, calcification and eruption of teeth. The incisor of the guinea-pig is so sensitive to vitamin C deficiency that it has been used to determine the vitamin C content of foodstuffs. Aschoff and Koch (1919) and Wolbach and Howe (1926) are of the opinion that vitamin C seems to play a primary role in the differentiation of connective-tissue cells, and therefore in the capacity of these cells to form and maintain intercellular substance.

Enamel.—Boyle (1938) found that atrophic changes in the enamel epithelium and enamel hypoplasia appear considerably later than do changes in the odontoblasts. He points out that the atrophy of the enamel epithelium is caused by traumatic injuries to the enamel organ. Fish and Harris (1934), on the other hand, are of the opinion that the atrophy of the enamel epithelium is caused directly by the vitamin C deficiency resulting in a primary premature degeneration of the ameloblasts.

Dentine.—Fish and Harris (1934, 1935) found that vitamin C deficiency results in an amorphous and irregular dentine in guinea-pigs and they believe that the dental changes are the direct result of a primary premature degeneration of the odontoblasts. Boyle *et al.* (1940) found that with a vitamin C deficiency in guinea-pigs, dentine formation is retarded and eventually ceases in advanced deficiencies.

Schour *et al.* (1941) found that the earliest dental effect of vitamin C deficiency is a disturbance in the histo-differentiation of the odontoblasts. These cells atrophy and become disorientated and are disturbed in function. This is distinguishable from the effect of vitamin A deficiency, in which there is a persistence and an increased rate of cell proliferation. Höjer and Westin (1925) observed a gradual change of the odontoblastic layer in the teeth of scorbutic guinea-pigs. The odontoblasts are transformed into osteoblasts and spongy, porous bone is formed instead of dentine. Westin (1931) found such pathologic calcification in the dentine in human teeth in scurvy. Wolbach (1937) states that there has been no human demonstration of the changes observed in the growing dental tissues in the guinea-pig.

Eruption.—Dalldorf and Zall (1930) have reported a retardation in the eruption of the incisors of scorbutic guinea-pigs. Schour and Massler (1945), however, found that scorbutic infants show an accelerated eruption of teeth.

Vitamin C Deficiency and Caries.—Westin (1925) states that as far as can be determined there is no relationship between vitamin C deficiency and dental caries. Grandison *et al.* (1942) found that the daily administration of 200 mgm. of vitamin C had no significant effect on dental caries in children. Hanke (1929), however, reported an association of caries with lack of vitamin C.

Vitamin D

Vitamin D plays an important role in calcium metabolism and the calcification of the hard tissues. Deficiencies of this vitamin result in disturbances in calcification of growing enamel and dentine.

Schour and Smith (1934) found that the response of the growing dentine to even a slight deficiency in vitamin D is so delicate that it can be used as an indicator of the adequacy of the vitamin D in the basal diet.

Enamel.—Weinmann and Schour (1945) found no changes in the enamel organ during enamel formation in the rat in vitamin D deficiency, but after the enamel is completely calcified cystic degenerations may occur. This effect takes place only after the enamel is completely formed and calcified.

Becks and Ryder (1931) produced enamel hypoplasia in rats subjected to vitamin D deficiency, but Gaunt and Irving (1940) are of the opinion that this deficiency was aggravated in their animals by some unknown factor. Klein (1931) observed that enamel hypoplasia results only when the vitamin D deficiency is aggravated by parathyroidectomy. Mellanby (1929) produced enamel hypoplasia in dogs when the vitamin D deficiency is aggravated by a direct or indirect deficiency in calcium.

Vitamin D deficiency is generally believed to cause enamel hypoplasia in man, but this has not been proved. (Sarnat and Schour, 1941, 1942.)

Dentine.—Weinmann and Schour (1945) found that the earliest effect of an acute deficiency in vitamin D is the appearance in the dentine of a line of disturbed calcification. In mild deficiencies the coalescence of the calcospherites is incomplete, so that instead of a homogeneously calcified dentine interglobular dentine results. The normally sharp demarcation between the predentine and calcified dentine disappears. The rate of dentine formation is retarded. In more severe chronic deficiencies of vitamin D, the predentine does not become calcified at all so that, as the dentine matrix continues to be deposited, the uncalcified predentine border becomes wider and wider. Similar observations were made by Karshan (1933). Becks and Ryder (1931), Irving (1941), and

Weinmann and Schour (1945) found that, in severe and prolonged deficiencies of vitamin D, pulpal inclusions may be found in the newly-formed dentine. Similar changes in the growing dentine observed in rats in vitamin D deficiencies have been found in dogs by Mellanby (1929, 1937), and Blackberg and Berke (1932), and by Howe *et al* (1940) in guinea-pigs. The histologic changes in human teeth are similar to those seen in experimental animals and consist of deficient calcification of the growing dentine. (Gottlieb, 1920, Wolfe 1935 and Jump, 1939.)

The effects of a vitamin deficiency are markedly aggravated by a deficiency in calcium and/or phosphorus and a disturbance in the Ca: P ration (Irving 1944, Lund and Armstrong 1942, Mellanby 1929 and Karshan and Rosebury 1933).

Irving (1941) and Weinmann and Schour (1945*a*) treated vitamin D deficient rats with vitamin D and observed an improvement in the calcification of the newly-formed dentine. The administration of phosphate to vitamin D deficient rats shows improved calcification in the dentine and a recovery to a normal rate of formation (Weinmann and Schour, 1945*b*).

Vitamin D Deficiency and Dental Caries.—Many investigations have been conducted to ascertain to what extent vitamin D deficiency affects caries, with conflicting results.

Mellanby (1934, 1937) and Mellanby and Coumoulos (1944, 1946) are of the opinion that optimal amounts of vitamin D will reduce the incidence of dental decay, but they were unable to produce caries in their experimental animals that were placed on a vitamin D deficient diet. McBeath and Verlin (1942) reported a lower incidence of decay in children receiving a daily addition of 800 units of vitamin D in their diet. Day and Sedwick (1934) found that supplements of vitamin D had no significant effect on the incidence of caries. Youmans *et al* (1944) observed no relation between extensive caries and vitamin D intake. Brekhus (1941) states that, in spite of the increased intake of vitamin D in children during the last two decades, the incidence of decay has not shown any decrease. Shaw (1932) is of the opinion that, in South Africa, the amount of sunshine is sufficient to produce adequate amounts of vitamin D for growth of teeth, and it is exceedingly improbable that in South Africa the prevalence of dental caries is due to lack of vitamin D. Hess and Abramson (1931) found that a group of children who had had mild or moderate rickets in infancy, showed slightly more caries than children who had not had rickets. Hess *et al.* (1934) observed that a group of children protected against rickets with cod-liver oil during the first year of life showed less caries than a group of unprotected children of the same ages. Mackay and Rose (1931) reported that the incidence among children with a definite history of rickets and among those with a negative history of rickets did not differ significantly. Eliot *et al.* (1934), on the other hand, found more caries among children with a known history of rickets. Shelling and Anderson (1936) found more caries in a rickets-free group of children than in a group with history of rickets and enamel hypoplasia. Rosebury (1938) states that the effect of vitamin D in mitigating caries or of its absence in aggravating it cannot be doubted.

Hypervitaminosis D.—Overdosage of vitamin D has deleterious dental effects.

Schour and Ham (1934) observed no effect of overdosage of vitamin D on growing enamel, but single massive doses of vitamin D showed a poorly calcified layer in the dentine. Harris and Innes (1931) also found irregular calcification of the growing dentine in rats given excessive doses of vitamin D. Schour *et al.* (1937) showed that massive doses of calciferol administered to parathyroidectomised rats resulted in an improved calcification of the dentine. Ziskin *et al.* (1943) observed a significantly increased rate of dentine appositon following the daily administration of large doses of vitamin D to rats for a period of two weeks.

Vitamin E

Davies and Moore (1941) found a loss of pigmentation of the enamel of the incisor of the rat in prolonged vitamin E deficiency. Irving (1942) observed in addition a premature and abnormal degeneration of the enamel organ.

(*d*) The Endocrines

Hypopituitarism

Experiments on hypophysectomized rats have shown a striking relationship between the hypophysis and growth, calcification and eruption of teeth.

Growth.—Schour and van Dyke (1932*a*, 1934) and Schour (1934) have shown that hypophysectomy effects retardation and distortion of growth of enamel and dentine in the rat.

Calcification.—Schour and van Dyke (1932*a*, 1934) found early atrophy of the enamel organ and an increased number of epithelial rests. The enamel becomes folded and hypoplastic. The dentine shows a state of over-calcification and the predentine shows many fine isolated calcium globules.

The effects of pituitary disturbance in man was found by Schour *et al.* (1934*a*) to be essentially similar to those in the rat.

Eruption.—Schour *et al.* (1934*a*) and Schour and van Dyke (1932, 1932*a*) observed that hypophysectomy and pituitary disturbance result in retardation of the eruption of the teeth, both in man and in the rat. The same retardation of eruption was found by Downs (1930) in hypophysectomized dogs.

Hypoparathyroidism

The parathyroids produce a hormone, parathormone, which regulates the blood calcium level and therefore also plays an important role in the calcification of teeth. According to Schour (1938*a*) the parathyroids seem to have little or no direct effect on growth and eruption.

Calcification.—Schour *et al.* (1937) found that in parathyroidectomized rats the enamel epithelium atrophies and the enamel matrix becomes stratified. There are vascular inclusions in the dentine, which is irregular and stratified. The post-operative dentine is interglobular. The primary effect of hypoparathyroidism is a deficient calcification of the dentine. Erdheim (1906) also found that the dentine, formed after rats had their parathyroids removed, was poorly calcified.

Hyperparathyroidism

Schour and Ham (1934) and Schour *et al.* (1934) observed that the primary effect of an injection of parathyroid extract in the normal rat is a rise in the blood calcium level and a corresponding hypocalcification of the dentine. Immediately following is a secondary reaction, a return of the blood calcium to its normal level and a corresponding hypercalcified stripe in the dentine. In a few animals there was also hypoplasia of the enamel. Similar results were obtained by Schour *et al.* (1937*a*) when vitamin D was administered instead of parathyroid extract.

Hypothyroidism

Very little is known of the effects of hypothyroidism on growth and calcification of teeth. According to Schour (1938*b*) thyroidectomy results in a retardation of eruption. Kranz (1934) in his examination of a number of cretins, also observed a marked retardation of eruption and defective tooth structure.

Adrenal Insufficiency

It appears that the adrenal secretion is also concerned with calcification of teeth. Schour and Rogoff (1936) found that in a small number of rats, after complete removal of the adrenals, the enamel-forming cells showed minute intracellular globules which stained prominently with haematoxylin. Characteristic disturbances in the calcification of the dentine were produced. The predentine is sprinkled with globular predentine, which suggests a premature calcification of the dentine. In addition an over-calcification of the post-operative dentine occurs.

Hypogonadism

A relationship appears to exist between the gonads and calcium metabolism. Schour (1936), in a study of the effects of gonadectomy in the ground squirrel, found typical and prominent disturbances in the calcification of the incisors. The dentine showed three distinct zones which were disturbed in calcification.

Hypergonadism

Not much is known about the effects of injections of gonadal or gonadotrophic hormones on growth, calcification and eruption of teeth.

Thymus

The function of the thymus and its effect on calcification are not clearly understood. The results of experimental work in this connection have been conflicting and highly confusing. Barratt (1935) found that rats injected with thymus extract showed general precocious development and eruption following treatment of successive generations.

Considering all this data, it appears that adequate amounts of minerals, especially calcium, phosphorus and magnesium, vitamins, and a normal endocrine equilibrium are necessary for the development of structurally sound and healthy teeth. The high incidence of dental caries among civilized people to-day, however, cannot be ascribed to deficiencies in minerals and vitamins and endocrine disturbances only. These deficiencies may result in a defective structure of the tooth, with the result that such a tooth will have less chance to survive the local caries-producing factors. (Mellanby 1928, 1929, 1930, 1934.) According to Rosebury (1938), no direct evidence of resistance to dental caries due to structure has been presented.

3. LOCAL CONTRIBUTORY CAUSAL FACTORS OF DENTAL CARIES

Notwithstanding the optimal conditions mentioned for the development of structurally sound teeth, dental caries can still develop as a result of a number of local factors. The active aetiological factor of dental caries appears to be acid produced by bacteria, chiefly *L. Acidophilus*. It is a chemico-parasitic process, in which the acid decalcifies the mineral structure of the teeth followed by a dissolution of the softened residue. This theory was originally formulated by Miller (1890), and has remained substantially unaltered. There are many contributory factors in the process of dental decay, which determine growth of the oral flora and affect the concentration and confinement of the acid produced and the resistance in the mouth and teeth to attack. Numerous theories as to how these contributory causal factors influence dental caries have been advanced, but as yet they are not clearly understood. In order to appreciate the problem of some of these secondary factors it is necessary to review these briefly.

(*a*) Carbohydrates

Carbohydrates appear to be one of the most important contributory causal factors of dental decay, and much has been written about the relationship of the carbohydrate content in the diet and dental caries. Miller (1890) was of the opinion that there is a direct relationship between dental caries and the carbohydrate content of the diet. Many hold the view that the high incidence of dental caries among civilized people is associated with increased carbohydrate consumption. The diet of the Eskimos, a primitive race with a low caries incidence rate, consists chiefly of proteins and fats and is very low in carbohydrates. Rosebury (1937), Waugh (1928, 1930, 1931) and Price (1934) found that when Eskimos adopt the white man's food habits (high in carbohydrates) they suffer a markedly increased incidence of decay. Schwartz (1946) found 38 cavities in 408 members of the primitive Masai tribe in the tropics. Their diet consists mainly of proteins and is very low in carbohydrates. It is observed in South Africa that when the Natives leave their primitive kraal environment and adopt the civilized food habits, the caries incidence rate increases.

Sprawson (1934) is of the opinion that the high carbohydrate food is responsible for the rampant caries at Pitcairn. Mellanby and Pattison (1932) hold the view that reduced carbohydrate in the diet may reduce caries.

The results of experiments by Boyd and Drain (1938) and Boyd *et al.* (1929) may also be interpreted that way. Hewat (1932) observed that the high consumption of sugar and confectionery seemed the most prominent factor associated with a high caries incidence. Koehne *et al.* (1934, 1934*a*) were likewise able to correlate the incidence of caries only with intake of artificially sweetened food.

Rosebury (1938) is of the opinion that caries results from an increase in amount or alteration in kind of the carbohydrate in the diet, directly permitting or encouraging the proliferation of acid-forming bacteria on the teeth or acting in some other way. He further postulates that dental caries in man is caused directly and primarily by the presence in the diet of certain specific foods and would expect these causation foods to be rich in carbohydrate. The physical character of these foods is capable of being impacted forcibly into the fissures of teeth. Friel and Shaw (1931) are of the opinion that among South African children in public government schools, high incidence of caries appears to be associated with excessive consumption of sugar and carbohydrates generally. It is very remarkable that Jones *et al.* (1934) believe that taro and sweet potatoes, high carbohydrate foods, are responsible for the sound teeth of the Hawaiians. Jay (1944), Day (1944), and Bunting (1935) consider that excessive amounts of fermentable carbohydrates promote the progress of dental decay. Osborn *et al.* (1937) have reported that although unrefined cereals and sugar are fermented in saliva as readily as the refined products, decalcification of enamel occurs less frequently with the former than with the latter. Schour and Massler (1947) reported that the prevalence of caries in four cities of post-war Italy is approximately two to seven times lower than that observed in the United States. The Italian diet is predominantly carbohydrate in character, pasta (spaghetti, macaroni, etc.) and bread forming the major portion of their intake. The refined portion of the carbohydrate intake, however, is low. This fact may be the decisive factor in explaining the low incidence of caries.

(*b*) Oral Bacteria and Immunology in Dental Caries

As previously mentioned, the exciting cause of dental decay is acid produced by oral bacteria, mainly *Lactobacillus acidophilus*. Factors which may inhibit or promote acid production by these organisms have been investigated, but the results are not clear and often conflicting. Jay *et al.* (1936), Jay (1936), and Becks *et al.* (1944) have shown that the acidophilus content of the saliva increases greatly when carbohydrate intake is increased, and results in greater caries activity. The oral lacto-bacilli may be inhibited by the depression of carbohydrates in the diet, or, as advocated by Odendaal (1947), by means of strong antiseptic mouthwashes. Koehne *et al.* (1934*a*) also reported a striking correlation between the carbohydrate content of the diet, the occurrence of *L. acidophilus* and the incidence of dental caries.

A high acidophilus count has been found in caries-free mouths, which may indicate an unknown factor in immunity to dental caries attack.

Howitt and Fleming (1930) found that, whereas a predominantly carbohydrate diet quantitatively increased the aciduric mouth flora, a diet with less carbohydrate and more protein diminished the number of organisms.

Speidel *et al.* (1939), on the other hand, observed that neither the incidence of *L. acidophilus* in large quantities nor the high acid-producing capacity showed marked correlation with activity or inactivity of dental caries.

Knighton (1942) found that when whole citrus fruits are added to the diet, bacterial fermentation is markedly reduced. Fosdick (1939) has shown that a highly refined sugar, such as glucose, is rapidly broken down by mouth organisms with the production of lactic, pyruvic, acetic, phosphoglyceric and possibly butyric and hexosephosphoric acids. Attempts to correlate the presence of bactericidal or other antagonistic substances in the mouth with absence of caries have been disappointing. Boyd *et al.* (1929) and Rosebury *et al.* (1933) have shown that in the rat mouth lacto-bacilli occur constantly, and Greenberg and Rosebury (1933), and Rosebury and Greenberg (1932), are of the opinion that, as far as can be determined, neither the number nor kind of lacto-bacilli can be correlated with the presence or absence of caries. Rosebury *et al.* (1934) found that the daily addition of a freshly isolated smooth strain of human mouth lacto-bacilli to deficient or adequate coarse rice diets did not in either instance, increase the incidence of caries in rats.

(*c*) Saliva and Dental Caries

The question as to whether saliva can inhibit or promote the process of dental decay has been investigated by a number of workers. It has been suggested that a preponderance of acid-forming foods impairs the acid-neutralizing properties either of the saliva, or of fluids within the tooth, and thus weakens an assumed normal protective mechanism. Other workers found no relationship between saliva and dental caries.

As the results of investigations of saliva and dental caries have been very conflicting, it appears that saliva is not a very important contributory factor in dental caries. It has not been established that changing the composition or pH of the saliva by dietary or other means will materially reduce, prevent, or promote caries.

Forbes and Gurley (1932) found that the pH of the saliva was lowered by a high cereal diet and increased by a high vegetable and fruit diet.

(*d*) Physical Character of Foods

Many investigators consider that the physical character of the diet is an important contributory factor in the problem of dental caries. Wallace (1900, 1929) is of the opinion that caries is induced by the soft refined foods of civilized people and that these foods have a tendency to lodge in the crevices of the teeth and directly cause the development of cavities. Caries is prevented by natural coarse fibrous foods, which not only do not lodge but exert an actively detergent effect, which may indeed counteract the effects of soft foods. Rosebury (1933), Pickerill (1924), Klatsky and Klatell (1943) and Day (1944) hold similar views. Schour and Massler (1945) consider that it may be concluded that the physical character of the diet is an important consideration in the problem of dental caries.

(*e*) Acid-base Character of Foods

Opinions concerning the effect of the acid-base character of foods on dental caries are conflicting. Jones (1935) considers that there is an association between an excessive intake of acid ash foods and dental caries. Kugelmass *et al.* (1934) reported an arrest in the progress of decay when children were placed on an alkalizing diet. Price (1935) has pointed out a relationship between acid-base balance of primitive and modern diets and the incidence of dental caries. Rosebury and Karshan (1935) altered the acid-base balance in rats by adding citric juices and synthetic mixtures to the diet, but found no effect on caries. Forshufvud (1938) produced rampant caries in rats by daily disturbances in the acid-base equilibrium of the blood, but Thomas and Bodecker (1942) who repeated the experiments, were unable to observe any effect on the incidence of caries.

(*f*) Acid Foods and Beverages

It has been found that acid foods and beverages can decalcify enamel surfaces but do not produce typical caries, as occurs in the carbohydrate degradation by bacteria. Kirkpatrick (1939) found that excessive use of lemon drinks decalcified enamel. Tooth decalcification by hard candies has been demonstrated by Miller and Neuwirth (1935). Experiments by West and Judy (1938) have shown that a 40 per cent. solution of acidified candies in water had a pH of 2·5 to 2·8 and dissolved enamel in six hours. McClure (1943*a*) has demonstrated the decalcifying action of dilute acids and acid drinks and beverages on rats' molar teeth, while McClure and Ruzicka (1946) have described the destructive effect of citrate *vs.* lactate ions on rats' molar tooth surfaces *in vivo*. Restarski *et al.* (1945) observed severe enamel destruction in rats allowed to drink a soft acid beverage. The presence of sucrose aggravated the effect of the acid.

The acids in these foods and beverages have a local decalcifying effect, but it is not known if they can accelerate the progress of decay once it has started.

(*g*) Malnutrition and Dental Caries

There is no clinical evidence yet to prove that there is a relationship between malnutrition and dental caries. (Schour and Massler, 1947.)

Black (1936) states that caries is often increased in conditions of malnutrition. Cunningham (1934), however, did not find this so.

Other investigators are of the opinion that an improvement in the nutritional value of the diet results in a lowering of the caries incidence in children (Boyd and Drain, 1928; Boyd *et al.* 1929; Bunting *et al.* 1932; McBeath 1932; Schoenthal and Brodsky 1933; McBeath and Zucker 1938).

(*h*) Lipoids and Dental Caries

Not much is known about the effects of lipoids on dental caries. Box (1938) and McCollum *et al.* (1939) believe that the lipoids in the diet may form a protective mechanical coating upon the teeth and thus prevent access by bacteria or their acids. Rosebury and Karshan (1935*a*, 1939*a*) have shown that vitamin-free corn oil, Wesson oil, Crisco and lard retarded the rate of progress of experimental caries in the rat.

(*i*) Proteins

The literature has very few references concerning the effect of proteins on growth and calcification of teeth. As regards the effect of proteins on dental caries, Schwartz (1946) found that the teeth of the Masai, whose diet consists mainly of protein, are remarkably free from caries. Jones (1930) found that the natives of the Island of Lewis have good teeth and little caries. She is of the opinion that this is due to the preponderance of acid-forming foods. The diet of these islanders consists mostly of proteins in the form of fish, liver and eggs.

According to Sprawson (1932) the islanders of Pitcairn have notoriously bad teeth and, as there are no cattle on the island, presumably the diet is low in proteins. The diet of the Eskimos is practically all proteins and their teeth are remarkably free from caries. Lilly (1938) found that when rats were placed on a Hoppert, Webber and Canniff (1931) diet in which the whole milk powder was replaced by casein, they developed very much less caries.

Rosebury and Karshan (1939) observed that the addition of protein to the basal deficient corn diet yielded an index of caries significantly lower than that of the control group. Cox and Levin (1942) observed, particularly in the case of rats which had been fed a high meat diet, that their offspring were distinctly more resistant to dental caries. Schweigert *et al.* (1946) found that when the casein content of the diet of the cotton rat was increased from 24 to 50 per cent. at the expense of the sucrose, some reduction in caries was observed.

4. EXPERIMENTAL DENTAL CARIES IN RATS

Investigations concerning the contributory causal factors of dental caries have proved most difficult in man, and means of using a suitable experimental animal were, therefore, explored. When McCollum *et al.* (1922) observed caries in the albino rat, and Hoppert *et al.* (1931, 1932), discovered a method of producing caries in the albino rat, it opened a promising field for experimental investigations of the aetiology of caries. According to McClure and Arnold (1941) the first production of caries-like lesions in the rat is attributed to Shibata (1929), who used a diet containing unground rice and greens mixed with various sugars, but McCollum *et al.* (1922) observed caries in the albino rat much earlier. Until recently the albino rat was regarded as being the most suitable and successful animal, but lately Shaw *et al.* (1944*a*, 1945) and Schweigert *et al.* (1945, 1945*a*, 1946, 1946*a*) have successfully used the cotton rat for experimental caries studies, while Arnold (1942) and Dale *et al.* (1944) have produced caries in the Syrian hampster. The albino rat has been used in numerous investigations to study the contributory causes and prevention of dental caries, and much valuable information has been added to our knowledge of the initiation, development and progress of the disease. It has been successfully used because it is like man, omnivorous and its molar teeth, while not quite identical to those of man, are similar in arrangement of their tissues. The dentition of the rat consists of two permanent incisors and six molars in each jaw. The rat reproduces and grows fast, thereby facilitating studies concerning the effect of different diets, heredity, etc., on calcification of the teeth and on caries. These effects can be studied both on the mature molars in which calcification stops, when they are fully developed, and on the incisors in which calcification and growth continue throughout life. The molars of the rat differ from those of man in that there are exposed surfaces of dentine in the summits of the cusps. The rat is easy to handle and takes up very little space. It is subject to several kinds of experimental dental lesions, some of which, however, differ fundamentally from dental caries in man.

(1) *Cusp Fracture Lesions.*—According to Rosebury *et al.* (1933) and Bibby and Sedwick (1933), coarsely ground raw hard cereal particles, which pass through a 20-mesh sieve, but not through a 30-mesh, frequently fracture the molar cusps, resulting in the exposed dentine becoming infected and caries follows. Since in its inception it is fundamentally unlike dental caries in man, this kind of caries cannot be considered analogous. Bodecker and Applebaum (1932), Klein and Shelling (1932), Rosebury and Foley (1932, 1934), and Arnim *et al.* (1936) have observed other lesions, which have their inception in the exposed dentine of the cusp summit. The caries in rat molars reported earlier by Macomber (1927), Marshall (1927), and Knowlton (1930) was probably also of the cusp fracture type.

(2) *Occlusal Fissure Caries.*—Caries lesions induced at the base of the occlusal fissures in the molars of the rat are considered to be essentially the same as occlusal fissure caries in man. Rosebury and Karshan (unpublished data) have made a careful comparative histologic study of these lesions in man and in the rat.

(3) *Proximo-gingival Caries.*—Rosebury (1937*a*, 1939) and Weisberger *et al.* (1940) have produced caries in the gingival areas of rat molars that may be analogous to proximal caries in man.

(4) *Interproximal Caries.*—Grieves (1922, 1923) and Klein and McCollum (1932) have described interproximal caries. Cox *et al.* (1939) have observed an opaque type of lesion on the distal buccal angle of the first lower molar and on the approximating mesial-buccal angle of the second molar.

Primary Aetiology of Caries in Rats

The primary causal factor in the production of experimental fissure caries in rats has generally been the presence in the diet of a coarse raw cereal, ground to pass through a 20-mesh sieve but not through a 30-mesh. This was first clearly shown by Rosebury *et al.* (1934*a*) and later by King (1935), and Cox (1937). Rosebury *et al.* (1933, 1934*a*), and Rosebury and Karshan (1935, 1935*a*) have produced fissure caries in the rat with diets containing coarsely ground raw rice, corn and dried lima beans. Sognnaes (1941*a*) extracted the upper molars of young rats and found that, after being fed on a coarse cereal diet for 100 days, no caries developed.

Experimental studies conducted by Rosebury and Karshan (1931, 1931*a*), Knowlton (1930), Lilly (1932), Lilly and Grace (1932), Lilly and Wiley (1934), Johnston *et al.* (1933), Agnew *et al.* (1933), Rosebury and Foley (1934), King (1935), and Day *et al.* (1935) have shown that when the cereal used in the caries-producing diet is cooked or ground to pass a 60-mesh sieve, no caries is induced. Furthermore, if high percentages of starch and sugars are incorporated in this diet there is also no development of caries. McClure (1945), however, found that rats developed a significant incidence of microscopic dental caries when fed synthetic non-caries-producing diets containing excessive quantities of sucrose and glucose. According to these investigators fissure caries in rats most frequently occurred in the first and second mandibular molars. Finn and Hodge (1941) found that the maxillary first molar is most frequently involved, and there is evidence that the teeth of the upper jaw are more often carious.

Mechanism of Fissure Caries in Rats

Fissure caries in the rat is probably produced by the impaction of coarse food particles into the fissure under the heavy occlusal pressure required to crush it. The starch of which these food particles largely consist is fermented by bacteria, and the acid thus formed initiates caries. This is in accordance with Miller's chemico-parasitic theory (Rosebury, 1933). Investigations conducted by Lilly and Grace (1932), King (1935), Day *et al.* (1935), Rosebury and Karshan (1935, 1939), and Cox (1944) are inclined to suggest that excessive intake of carbohydrates does not initiate caries in the rat, but rather increases the rate of decay already present. Other factors in the aetiology of caries in rats have been reviewed elsewhere.

Comparison of Aetiology of Dental Caries in Man and Rat

Briefly summarizing, therefore, we may conclude that the histopathology of dental caries in the rat and in man appear to be identical. The enamel is first decalcified, followed by decalcification, bacterial invasion, decomposition and cavitation of dentine. In man the chief bacteria concerned appear to be lacto-bacilli, but the evidence in rats on this point is not quite clear, although lacto-bacilli are always present in the mouth. Although

cusp fracture lesions in rats appear not to be analogous to caries in man, the progress of the decay which results is similar to the progress in man. In man, as in the rat, improvement of the nutritional adequacy of the diet diminishes caries. Excess of carbohydrates in the diet, on the other hand, may aggravate the incidence of caries.

Other contributory causal factors in man and rats have been reviewed elsewhere.

5. FLUORINE AND DENTAL CARIES

It has been proved that fluorine has an inhibitory effect on dental caries. Excessive amounts of fluorine ingested during calcification of the teeth, however, cause undesirable mottling, pitting and hypoplasia. Posteruptively they may also affect the system generally, more especially the skeletal and muscular systems.

(*a*) Intake and Excretion of Fluorine

Fluorine usually enters the system through drinking water. Certain geological formations contain fluorides, and these are the main sources of the fluorine in water supplies. It may also enter the system through foods and by inhalation of dust containing fluorides in cryolite and rock phosphate mines and factories. Only large doses appear to affect the blood calcium. McClure and Mitchell (1931, 1931*a*) found that the calcium balance of the blood was not affected in rats by sodium or calcium fluoride up to 313 p.p.m., but both salts lowered calcium retention at 623 p.p.m. Foit (1931) observed that the blood calcium in rabbits was diminished by the injection of sodium fluoride. Irving (1943*a*) is of the opinion that the action of sodium fluoride on normal rats must be associated with a lowering of the blood calcium. Roholm *et al.* (1937, 1938), and Smith and Lantz (1935) found no change in the blood phosphatase. Some of the fluorides ingested from food and water is stored in the bones and teeth, but most is eliminated in the urine (Roholm 1937; Shortt *et al.* 1937; Smith *et al.* 1942; Machle and Largent 1943; Machle 1942; Largent and Ferneau 1944; Machle *et al.* 1942).

(*b*) Maternal Transference of Fluorine

The question of transference of fluorine from the milk and through the placenta is of great importance in investigations of fluorine inhibition of caries, but has not been definitely settled. Murray (1936) found that fluorine is transmitted in milk and through the placenta in the rat. Evans and Phillips (1939) observed that the addition of fluorine to the basal diets of rats increased placental transfer, but up to 20 p.p.m. had no effect on mammary transmission of fluorine. Smith (1936) fed 0·05 per cent. sodium fluoride to pregnant rats, and found the teeth of their offspring normal at the time of weaning. Cox *et al* (1939*b*), Cheyne (1940) and Norvold and Armstrong (1943) administered fluorine to rats pre-natally and found a lower caries incidence rate in their offspring. This suggests that apparently fluorine does pass through the placenta. Phillips and Hart (1934) found no increase of fluorine in milk when cows were given water containing 500 p.p.m.F. Evans *et al.* (1938) states that in cows, small amounts of fluorine are transferred through the placenta under normal conditions and also that small amounts pass into the milk. As mottled enamel is seldom seen in deciduous teeth Smith and Smith (1935) are of the opinion that the fluorine is probably filtered out by the placenta. Silva *et al.* (1940) hold similar views. Roholm (1937), on the other hand, maintains that small amounts of fluorine do pass through the placenta. He concludes that, as children of female cryolite workers had mottling of the permanent teeth, although these children never drank water containing fluorine, it is excreted in the milk of the human female.

(*c*) The Effect of Fluorine on Growth

There is no definite evidence that fluorine is essential to body growth and to tooth development. Shills and McCollum (1942) are of the opinion that more refined nutritional experiments, or the elucidation of its role in bone and tooth structure, may show that fluorine is necessary. Fluorine is an enzymatic inhibitor and a protoplasmic poison and, when ingested in large doses, inhibits growth, weakens and bows bones (McClure and Mitchell, 1931; Smith and Lantz, 1933*a*; Smith and Leverton, 1933, 1934; Sharpless, 1936, 1936*a*.). The developing enamel is the first structure to react to the ingestion or injection of fluorine. Schour and Smith (1934*a*) have shown that abnormal globules are formed in the ameloblasts as early as one hour following a single injection of 0·3 cc. of a 2·5 per cent. solution of sodium fluoride. The odontoblasts are less sensitive to fluorine than the ameloblasts.

(*d*) The Effect of Fluorine on Calcification of Enamel

Fluorine causes a disturbance in the calcification of the enamel. The degree of disturbance depends upon the dosage and duration of ingestion and injection. The mechanism is obscure.

Chase (1940), and Schour and Smith (1942) found that fluorine disturbs the cellular function of the ameloblasts, resulting in a defective structure of the enamel. Schour and Smith (1934) are of the opinion that when the fluorine enters the bloodstream it unites with calcium, forming calcium fluoride, which may be taken up by the ameloblasts, injuring them by acting in the nature of a foreign body. The ameloblasts and odontoblasts are so sensitive to fluorine that Schour and Smith (1934, 1934*a*), and Schour and Poncher (1937) used injections of sodium fluoride to measure quantitatively the rate of growth of enamel and dentine in man and animals. De Eds (1941) thinks that fluorine may disturb the enzymatic phosphatase system concerned in calcification. In man, ingestion of fluorine in excessive amounts, usually from water, during calcification of the permanent teeth, i.e., from birth to eight years, causes mottling of the enamel. The degree of mottling depends on the amount of fluorine present in the water. According to Dean (1934):—

0·8 p.p.m. fluorine causes no mottling.
0·9 p.p.m. fluorine causes questionable mottling.
1 to 2 p.p.m. fluorine causes very mild mottling.
2 to 3 p.p.m. fluorine causes mild mottling.
3 to 6 p.p.m. fluorine causes moderate mottling.
6 and more p.p.m. fluorine causes severe mottling, with pitting, hypoplasia and chipping.

Dean (1938) has classified mottled enamel as follows, the figures indicating the degree of mottling:—

0 Normal.
0·5 Questionable. A few white flecks to occasional white spots.
1·0 Very mild. Less than 25 per cent. of the tooth's surfaces covered by small, opaque, paper-white areas.
2·0 Mild. 50 per cent. of the tooth's surfaces covered by white, opaque areas.
3·0 Moderate. Nearly all the tooth's surfaces are involved; minute pitting and brown stain.
4·0 Severe. Smoky-white appearance of all the teeth. Pitting frequent and on all tooth's surfaces, hypoplasia, chipping and large stains which vary from chocolate-brown to black.

Mention of "mottled enamel" was probably first made by Kühns (1888). He observed black spots in the teeth of people who lived in Mexico. The condition was later described by Eager (1902) of the United States Marine Hospital Service, when he noticed the stained teeth ("denti di Chiaie") of Italian emigrants embarking at Naples. It was first described in the United States by Fynn (1910) as occurring in Colorado Springs, Col. McKay and Black (1915, 1916, 1916*a*, *b* and *c*) established that the aetiologic factor was some rare element in the drinking water used during the calcification of the teeth, because after the water supply of Colorado Springs had been changed, no more cases of mottling occurred. It was not until 1931, however, that Smith *et al.* (1931), Churchill (1931), and Velu (1931) independently proved beyond doubt that toxic amounts of fluoride in drinking water caused mottled enamel.

Its occurrence has now been reported from practically every country in the world, and the affected areas are termed "endemic areas". Dean and McKay (1939) report 375 known "endemic areas" in the United States. Cases have been reported by Walker and Spencer (1937) from Canada, and by Kühns (1888) and Mazzotti and Rivera (1939) from Mexico; Chaneles (1932), Munoz (1934) and Erausquin (1934, 1935) place the "endemic areas" in the Argentine Republic at well over 175. Mottled enamel is there called "dientes veteados" and "dientes machados." Damon (1930), an American dentist practising in Buenos Aires, found mottled enamel not only in the Argentine but also scattered over adjoining countries in South America. Velu (1932, 1933, 1933*a*, 1934, 1934*a*, 1938) has written much about the extensive occurrences in the phosphate zones of Morocco, Tunis, and Algiers in North Africa, where it affects man and animal and is called "darmous". In man it is caused by drinking water, which is polluted with small particles of dust containing fluorides from the phosphate deposits. In animals it is caused by ingestion of fluorides in this dust, which is deposited on grass, shrubs, soil and in the drinking water. I have seen several severe cases of "mottling" in children from Moshi, Ngare Nanyuki and Arusha in the Tanganyika Territory, Central Africa. Samples of drinking water from Arusha contained 11·8 and 6·4 parts per million fluorine. Mottled enamel occurs extensively in South West Africa, and many water samples showed excessive amounts of fluorine, the highest of which (20·62 p.p.m.) came from the Omaruru district. Drinking water from Maun, N'Gamiland, contained 8·40 and 8·50 parts per million fluorine. Masaki (1931) has written about the geographical distribution of mottled enamel in Japan, while Anderson and Stevenson

(1930) and Ni (1937) have reported on the occurrence of " mottled enamel " among the Chinese. Ouw Eng Liang (1939) has described cases occurring in West Krawang, Java. Extensive endemic areas have been reported in India by Shortt *et al.* (1937), Pillai (1938), Wilson (1939), Day (1940), Raghavachari and Venkataramanan (1940), and Pandit *et al.* (1940). Ainsworth (1933), Morgan (1939), and Wilson (1939) have written about cases occuring in England, and Ricci (1933) about those occurring in Italy, where it is called " denti scritti ", " denti screziati " and " denti macchiati ". Straub (1940) has reported cases in Hungary. McKay (1930) has information about affected areas in the Bahama Islands, the Barbadoes, Cape Verde Islands, Spain and Holland. Lambadarides (1940, 1941) found numerous cases occurring in several districts of Greece on the mainland and on the islands, while Koutsouveli (1940) has described cases of mottling among the inhabitants of the city of Laurion near Athens. Clements (1939) describes cases occurring in Australia, while Clawson *et al.* (1940) describe occurrences in Western Asia.

The occurrence of mottled enamel in South Africa was first reported by Maughan Brown (1935). A few cases have also been recorded by Staz (1938). Steyn (1938) and Steyn and Reinach (1939), and van der Merwe (1940, 1940*a*, 1940*b*) are greatly interested in the problem, and have discussed the toxic effects of fluorine in drinking water in this country, and have made certain suggestions concerning its prevention.

Mottled Enamel in Rats

McCollum *et al.* (1925) fed sodium fluoride to rats and observed that the incisors were of a dull opaque white colour. Bergara (1927, 1927*a*) produced alternate white and chocolate-coloured striations in the enamel of both upper and lower incisors of rats fed sodium fluoride for four months. Afterwards, when it was discovered that fluorine in drinking water caused mottling in man, mottled enamel has been produced in rats by many investigators (Smith *et al.*, 1931; Pachaly, 1932; Bethke *et al.*, 1933; Smith and Lantz, 1933; Smith and Leverton, 1934; Marcovitch *et al.*, 1937; Cheyne, 1942). Cox *et al.* (1939*a*) fed daily doses of fluorine directly to suckling rats until they were weaned at 21 days of age. They produced mottled enamel in the first two permanent molars. When high doses of fluorine were fed from 21 days of age, only the third molars were mottled (Dixon and Cox, 1939).

(*e*) The Effect of Fluorine on Calcification of Dentine

As previously mentioned, the odontoblasts are less sensitive to fluorine than the ameloblasts. Large doses affect the calcification of the dentine, which becomes interglobular and stratified. After prolonged ingestion, disturbances in the formation of the dental matrix occur (Bergara, 1929; Chaneles, 1930; Pachaly, 1932; Bethke *et al.*, 1933; Schour and Smith, 1934). Calcium appears to have a protective action against fluorine intoxication. Lawrenz and Mitchell (1941), and Irving (1943*a*) found that variations in the calcium and phosphorus content of the diet may modify the degree of response of the dentine, but Smith (1936) found that they failed to nullify the effect of the fluorine.

(*f*) The Effect of Fluorine on Eruption

Not much is known as to what extent excessive amounts of fluorine affect eruption of teeth in man. Masaki (1931) observed that the eruption of the permanent teeth, especially the first molar, of children with mottled enamel is very late. Schour and Smith (1934) found that fluorine retards the rate of eruption in rats.

(*g*) Fluorine and Dental Caries in Man

Many attempts have been made to control and prevent dental caries. When the inhibitory action of fluorine on caries was established, the attention of many investigators became centred on this aspect of caries prevention, and much has lately been written about its importance and possibilities in dentistry. Different theories have been advanced regarding the exact mechanism of fluorine in reducing caries, but it is as yet not clearly understood. As a number of factors may be associated in the process, it will be necessary to review some of the more important findings.

From numerous field studies conducted in different parts of the world and from experimental investigations, it has definitely been established that fluorine in drinking water is associated with a lower caries incidence rate. Masaki (1931) in Japan was probably the first to observe that the incidence of dental caries is comparatively small among those who suffer from mottled enamel. Dean (1938, 1938*a*, 1940, 1943) and his co-workers (1939, 1941, 1941*a*, 1942) have studied the relationship between dental caries and fluorine in great detail in the United States. They have shown that a lower rate of dental caries is associated with mottled enamel, and also with the presence of fluorine in water supplies. Deatherage (1942, 1943, 1943*a*), Klein and Palmer (1937), Messner *et al.* (1936), Jay (1942), and Bull (1943) have reported similar findings.

Wilson (1941), Murray and Wilson (1942), Ainsworth (1943), King (1944), and Weaver (1944*a*) have observed a lower caries incidence rate in endemic fluorosis areas in England. Erausquin (1935), Chaneles (1932), Silva *et al.* (1940), and Pasqualine and Celli (1940) have reported less caries in mottled teeth than in normal teeth in the Argentine. Day (1940, 1944*a*) found that the caries incidence rate among children in the endemic fluorosis districts of Kasur and Hissar was very much lower than in the non-endemic area of Lahore City. Shourie (1946) made similar observations in other parts of India. Sognnaes (1941) noted that the teeth of the inhabitants of Tristan da Cunha were excellent and that mild mottling was widespread. Brown (1935) observed that the mottled teeth of the children in South Africa were strong and resistant to caries.

An exception to the above findings may be mentioned. Agnew and Agnew (1943) state that in West China and Eastern Tibet the incidence of caries among groups who have mottled enamel, or who live in areas where this condition is endemic, was equal to the incidence among those not suffering from mottled enamel or living in areas where this condition is not endemic. This would be very difficult to explain. Boyd (1943) found no correlation between annual increment of caries in 55 children and either the maximum or minimum regional fluorine content of waters. Cox (1940) is of the opinion that posteruptive exposure to fluoride-bearing water-supplies has no effect on the course of dental caries. Reduction of caries by topical application of fluorides to teeth, however, has recently been successfully applied to clinical experiments in man by Cheyne (1942), Bibby (1942, 1942*a*, 1943, 1944, 1944*a*, 1947), Largent and Moses (1943), McClendon and Foster (1943), Atkins (1944), Arnold *et al.* (1944), Knutson and Armstrong (1943, 1944, 1945, 1946), Knutson *et al.* (1947), Galagan and Knutson (1947), and Bibby and Turesky (1947).

(*h*) Fluorine and Dental Caries in Rats

When the caries inhibitory action of fluorine was observed in man, numerous experiments have since been conducted with rats, and the results have confirmed these findings Miller (1938) was probably the first investigator to demonstrate the inhibitory action of fluorine on rat caries He added fluoride to the ration of animals whose teeth were already calcified Hodge and Finn (1939), Finn and Hodge (1941), Cheyne (1940, 1940*a*), Sognnaes (1940, 1941*b*), Arnold and McClure (1941), McClure (1941), McClendon and Foster (1942) and Norvold and Armstrong (1943) have conducted experiments on similar lines by adding fluorides to coarse particle diets or to water and observed various reduced caries-incidence rates in rats. Dale *et al.* (1944) have reported a reduction of 80 per cent. caries in Syrian hampsters with added fluorine. Cox *et al.* (1939*b*), Cheyne (1940*a*), and Norvold and Armstrong (1943) gave added fluorides to rats during pregnancy and lactation, and found that their offspring showed a reduced caries-incidence rate.

Sognnaes (1940) and Cheyne (1940*a*) administered fluorine by stomach-tube to desalivated rats and obtained a moderate reduction in the number of molar lesions. Cheyne (1940*a*) also found that fluorine inhibited experimental dental caries in the rat even when the salivary secretion was decreased to a minimum by removal of the salivary glands. Sognnaes (1940, 1941*b*) applied fluoride solutions topically to rat molar teeth and obtained a limited reduction of caries. McClure (1943) gave 100 p.p.m. fluorine for 40, 60 and 85 days prior to the 100 days on the caries-producing diet, and obtained reduction in the molar lesions of 20, 30 and 75 per cent. respectively.

(*i*) The Mechanism of Inhibition of Caries by Fluorides

There is no clear-cut evidence as to how fluorine acts in reducing caries, but two theories of any importance have been advanced concerning the mechanism. In the first place, some workers are of the opinion that mottled

enamel and dentine contain more fluorine than normal teeth (Armstrong and Brekhus, 1938), which makes them more resistant to caries. Others, on the other hand, believe that fluorides inhibit bacterial activity in the mouth. Armstrong (1937), and Armstrong and Brekhus (1938*a*) actually found that the fluorine content of carious enamel is much lower than the fluorine content of sound enamel. This would suggest that optimum quantities of fluorine in enamel might be associated with an increased resistance to caries, without any deleterious effect on the appearance and the structure (Volker and Bibby, 1941; Bibby, 1944*a*, and Odendaal, 1947).

Local applications of a solution of fluorides to teeth *in situ* suggest that sufficient fluorine is adsorbed to the enamel surface to decrease its susceptibility to dental caries (Volker *et al.*, 1940). Because the adsorption of fluorine to fully formed teeth does not necessarily prevent decay, Cox and Levin (1942) are not in favour of the chemical theory. They postulate that fluorine must be present during calcification in order to alter the teeth structurally. Only such teeth would appear to withstand decay. It has been demonstrated that enamel containing more fluorine than normal is less soluble in acids (Volker 1939, 1940, 1940*a*, 1943, and Bibby *et al.*, 1942).

Restarski *et al.* (1945) found that soft acid beverages caused destruction of enamel in rats, but when 1 to 20 p.p.m. of fluorine was included, the enamel destruction was decreased, although not completely prevented. From experimental studies with animals it has been shown that fluorine can be adsorbed by enamel posteruptively by direct exposure of the teeth to fluorides (Volker *et al.*, 1940; Arnold and McClure, 1941; Norvold *et al.*, 1941; McClure, 1943; Perry and Armstrong, 1941; and McIntire *et al.*, 1944.). Fluorine can also be taken up in the enamel and dentine through the bloodstream during and after calcification (Bowes and Murray, 1936; Glock *et al.*, 1941; Volker, *et al.*, 1941; Arnold and McClure, 1941; McClure and Arnold, 1941; Perry and Armstrong 1941.). Arnold and McClure (1941) found that the increased fluorine content in the enamel and dentine in rats, from posteruptive subcutaneous injections of sodium fluoride did not increase their resistance to induced dental caries. When the enamel and dentine are exposed to fluorides it appears that the fluorine reacts with the apatite. The general formula given to tooth substance is $3Ca_3(PO_4)_2Ca(OH)_2$ or $3Ca_3(PO_4)_2CaCo_3$, and with fluorine, fluorapatite ($3Ca_3(PO_4)_2CaF_2$) is formed. It is because this fluorapatite is stable and therefore less soluble in acids than apatite itself, that the presence of fluoride has such a marked effect on the behaviour of tooth substance. It reduces acid solubility and caries susceptibility (Leicester, 1946). When optimal amounts of fluorine are present in the blood stream during calcification of teeth it also appears that a similar interaction takes place. Enamel is deposited as fluorapatite and not as hydroxyapatite.

Inhibitory Action of Fluorine on Bacterial Activity in the Mouth.—Fluorine in large doses is a protoplasmic poison and is a powerful inhibitor of certain enzymes. It probably inhibits the bacterial enzymatic process, and since the production of acids from carbohydrates is an enzymatic process, it naturally follows that fluorine will inhibit the process and thus the initiation and spread of caries (Odendaal, 1947). According to Lipmann (1930) it blocks the formation of lactic acid by preventing the transformation of glycerol-phosphoric acid into phosphopyruvic acid. Fosdick (1942) explains that as the teeth absorb large quantities of the fluoride ion, it appears possible that the concentration in the teeth is sufficient to inhibit the local formation of lactic acid. Thus the natural neutralizing or immunizing influences of the mouth will cause immunity to caries. Bibby and van Kesteren (1940) found that fluorine concentrations of less than 1 p.p.m. limit acid production by bacteria, but concentrations in excess of 250 p.p.m. are needed to affect bacterial growth. Bibby (1941) has shown that fluorides cause a reduction in acid formation by oral streptococci. Cox and Levin (1942) also state that extremely small amounts of fluorine have a marked effect on bacterial acid production. Dean *et al.* (1939, 1942) obtained significant differences in both the negative and high lacto-bacillus counts, between communities using fluoride water and the neighbouring control population.

Fluorine is probably not a salivary enzyme inhibitor. McClure (1939) could find no effect of various amounts of fluorides on salivary amylase. Neither did Clifford (1936) find any acceleration by fluorides on the hydrolysis of starch by pancreatic and salivary amylases. Chlorides, bromides and iodides generally accelerated these processes.

It appears that fluorine ingested from food and water is not found in the saliva (Bossevaiin and Drea 1933; Cox 1930; McClure 1941*a*).

(*j*) Prevention of Caries by Fluorides

From the above observations it appears that the incidence rate of dental caries can be reduced by applying fluorides topically to the teeth or by adding optimal amounts of fluorine to the drinking water. This latter aspect of caries reduction has been fully discussed by Cox (1939, 1940), Cox *et al.* (1939*b*), Chapin and Mills (1942), McClendon *et al.* (1942), Arnold (1943), Dean (1943), Ast (1943, 1943*a*) and Faust (1944). Experiments on these lines are now being conducted in New York State (Ast, 1944), and in Brantford, Ontario, Canada.

In view of the fact that very little research work has been done in connection with the incidence of caries in different parts of South Africa, and that no direct research has been carried out here on caries in rats, the following studies have been undertaken to ascertain—

(i) the epidemiological and geographic distribution of dental caries among European schoolchildren;

(ii) to what extent the application of the dietary habits and other factors found in different parts of South Africa would affect rat caries;

(iii) which measures should be taken to prevent caries.

The experimental investigations have been carried out in the Department of Physiology, University of Pretoria.

II. MATERIALS AND METHODS

1. CLINICAL SURVEY

(*a*) Description of South Africa

(1) *Area.*—South Africa is situated between latitudes 22° 10′ and 34° 50′ south, and between longitudes 16° 30′ and 33° east. It covers an area of 472,550 square miles, or more than five times the area of Great Britain.

(2) *Divisions.*—South Africa is divided into four provinces, the areas of which are:—

Cape Province	277,169 square miles.
Natal Province	35,284 square miles.
Transvaal Province	110,450 square miles.
Orange Free State Province	49,647 square miles.

Each province is again sub-divided into magisterial districts.

As statistics of the population of South Africa are also given in regional divisions, the incidence of dental caries is given in these regional divisions as well as in the provinces.

These regional divisions are non-administrative, but each comprises a group of magisterial districts. The boundaries, although somewhat arbitrarily drawn, follow closely the different climatic conditions found in the Union due to altitude, varying rainfall, etc.

Regional Divisions

The following are the regional divisions:—

(1) *South-Western Coastal.*—Extending from Malmesbury district to Alexandria district inclusive.

(2) *South-Eastern Coastal.*—Mainly the coastal districts of Transkei, Natal and Zululand, from Bathurst district to the border of Portuguese East Africa.

(3) *Karroo–Cape Central.*—Bounded on the south by Division I, on the east by Division V, and on the north by Divisions IV and VII. Clanwilliam and Piquetberg are the only districts in this division on the Atlantic coast.

(4) *Highveld.*—This embraces the greater portion of the inland plateau land, and includes the whole of the Orange Free State, the inland districts of Natal, that portion of Transvaal lying approximately south of the 25° of south latitude, the districts of the Cape Province adjacent to the western boundary of the Transvaal and the western and south-western boundary of the Orange Free State.

Vegetation

The natural vegetation of the Union consists of forest, parkland, grassland and desert shrub.

Forest occupies a very small proportion of the country, and is chiefly confined to the region of constant rainfall in the south and to the seaward slopes and deep kloofs.

Parkland vegetation covers the central and north-eastern portion.

Thornbush characterizes the central portion, while deciduous and evergreen trees are one of the main features of the north-eastern portion. Grassland covers the eastern portion of the Union.

Desert shrub, composed of perennial succulents and woody shrubs, covers a large portion of the western half of South Africa. A vegetation map of South Africa will be found at the end of the text.

Farming Activities

Stock raising (sheep, cattle, goats), wool, mealie-, wheat-, fruit-, tobacco growing, poultry and dairying are the chief farming activities in South Africa. The distribution of these activities is shown on the map at the end of the text.

Population

The European population of South Africa, according to the census of 1946, was 2,372,690, of which 1,194,626 were males, and 1,178,064 females. There are 413,884 schoolchildren.

The stock is largely of English, Dutch and French origin. According to the South African Year Books it is estimated that about 57·5 per cent. of the children are of Dutch South African parentage, 33·7 per cent. of English and the remainder of other European races. In the urban areas 53 per cent. of the people speak English and 41 per cent. Afrikaans, while in the rural areas, 14 per cent. of the people speak English and 84 per cent. Afrikaans. The average number of children per family is three.

(*b*) Selection of Material

European schoolchildren between the ages of six and seventeen were selected for this survey because of the easy access to large groups for dental examinations. The children were divided into three groups:—

(1) Those born and bred in urban areas.

(2) Those born and bred in rural areas.

(3) Those from other areas, i.e., those who were not domiciled continuously in either a particular urban or rural area.

(*c*) Dental Examinations

The children were examined with probe and mirror for dental caries in good daylight. Children " with caries " means those children who suffer or have suffered at some time from one or more carious teeth.

Symptoms of chronic dental fluorosis or mottled enamel were also looked for during these dental examinations. When cases of mottling among these children occurred, detailed particulars about the degree of mottling and caries as well as the area where these children were born and lived up to the age of eight years, were carefully recorded. The cases of mottling were classified according to Dean's (1938) classification.

(*d*) Teeth Analysis

Many teeth, extracted by dentists and doctors from children living in different areas, were collected and prepared for chemical analysis to determine the percentage CaO, MgO, P_2O_5 and F. The decay, when present, was first removed with burrs. The crowns were then broken into small bits, the pulps removed and the enamel and dentine separated by the flotation method, as described by Manly and Hodge (1939).

(*e*) Water Analysis

Quart samples of drinking water were collected in different areas for a detailed chemical analysis to determine a possible relationship between the pH values and the mineral content and the caries incidence rate.

The pH values and the mineral content of the drinking water of the cities and towns were all determined on representative samples collected. The pH values and the mineral content and total hardness of the drinking water of the districts were calculated from the numerous analyses supplied by the South African Railways and Harbours Administration, Division of Chemical Services, Department of Agriculture and Forestry, and from analyses of samples submitted personally.

Information about the diets of the schoolchildren was mostly obtained from their teachers, as it was found that they are well acquainted with the social and economic conditions in the homes of their pupils.

2. EXPERIMENTAL INVESTIGATIONS

A preliminary analysis of the clinical survey showed a great variation of the incidence of dental caries in different districts, and it was decided to conduct experimental investigations on the effects of the different diets found in these districts on rat caries. Groups of 25 albino rats (*Mus norvegicus albinus*), of the Wistar Institute strain, of the same age were placed on different diets for 80 days. The experiments were started after the third molar was fully erupted. In a preliminary study, the eruption time of the molars of rats was determined, and it was found that the eruption of the first molar begins on the 19th day, the second molar on the 21st day, and the third molar on the 31st day, and that the first molar was fully erupted on the 25th day, the second molar on the 27th day, and the third molar on the 35th day. From these findings it was decided to start the experiments on 35-day-old rats. Originally experiments were conducted with the caries-producing diet of Hoppert *et al.* (1931, 1932). It was felt, however, that this diet is not completely balanced and it was therefore decided to alter the balanced stock laboratory diet (Kellerman, 1943) into a caries and non-caries one. The balanced diet consisted of:—

68 parts yellow corn meal.
15 parts linseed meal.
7 parts crude casein.
3 parts dried brewer's yeast.
2 parts lucerne meal.
2 parts bone meal.
2 parts fish liver oil.
0·5 parts NaCl.
0·5 parts $CaCO_3$.

The fish liver oil contained 10,000 I.U. of vitamin A and 250 I.U. of vitamin D per gram. The caries-producing diet, used in all the experiments, was the same as the balanced diet, except that the fine corn meal was replaced by coarse corn meal, which passed through a 20-mesh sieve but not through a 30-mesh. In the non-caries producing diet the corn meal was ground to pass a 60-mesh sieve. From the time of weaning at 21 days until 35 days all the rats were placed on the non-caries producing diet.

(*a*) Post-natal Experiments

Table III gives the different diets and drinking water on which the groups of 25 rats were placed for 80 days:—

Table III

Group.	Diet.	Water.
I	Non-caries producing	Tap.
II	Caries-producing	Tap.
III	Non-caries producing and boiled sweet potatoes	Acidified.
IV	Caries-producing and boiled sweet potatoes	Acidified.
V	Non-caries producing and sweets	Tap.
VI	Caries-producing and sweets	Tap.
VII	Non-caries producing	Orange juice.
VIII	Caries-producing	Orange juice.
IX	Caries-producing and meat	Tap.
X	Caries-producing	Distilled plus 5 p.p.m. F.
XI	Caries-producing	Distilled plus 100 p.p.m. F.

The rats in Group I served as controls for the non-caries producing groups, while those in group II served for the caries-producing groups. The rats in Group III were placed on a non-caries-producing diet, with added carbohydrates in the form of boiled sweet potatoes and an acid drinking water. This diet and drinking water are found in the high caries incidence areas of the southern coastal areas and were used to ascertain if they could initiate caries in the rat. The acidity of the waters in these areas is caused by organic acids. As none of these acids was obtainable, the water used was acidified by adding 0·3 gms. tannic acid to one litre of distilled water. This gave it a pH of 5·2. In group IV the boiled sweet potatoes and acid drinking water were added to the caries-producing diet to determine to what extent caries in the rat is aggravated or accelerated. The addition of sweets to the diets in groups V and VI was to ascertain if sugar in the form of sweets can initiate caries with a non-caries-producing diet, or accelerate the progress of decay with a caries-producing diet. Some investigators are of the opinion that the high caries incidence rate in the big cities is caused partly by high sugar consumption. The sweets used were Lion Creamy Toffees, ground into small pieces in a mortar and pestle. Four to five teaspoonsful of this were added daily to the caries and non-caries producing diets of each group. From the clinical survey it was noticed that the caries incidence rate among the children living in the citrus-growing areas was high, and the rats in groups VII and VIII were therefore placed on a caries and non-caries producing diet with orange juice to determine its effect on rat molars and caries. In preliminary experiments it was ascertained how rats would react to orange juice. Both tap water and orange juice were given simultaneously in two separate bottles to ten rats. After a few weeks it was found that the rats preferred the orange juice to the water and that they thrived on it. All the rats in groups VII and VIII were given only orange juice to drink and all survived the 80 days experimental period. The pH of the orange juice was found to range from 3·5 to 3·7. Meat was added to the caries-producing diet of rats in group IX to establish its effect on caries. Some investigators are of the opinion that the chewing of meat has a detergent action on the teeth, and may be associated with a lower caries incidence rate among people who eat much meat. The meat, mostly roast beef, was given *ad lib.* in pieces the size of a walnut. To determine the inhibitory effect of fluorine on dental caries in the rat, the rats in group X were placed on a caries-producing diet with drinking water containing approximately 5 p.p.m. fluorine as sodium fluoride, and those in group XI on a caries-producing diet with drinking water containing approximately 100 p.p.m. fluorine as sodium fluoride.

(*b*) Pre-natal Experiments

Female rats were placed on the following diets after copulation and during lactation to determine the effect on caries in their offspring:—

Group.	Diet.	Water.
XII	Caries-producing	100 p.p.m. fluorine.
XIII	High carbohydrate, low protein	Tap.
XIV	Low carbohydrate, high protein	Tap.

In the high carbohydrate and low protein diet the coarse corn meal was increased to 72 parts and the casein reduced to three parts, while in the low carbohydrate and high protein diet, the coarse corn meal was reduced to 35 parts and the casein increased to 40 parts. In addition the protein intake was augmented by meat. From the litters of each group, twenty-five rats, 35 days old, were selected and placed on the stock caries-producing diet for 80 days. The offspring of the first group served as controls.

(*c*) Heredity and Pre-natal Experiments

To ascertain whether heredity in conjunction with the feeding of high and low carbohydrate diets and fluorine during pregnancy and lactation can influence caries susceptibility in the rat, the following experiments were conducted.

A number of male and female rats, 35 days old, were placed on the stock caries-producing diet for 100 days. Their mouths were then examined for caries and those with the worst caries were selected for breeding. Five to seven females were mated with one male. The females, offspring of each litter, were then mated with a male

of the same litter. This was repeated. It was found that in the fourth generation a number of the females was sterile. Those not sterile were mated with a litter mate and left on the stock caries-producing diet. Of the different litters 25 were selected when 35 days of age and placed on the caries-producing diet for 80 days as controls. (Group XV.) When the mothers of the fourth generation were mated a second time they were placed on the high carbohydrate and low protein diet during pregnancy and lactation. Many of the offspring were eaten by their mothers. Two groups of 25 each were selected from those which survived. At 35 days of age, one group (XVI) was placed for 80 days on a non-caries-producing diet with added carbohydrates in the form of boiled sweet potatoes to ascertain if this diet could initiate caries. Those in the second group (XVII) were placed for 80 days on a non-caries-producing diet with added carbohydrates in the form of sweets. These mothers of the fourth generation were mated a third time, and were placed on the high carbohydrate and low protein diet during gestation and lactation. Many of the offspring were again eaten by their mothers. Of their offspring 25 rats were selected and at 35 days of age were placed, for 80 days, on the caries-producing diet (group XVIII). When these mothers were mated a fourth time they were placed on the low carbohydrate and high protein diet during gestation and lactation. Of their offspring, which all survived, 25 rats were selected and, at 35 days of age, were placed on the caries-producing diet for 80 days (group XIX). When the mothers were mated a fifth time, they were placed on the high carbohydrate low protein diet with drinking water containing approximately 100 p.p.m. fluorine as sodium fluoride, during pregnancy and lactation. Many of the offspring were again eaten by the mothers. Of the survivors 25 were selected and, at 35 days of age, were placed on the caries-producing diet for 80 days (group XX). When the mothers were mated for the sixth time, they were placed on the low carbohydrate and high protein diet with drinking water containing 100 p.p.m. fluorine. Of their offspring, most of which survived, 25 rats were selected and, at 35 days of age, were placed on the caries-producing diet for 80 days (group XXI).

All the rats on the experimental diets were weighed at the beginning and end of the 80-day period. After the rats were sacrificed the jaws were removed and preserved in 10 per cent. formalin for future examination.

Recording of Caries

In order to facilitate the examination of cavities and determining the extent of caries, numerous stains and methods were first tried out (Schamp and Leicester, 1943; Gomori, 1940, etc.). Of all the stains used, methylene blue proved the most successful. The dissected upper and lower jaws were first cleared of soft tissue, stained with a 1 per cent. solution of methylene blue for a few minutes and then left to dry. The cavities and food debris in the grooves and fissures are stained a dark blue, while the unaffected enamel and dentine remain white. Before examination under the dissecting microscope, the occlusal surfaces of the teeth were first cleaned with a Robinson brush and as much as possible of the food debris removed. The cavities were explored with a very fine needle, held in a broach holder. The following system of scoring caries was used, the numbers indicating the extent of the decay:—

1..... Small cavity affecting the enamel and dentine.
2..... Large cavities affecting the enamel, dentine and pulp.
3..... Half of the crown destroyed by caries.
4..... More than half of the crown destroyed.
5..... Total crown destroyed.
6..... Total crown destroyed, and the decay extending into the roots.

This scoring gave a fairly accurate extent of the caries for comparison in the different groups. The caries index of each group was calculated by adding the number of points of each cavity in the group and then dividing this number by twenty-five.

After the examination of the teeth, the jaws were mounted on glass slides and each group photographed.

III. FINDINGS

1. CLINICAL SURVEY

(*a*) The Incidence of Dental Caries Among Schoolchildren

Table IV gives the number of European schoolchildren examined in each province and the *number with caries.*

Table IV

THE INCIDENCE OF DENTAL CARIES IN PROVINCES

Province.	No. of Children Examined.	No. of Children with Caries.	Per cent.
Cape	31,212	25,614	82
Natal	4,483	4,288	96
Orange Free State	11,192	9,192	82
Transvaal	31,676	28,032	88
Totals	78,563	67,063	85

These figures show that approximately 85 per cent. of the schoolchildren in South Africa suffer, or have suffered at some time, from one or more carious teeth.

The highest caries incidence rate was found among the children in Natal (96 per cent.), the figures for the Transvaal, Cape and Orange Free State being 88 per cent., 82 per cent. and 82 per cent. respectively.

Table V gives the number of children with caries in the regional divisions.

Table V

THE INCIDENCE OF DENTAL CARIES IN REGIONAL DIVISIONS

Regional Division.	No. of Children Examined.	No. of Children with Caries.	Per cent.
South-western Coastal	12,551	12,082	97
South-eastern Coastal	4,546	4,425	97
Karroo Cape Central	6,988	5,322	76
Highveld	38,047	33,840	89
Cape Thornveld	2,090	1,856	93
Transvaal Bushveld	8,424	6,610	78
North-West Cape	5,917	2,928	49

The highest caries incidence rate was found in the south-western and south-eastern coastal divisions (97 per cent.), while the lowest was found in the north-west Cape (49 per cent.). A map showing the distribution of dental caries in the regional divisions is found at the end of the text.

Table VI gives the number of children examined, the number of children with caries, etc., in 117 districts in South Africa:—

TABLE VI

THE INCIDENCE OF DENTAL CARIES IN DISTRICTS

Name of District.	No. of Children Examined.	No. with Caries.	Per cent.	No. of Children Examined in Urban Areas.	No. with Caries.	Per cent.	No. of Children Examined in Rural Areas.	No. with Caries.	Per cent.	Approx. per cent. Caries in District.
Aberdeen	225	168	75	142	105	74	74	40	54	61
Albert	392	313	80	153	129	84	156	118	76	83
Bredasdorp	470	462	98	202	201	99·5	213	209	98	99
Beaufort West	1,069	779	73	386	316	82	159	103	65	65
Caledon	335	334	99	191	191	100	87	87	100	100
Calvinia	947	431	45	154	87	56	304	118	39	43
Cape Town	1,712	1,661	97	1,185	1,167	98	—	—	—	—
Carnarvon	561	245	44	53	27	62	334	131	36	38
Cathcart	200	186	93	77	74	96	23	22	96	96
Clanwilliam	355	312	88	64	61	95	235	202	88	88
Colesberg	173	104	60	108	67	62	65	37	57	60
Cradock	725	571	79	250	201	80	188	130	69	75
De Aar	462	356	77	293	231	78	149	105	70	76
East London	468	453	97	319	308	97	—	—	—	97
George	842	807	96	343	343	100	499	464	93	96
Gordonia	72	20	28	—	—	—	72	20	28	28
Graaff-Reinet	557	443	80	239	204	85	121	81	67	67
Hanover	130	70	54	63	38	60	39	16	41	53
Hopetown	299	200	67	134	116	87	174	83	48	64
Humansdorp	1,664	1,631	98	137	137	100	1,347	1,327	98	98
Jansenville	423	293	69	—	—	—	196	129	67	67
Kenhardt	519	119	23	—	—	—	441	88	20	20
Kimberley	1,234	1,132	92	473	446	94	87	79	91	91
Kingwilliamstown	225	223	99	132	132	100	32	31	99	99
Knysna	673	667	99	332	330	99	341	337	99	99
Komgha	116	112	97	41	41	100	38	37	99	99
Kuruman	715	505	70	94	86	91	543	360	67	70
Laingsburg	289	226	78	104	89	86	107	67	63	74
Malmesbury	418	378	90	192	178	93	139	118	85	90
Maraisburg	223	188	76	76	73	96	126	76	60	73
Molteno	336	302	90	104	98	94	150	128	85	88
Mossel Bay	642	633	99	385	377	99	257	256	99	99
Murraysburg	242	131	54	54	32	59	121	61	50	53
Namaqualand	1,200	313	26	—	—	—	1,142	284	25	25
Oudtshoorn	882	828	94	245	240	98	424	384	90	93
Paarl	756	724	96	673	650	96	—	—	—	—
Pearston	226	168	74	67	59	88	100	66	66	75
Piquetberg	350	300	86	102	96	94	189	152	80	85
Port Elizabeth	1,794	1,741	97	1,057	1,045	99	—	—	—	99
Prieska	333	197	59	196	109	55	137	88	64	59
Prince Albert	172	121	70	63	55	87	75	44	60	72
Queenstown	521	497	95	239	232	97	57	54	95	96
Richmond	250	141	56	91	52	57	78	40	51	54
Riversdale	437	427	98	194	192	99	91	89	98	99
Sterkstroom	283	239	84	104	88	84	60	45	75	80
Steynsburg	186	145	78	79	66	84	60	45	75	80
Stockenstroom	79	78	99	—	—	—	—	—	—	99
Stutterheim	238	236	99	58	58	100	80	78	97	98
Sutherland	183	98	54	65	46	71	105	45	43	54
Swellendam	377	367	97	180	178	99	117	116	99	99
Uitenhage	1,497	1,439	96	673	664	99	272	254	94	97
Umtata	522	512	98	196	193	99	155	152	98	99
Uniondale	564	553	98	181	172	95	383	381	99	99
Van Rhynsdorp	570	408	71	96	69	72	456	339	74	74
Venterstad	120	83	69	39	30	77	42	28	67	71
Victoria West	279	196	70	100	75	75	76	55	72	74
Vryburg	664	521	80	205	177	86	289	209	72	78
Williston	257	114	44	48	33	69	144	48	33	42
Willowmore	352	257	73	78	73	94	201	153	76	81
Worcester	570	560	98	391	384	98	143	141	98	98

TABLE VI—(*continued*).

THE INCIDENCE OF DENTAL CARIES IN DISTRICTS

Name of District.	No. of Children Examined.	No. with Caries.	Per cent.	No. of Children Examined in Urban Areas.	No. with Caries.	Per cent.	No. of Children Examined in Rural Areas.	No. with Caries.	Per cent.	Approx. per cent. Caries in District.
NATAL										
Alfred	187	171	92	—	—	—	62	58	93	92
Durban	1,267	1,228	97	598	584	98	54	51	94	98
Eshowe	120	117	98	—	—	—	42	41	98	98
Ixopo	196	190	97	30	28	93	53	52	98	97
Lower Tugela	137	132	96	—	—	—	70	68	97	96
Lower Umfolosi	202	195	97	47	46	98	33	33	100	97
New Hanover	183	178	97	23	23	100	102	99	98	97
Pietermaritzburg	320	311	97	139	135	97	26	25	96	97
Polela	63	59	94	—	—	—	25	23	92	94
Port Shepstone	377	365	97	39	38	97	95	93	98	97
Richmond	67	66	99	—	—	—	29	29	100	99
Umzinto	178	172	97	—	—	—	24	23	96	97
Underberg	31	30	97	—	—	—	19	18	95	97
Vryheid	1,061	989	93	274	259	95	278	222	93	93
Weenen	92	85	92	43	43	100	—	—	—	92
ORANGE FREE STATE										
Bethulie	673	528	78	399	323	81	182	138	76	80
Bloemfontein	1,247	997	80	677	598	88	219	176	80	70
Bothaville	388	302	78	98	83	85	290	219	76	78
Brandfort	203	177	87	—	—	—	—	—	—	—
Dewetsdorp	329	231	70	200	138	69	129	93	72	70
Edenburg	90	67	74	—	—	—	—	—	—	—
Fauresmith	289	241	83	74	68	92	95	77	81	83
Harrismith	915	828	90	319	294	92	329	281	85	93
Heilbron	622	534	86	182	154	85	235	206	88	86
Kroonstad	307	213	69	—	—	—	307	213	69	69
Ladybrand	710	601	85	628	535	85	82	66	80	85
Phillipolis	268	193	72	99	78	79	95	67	71	74
Reitz	452	415	92	223	208	93	229	207	90	92
Rouxville	408	295	72	160	131	82	248	164	66	72
Senekal	337	266	79	48	96	81	125	102	82	81
Smithfield	371	290	78	186	159	85	82	60	73	79
Thaba 'Nchu	204	171	84	—	—	—	135	110	82	84
Trompsburg	236	172	73	123	97	79	113	75	67	82
Ventersburg	361	291	81	117	89	76	244	202	83	81
Vrede	361	334	93	131	124	95	155	143	86	93
Vredefort	256	218	86	48	43	89	156	137	88	88
Wepener	350	298	85	182	161	88	168	137	82	84
Winburg	874	713	81	209	182	87	264	225	85	84
Zastron	533	459	86	175	161	92	139	107	77	85
TRANSVAAL										
Barberton	349	330	95	26	26	100	192	180	94	94
Bloemhof	393	291	77	162	121	75	82	55	67	72
Christiana	436	362	83	166	145	87	41	38	81	86
Johannesburg	1,679	1,617	96	887	858	97	—	—	—	—
Klerksdorp	853	777	91	359	328	91	125	111	89	91
Lichtenburg	635	542	85	227	206	90	217	168	78	84
Lydenburg	547	510	93	157	151	96	275	254	93	94
Marico	1,021	824	81	212	174	82	604	494	81	81
Nelspruit	311	283	91	65	62	95	97	86	87	91
Pietersburg	1,293	1,020	79	217	177	82	804	608	76	76
Potchefstroom	1,090	968	89	457	413	90	277	236	85	88
Potgietersrust	1,914	1,426	75	225	204	91	1,102	741	67	67
Pretoria	15,041	14,149	94	14,057	13,457	96	957	763	80	80
Rustenburg	2,193	1,697	77	277	247	90	1,326	973	73	69
Schweizer Reneke	358	290	81	116	101	87	180	146	81	84
Standerton	410	367	90	113	108	96	103	83	81	93
Vereeniging	254	238	93	—	—	—	—	—	—	93
Waterberg	1,661	1,302	79	103	98	95	1,126	819	77	77
Zoutpansberg	1,338	1,039	78	299	271	90	753	498	66	66

This table shows that the incidence of dental caries varies from 20 per cent. in the Kenhardt district to 100 per cent. in the Caledon district. Although it was found that the caries incidence rate was 100 per cent. in Caledon, the highest D.M.F.* teeth per child was found among the children in the George, Humansdorp and Knysna districts, where the caries incidence rate was 96 per cent., 98 per cent. and 99 per cent. respectively.

A map showing the distribution of the incidence of dental caries in the districts will be found at the end of the text.

Table VII gives the incidence of dental caries among children in the urban and rural areas in the four provinces:—

TABLE VII

INCIDENCE OF DENTAL CARIES AMONG CHILDREN IN URBAN AND RURAL AREAS

Province.	No. of Children in Urban Areas Examined.	No. with Dental Caries.	Per Cent.	No. of Children in Rural Areas Examined.	No. with Dental Caries.	Per Cent.
Cape	11,832	10,868	92	11,466	9,039	79
Natal	1,193	1,156	97	852	817	96
Orange Free State	4,417	3,783	86	3,820	3,052	80
Transvaal	18,123	17,147	95	8,267	6,253	76
TOTALS	35,565	32,954	93	24,405	19,161	79

From this table it will be seen that the incidence of dental caries is invariably higher among the urban children (93 per cent.) than among the rural children (79 per cent.).

Table VIII gives the number of children (boys and girls) examined and the number of children with caries in the three age groups of 6 to 8, 9 to 11, and 12 to 14 years.

TABLE VIII

INCIDENCE OF DENTAL CARIES IN AGE GROUPS

Age Groups.	No. Examined.	No. with Caries.	Per cent.	Age Groups.	No. Examined.	No. with Caries.	Per cent.
6–8	13,739	12,142	88·4	12–14	18,681	14,815	79·3
Boys	7,100	6,308	88·8	Boys	9,566	7,476	78·2
Girls	6,639	5,834	87·9	Girls	9,115	7,339	80·5
9–11	19,314	16,571	85·8	All ages	51,734	43,528	84·0
Boys	10,129	8,737	86·3	Boys	26,795	22,521	84·1
Girls	9,185	7,834	85·3	Girls	24,939	21,007	84·2

This table shows that the percentage number of children with caries varies to some extent in the three age groups, namely, 88·4 per cent. in the 6 to 8 group, 85·8 per cent. in the 9 to 11 group, and 79·3 per cent. in the 12 to 14 group.

* Decayed, missing, filled.

Table IX gives the analysis of the caries data in the three age groups in the four provinces.

TABLE IX

Age Groups.	No. Examined.	No. with Caries.	Per cent.	Age Groups.	No. Examined.	No. with Caries.	Per cent.
CAPE				ORANGE FREE STATE			
6–8	6,254	5,535	88·5	6–8	2,234	1,926	86·2
Boys	3,197	2,857	89·4	Boys	1,145	986	86·1
Girls	3,057	2,678	87·6	Girls	1,089	940	86·3
9–11	8,387	7,163	85·4	9–11	3,044	2,507	82·3
Boys	4,215	3,605	85·5	Boys	1,538	1,278	83·1
Girls	4,172	3,558	85·3	Girls	1,506	1,229	81·6
12–14	8,631	6,657	77·1	12–14	3,050	2,403	78·8
Boys	4,368	3,350	76·7	Boys	1,496	1,139	76·1
Girls	4,263	3,307	77·6	Girls	1,554	1,264	81·3
NATAL				TRANSVAAL			
6–8	991	945	95·4	6–8	4,260	3,726	87·7
Boys	575	551	95·8	Boys	2,183	1,914	87·7
Girls	416	394	94·7	Girls	2,077	1,822	87·7
9–11	1,518	1,460	96·2	9–11	6,365	5,441	85·5
Boys	976	937	96·0	Boys	3,400	2,917	85·8
Girls	542	523	96·4	Girls	2,965	2,524	85·1
12–14	1,467	1,405	95·8	12–14	5,533	4,350	78·6
Boys	788	747	94·8	Boys	2,914	2,240	76·9
Girls	679	658	96·9	Girls	2,619	2,110	80·6

Table X gives an analysis of the caries data based on the examination of 2,314 European children living in the north-west Cape. The percentage of children affected by dental caries is 40 per cent., which is the lowest in South Africa, and means that only 40 out of every 100 children examined suffer, or have suffered at some time, from one or more carious teeth.

TABLE X

Age Groups.	No. Examined.	No. with Caries.	Per cent.	Age Groups.	No. Examined.	No. with Caries.	Per cent.
6–8	513	282	55·0	12–14	950	274	28·8
Boys	257	147	57·2	Boys	475	132	27·8
Girls	256	135	52·7	Girls	475	142	29·9
9–11	851	361	42·4	All ages	2,314	917	39·6
Boys	434	186	42·9	Boys	1,166	465	39·9
Girls	417	175	42·0	Girls	1,148	452	39·4

This table also shows conclusively that the number of children affected by dental caries decreases very considerably in the older age groups, from 55 per cent. in the 6 to 8 age group to 42 per cent. in the 9 to 11 group, and to 29 per cent. in the 12 to 14 age group.

Table IX gives the analysis of the caries data in the three age groups in the four provinces.

TABLE IX

Age Groups.	No. Examined.	No. with Caries.	Per cent.	Age Groups.	No. Examined.	No. with Caries.	Per cent.
CAPE				ORANGE FREE STATE			
6–8	6,254	5,535	88·5	6–8	2,234	1,926	86·2
Boys	3,197	2,857	89·4	Boys	1,145	986	86·1
Girls	3,057	2,678	87·6	Girls	1,089	940	86·3
9–11	8,387	7,163	85·4	9–11	3,044	2,507	82·3
Boys	4,215	3,605	85·5	Boys	1,538	1,278	83·1
Girls	4,172	3,558	85·3	Girls	1,506	1,229	81·6
12–14	8,631	6,657	77·1	12–14	3,050	2,403	78·8
Boys	4,368	3,350	76·7	Boys	1,496	1,139	76·1
Girls	4,263	3,307	77·6	Girls	1,554	1,264	81·3
NATAL				TRANSVAAL			
6–8	991	945	95·4	6–8	4,260	3,726	87·7
Boys	575	551	95·8	Boys	2,183	1,914	87·7
Girls	416	394	94·7	Girls	2,077	1,822	87·7
9–11	1,518	1,460	96·2	9–11	6,365	5,441	85·5
Boys	976	937	96·0	Boys	3,400	2,917	85·8
Girls	542	523	96·4	Girls	2,965	2,524	85·1
12–14	1,467	1,405	95·8	12–14	5,533	4,350	78·6
Boys	788	747	94·8	Boys	2,914	2,240	76·9
Girls	679	658	96·9	Girls	2,619	2,110	80·6

Table X gives an analysis of the caries data based on the examination of 2,314 European children living in the north-west Cape. The percentage of children affected by dental caries is 40 per cent., which is the lowest in South Africa, and means that only 40 out of every 100 children examined suffer, or have suffered at some time, from one or more carious teeth.

TABLE X

Age Groups.	No. Examined.	No. with Caries.	Per cent.	Age Groups.	No. Examined.	No. with Caries.	Per cent.
6–8	513	282	55·0	12–14	950	274	28·8
Boys	257	147	57·2	Boys	475	132	27·8
Girls	256	135	52·7	Girls	475	142	29·9
9–11	851	361	42·4	All ages	2,314	917	39·6
Boys	434	186	42·9	Boys	1,166	465	39·9
Girls	417	175	42·0	Girls	1,148	452	39·4

This table also shows conclusively that the number of children affected by dental caries decreases very considerably in the older age groups, from 55 per cent. in the 6 to 8 age group to 42 per cent. in the 9 to 11 group, and to 29 per cent. in the 12 to 14 age group.

(*b*) Chronic Endemic Dental Fluorosis

The following table gives the number of endemic fluorosis areas found in South Africa, the number of children with mottled enamel, and the number of children with mottling and caries.

Table XI

Province.	No. of Endemic Areas.	No. of Children with Mottling.	No. of Children with Caries.	Per cent.	No. of Children with Mottling of Deciduous Teeth.
Cape	580	2,183	457	21	25
Transvaal	144	506	218	43	9
Orange Free State	81	378	198	52	4
Natal	0	0	0	0	0
Totals	805	3,067	873	28	38

A map showing the endemic fluorosis areas in South Africa will be found at the end of the text.

Of the 3,067 children with mottled enamel, 1,686, or 55 per cent., were boys and 1,381, or 45 per cent., were girls. It will be noted that no endemic fluorosis areas and no cases of mottled enamel were found in Natal. Of 9,806 children with no mottling examined in the endemic areas 6,749, or 69 per cent., had caries. Figs. 1 to 4 show mottling, pitting and hypoplasia found in the endemic areas.

Fig. I. Mild mottling.

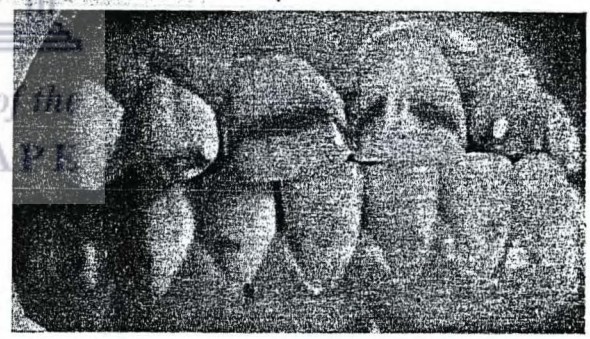


Fig. II. Moderate mottling.

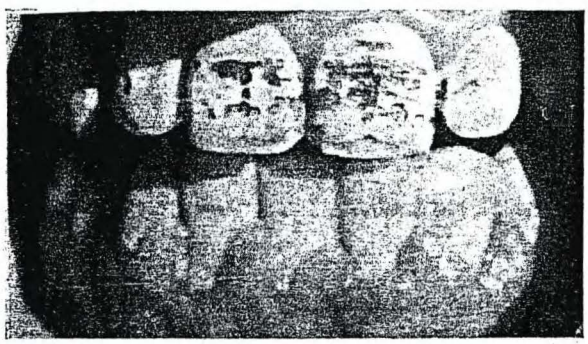


Fig. III. Pitting.

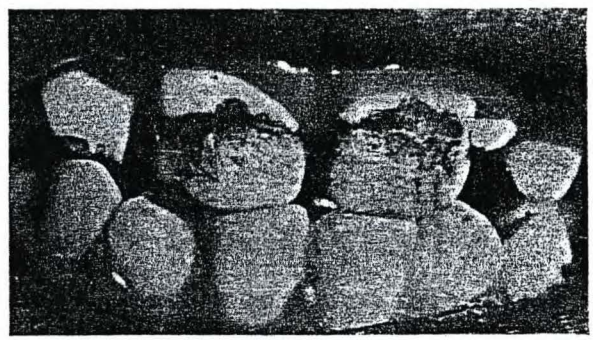


Fig. IV. Hypoplasia.

(*c*) THE CHEMICAL COMPOSITION OF TEETH OF EUROPEAN CHILDREN IN DIFFERENT AREAS IN SOUTH AFRICA

The following table XII gives the percentage CaO, MgO, P_2O_5 and F p.p.m. of carious and non-carious enamel and dentine of teeth in children in different areas.

The percentage incidence of dental caries and the approximate fluorine content in parts per million of the drinking water is also given.

The details are given on page 64.

TABLE XII

District.	Incidence of Dental Caries.	F Content Water.	Carious Enamel.				Non-Carious Enamel.			
			CaO.	MgO.	P_2O_5.	F.	CaO.	MgO.	P_2O_5.	F.
	Per cent.	p.p.m.	Per cent.	Per cent.	Per cent.	p.p.m.	Per cent.	Per cent.	Per cent.	p.p.m.
Calvinia	43	1·30	—	—	—	—	51·20	0·72	40·1	250
Carnarvon	38	1·12	—	—	—	—	48·7	0·69	39·7	660
Humansdorp	98	0·17	50·99	0·71	N.D.	130	51·21	0·77	N.D.	165
Kenhardt	20	3·25	—	—	—	—	49·27	0·77	N.D.	750*
Knysna	99	0·30	50·49	0·71	40·6	160	50·46	0·72	N.D.	130
Potchefstroom	88	0·26	49·30	0·58	39·4	120	50·2	0·70	40·7	70
Sutherland	54	2·70	—	—	—	—	50·3	0·68	39·9	175
Waterberg	77	0·80	49·6	0·70	39·7	200	49·90	0·79	40·6	146
							49·7	0·50	35·4	1,104*

District.	Incidence of Dental Caries.	F Content Water.	Carious Dentine.				Non-Carious Dentine.			
			CaO.	MgO.	P_2O_5.	F.	CaO.	MgO.	P_2O_5.	F.
	Per cent.	p.p.m.	Per cent.	Per cent.	Per cent.	p.p.m.	Per cent.	Per cent.	Per cent.	p.p.m.
Calvinia	43	1·30	—	—	—	—	37·7	2·18	29·2	500
Carnarvon	38	1·12	—	—	—	—	38·2	1·62	30·6	953
Humansdorp	98	0·17	37·73	1·53	29·8	222	38·83	1·59	30·0	255
Kenhardt	20	3·25	—	—	—	—	39·35	1·30	N.D.	1,700*
Knysna	99	0·30	38·74	1·51	30·9	296	38·10	1·50	N.D.	320
Potchefstroom	88	0·26	38·10	1·36	29·9	216	37·2	1·41	29·2	365
Sutherland	54	2·70	—	—	—	—	38·1	1·11	29·4	240
Waterberg	77	0·80	39·10	1·40	30·4	205	38·8	1·43	30·5	240
							36·5	2·10	27·4	2,347*

* Mottled teeth. N.D.: Not determined.

The results of these chemical analyses of the carious and non-carious enamel and dentine from several areas show no significant differences in the percentage CaO, MgO and P_2O_5.

It was found that mottled enamel and dentine contain more fluorine than normal enamel and dentine:—

Mottled enamel.....	0·075 per cent.	Normal enamel.....	0·02 per cent.
Mottled dentine.....	0·17 per cent.	Normal dentine.....	0·026 per cent.

(*d*) THE pH AND MINERAL CONTENT OF DRINKING-WATER IN DIFFERENT AREAS IN SOUTH AFRICA

The pH values, fluorine and total hardness of a number of places and districts are given on page 65, etc. They indicate that the reaction of the majority of the waters is alkaline, of a few, neutral, and of those from the southern coastal areas generally, acid. The drinking waters with a low mineral content are usually acid, while those with a high mineral content are usually alkaline.

The drinking water in the high-caries incidence areas of the southern coastal region, mostly obtained from springs and streams, especially in the Knysna, Humansdorp and George districts, are very acid, some having a pH value as low as 4·5. The mineral content of drinking waters in the more arid regions, such as the north-west Cape, Northern Transvaal, etc., is very much higher than of those in the higher rainfall areas. The water in these areas is mostly obtained from boreholes. The total hardness is also v ry much higher in the low rainfal areas than in the high rainfall areas. Statistical analysis of the caries data will be found on page 68.

(*e*) Dental Caries and Diet

The lowest caries incidence rate was found among the children living in the sheep-raising areas of the Karroo, the north-west Cape and northern Transvaal, where meat and bread form the principal items of their diet (Fig. V).

Fig. V. The diet, consisting mostly of meat and bread, of children in the north-west Cape.

Meat is eaten three times a day, and their wholemeal bread is made from locally-grown wheat and usually ground in small wind-driven mills (Fig. VI).

Many families in the north-west Cape, especially in Namaqualand, lead a nomadic life, trekking from place to place in their donkey wagons, seeking grazing for their sheep. While on trek they live in their wagons, but when they have found grazing, they live in a " hartebeesthuisie " made of reeds until the grazing is exhausted, after which the same process is repeated.

Until recently meat (mostly game) was the principal item of the diet of the people living in the northern Transvaal. Now, because of the increasing scarcity of the game, this no longer holds and more carbohydrates have taken its place. The teeth and jaws of the children living in the low caries areas are well developed, strong and clean in appearance. The teeth are lustrous and of a light-cream colour (Figs. VII and VIII). The cusps are low and flat. The grooves and fissures are shallow and exhibit no defective enamel structure.

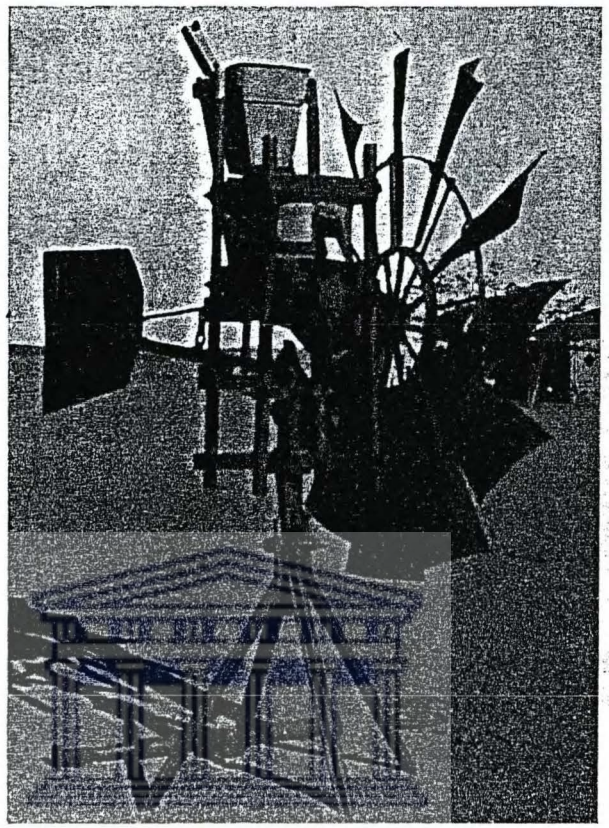


Fig. VI. Wind-driven mill, used for grinding wheat in the north-west Cape.

The highest caries incidence rate was found among the children living in the George, Humansdorp, Uniondale and Knysna districts, where bread and sweet potatoes form the principal items of their diet. Very little meat is eaten and sweet potatoes are eaten three times a day. (Fig. IX.) Schoolchildren were seen eating sweet potatoes, with bread during playtime. The teeth of the children in these districts have a dull white, chalky appearance. The cusps are high and sharp. The grooves and fissures are deep and often show a defective enamel structure.

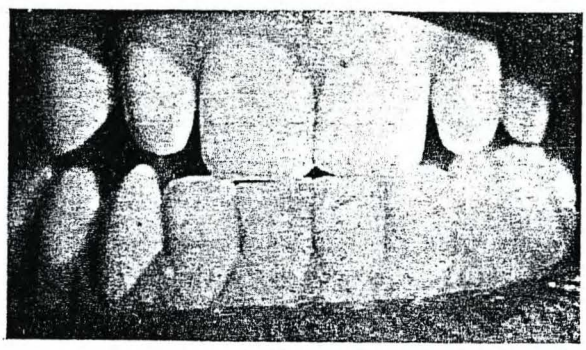


Fig. VII.

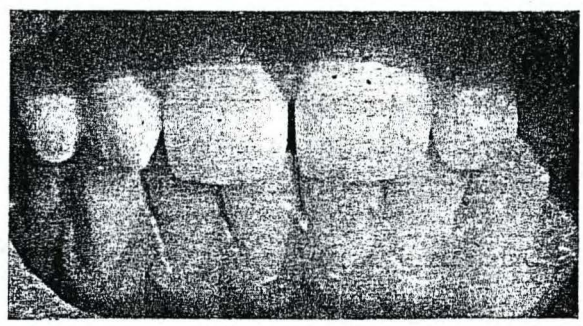


Fig. VIII.

Figs. VII and VIII. Typical caries-free teeth of children living in the north-west Cape.

Dental caries is rampant, and one is impressed by the pathetic sight of wide gaps in the anterior part of the mouths of so many young children when they talk or laugh. There are many interproximal cavities in the anterior lower teeth. Figs. X to XIII show typical mouths of children living in these areas.

The permanent upper and lower anterior teeth have, in many instances, been extracted while quite young. the majority of the people become edentulous before they reach the age of 15 years and, wearing no dentures, they provide a queer and comical sight when nose and chin nearly meet during masticatory, laughing and talking movements.

Fig. IX. Usual diet of children in Knysna and George areas. Note sweet potatoes, rice and black coffee.

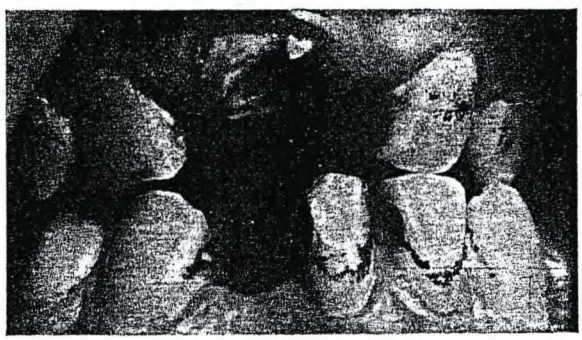


Fig. X.

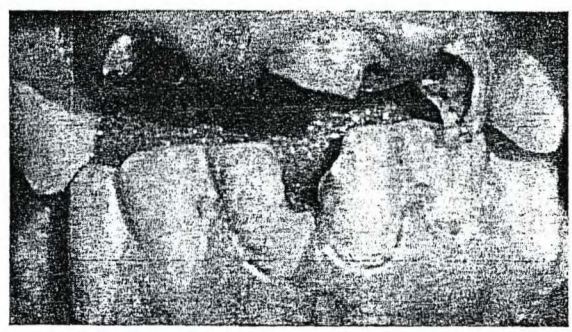
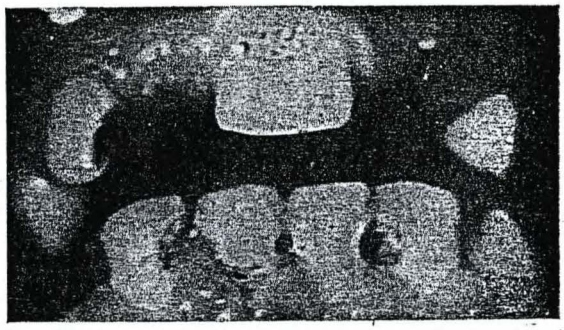
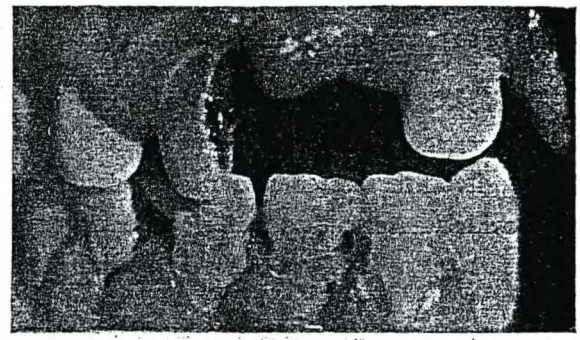


Fig. XI.

As the urban children have easier access to sweets and cakes than the rural children, the consumption of these by the urban children is higher than by the rural children. From the dietary investigation it was found that the consumption of carbohydrates is high and proteins low in the cities, towns and southern Cape coastal areas, while in the Karroo, north-west Cape and Northern Transvaal the consumption of carbohydrates is low and proteins high.





Figs. X to XIII. Typical carious teeth in mouths of children in the south Cape coastal areas.

(*f*) The Incidence of Dental Caries in some of the Citrus-growing Areas in South Africa

The following table XIII gives the incidence of dental caries in some of the citrus-growing areas:—

Table XIII

Area.	No. of Children Examined.	No. of Children with Caries.	Per cent.	Area.	No. of Children Examined.	No. of Children with Caries.	Per cent.
Transvaal				Cape.			
Brits, Rustenburg	362	339	94				
Barberton	271	265	98	Gamtoos Valley	363	351	97
Nelspruit	591	580	97	Uniondale	383	376	98
White River	312	302	97	Stockenstroom	158	156	98
				Uitenhage	272	254	97

2. EXPERIMENTAL WORK

(*a*) Post-natal Investigations

Table XIV shows the effect of the different diets on rat molar teeth of each group of 25 rats:—

Table XIV

Group.	Diet.	No. of Rats with Caries.	No. of Molars with Caries.	No. of Upper Molars with Caries.	No. of Lower Molars with Caries.	Caries Index.
I	Non-caries	None	None	None	None	Nil
II	Caries	19	76	None	76	5·7
III	Non-caries and sweet potatoes	None	None	None	None	Nil
IV	Caries and sweet potatoes	22	92	8	84	11
V	Non-caries and sweets	10	20	None	20	1·2
VI	Caries and sweets	25	116	None	116	9·6
VII	Non-caries and orange juice	None	None	None	None	Nil
VIII	Caries and orange juice	24	88	None	88	8·0
IX	Caries and meat	22	62	5	57	4·4
X	Caries and 5 p.p.m.	24	77	None	77	4·3
XI	Caries and 100 p.p.m.	2	2	None	2	0·1

Figs. XIV to XXIV are the photographs of the upper and lower molars of the rats of the different groups.

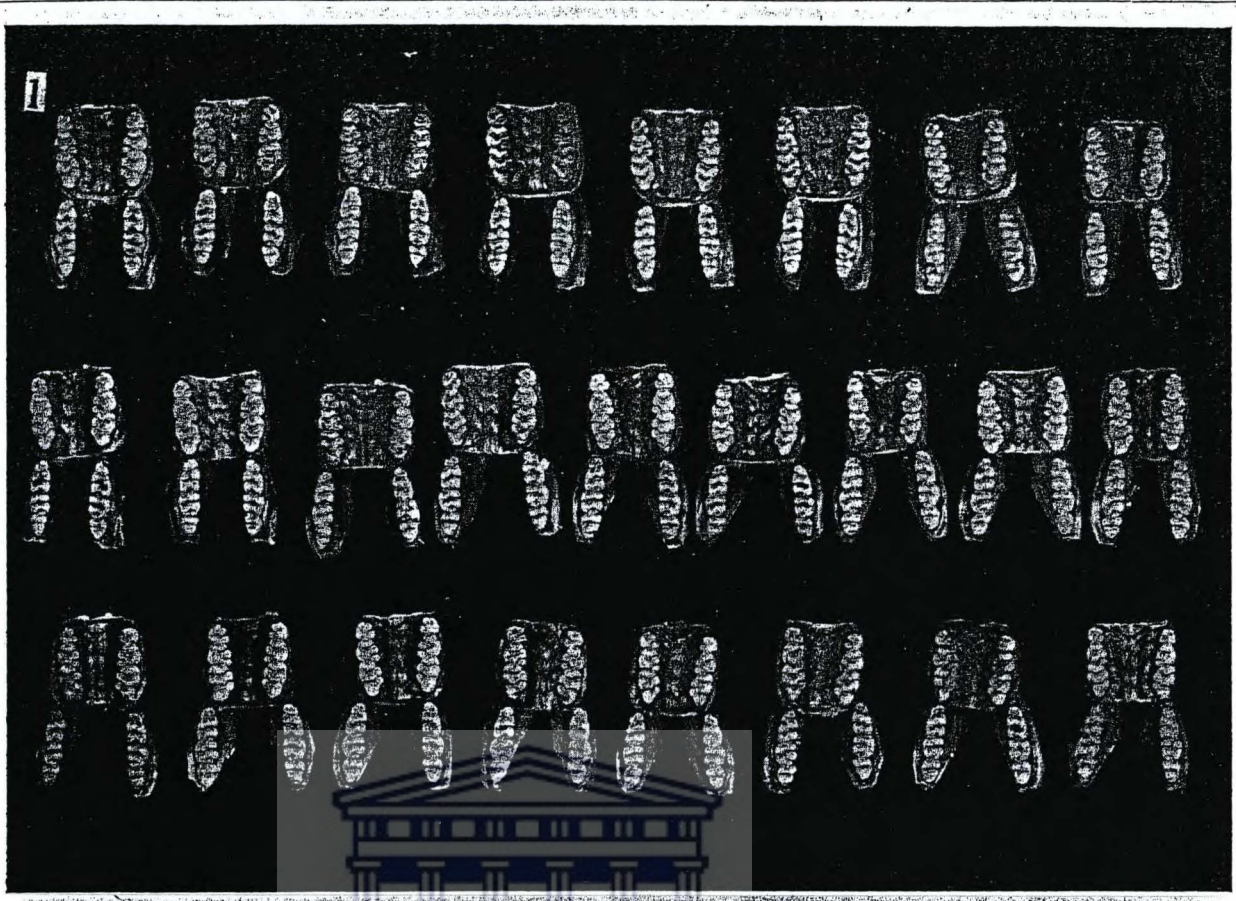


Fig. XIV. Molars of rats in group I showing no caries.

Fig. XV. Molars of rats in group II showing extensive caries in lower molars.

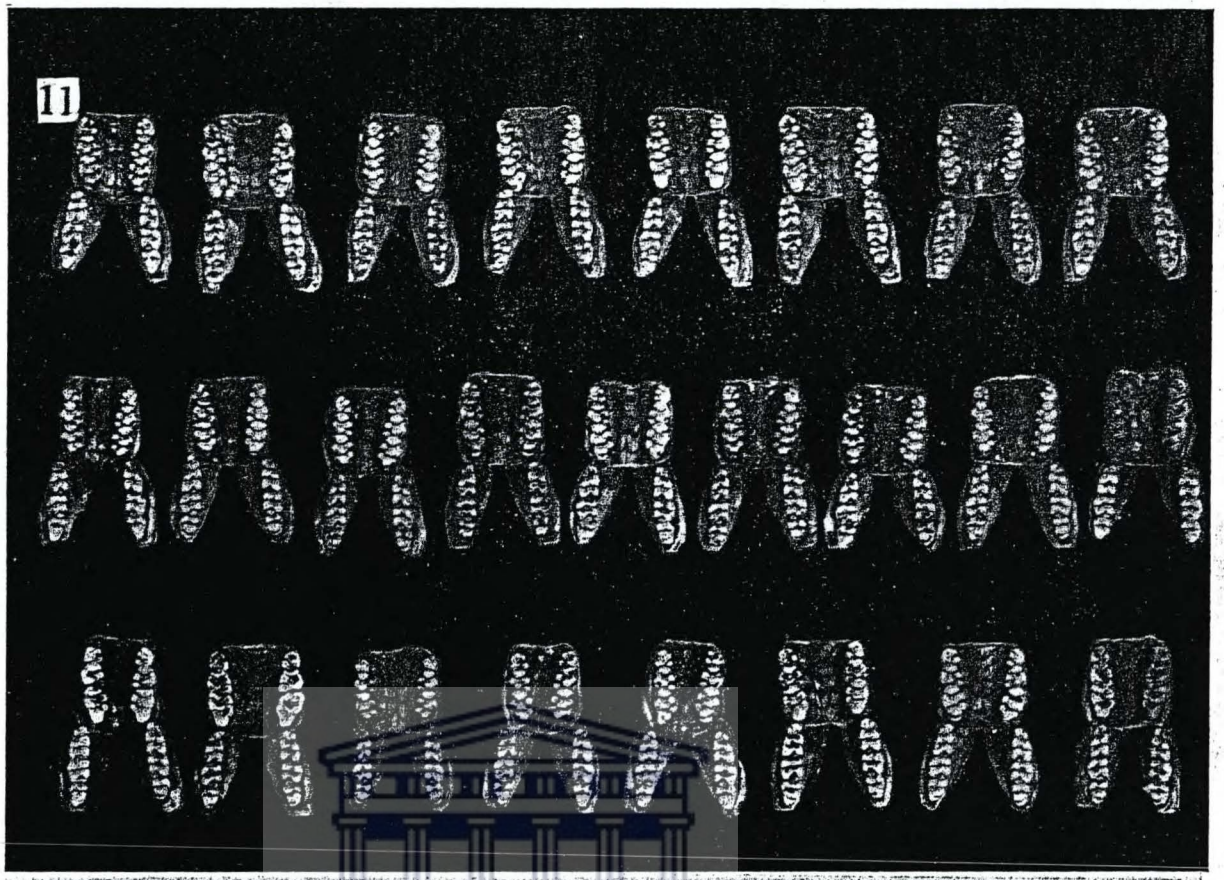


Fig. XVI. Molars of rats in group III showing no caries.

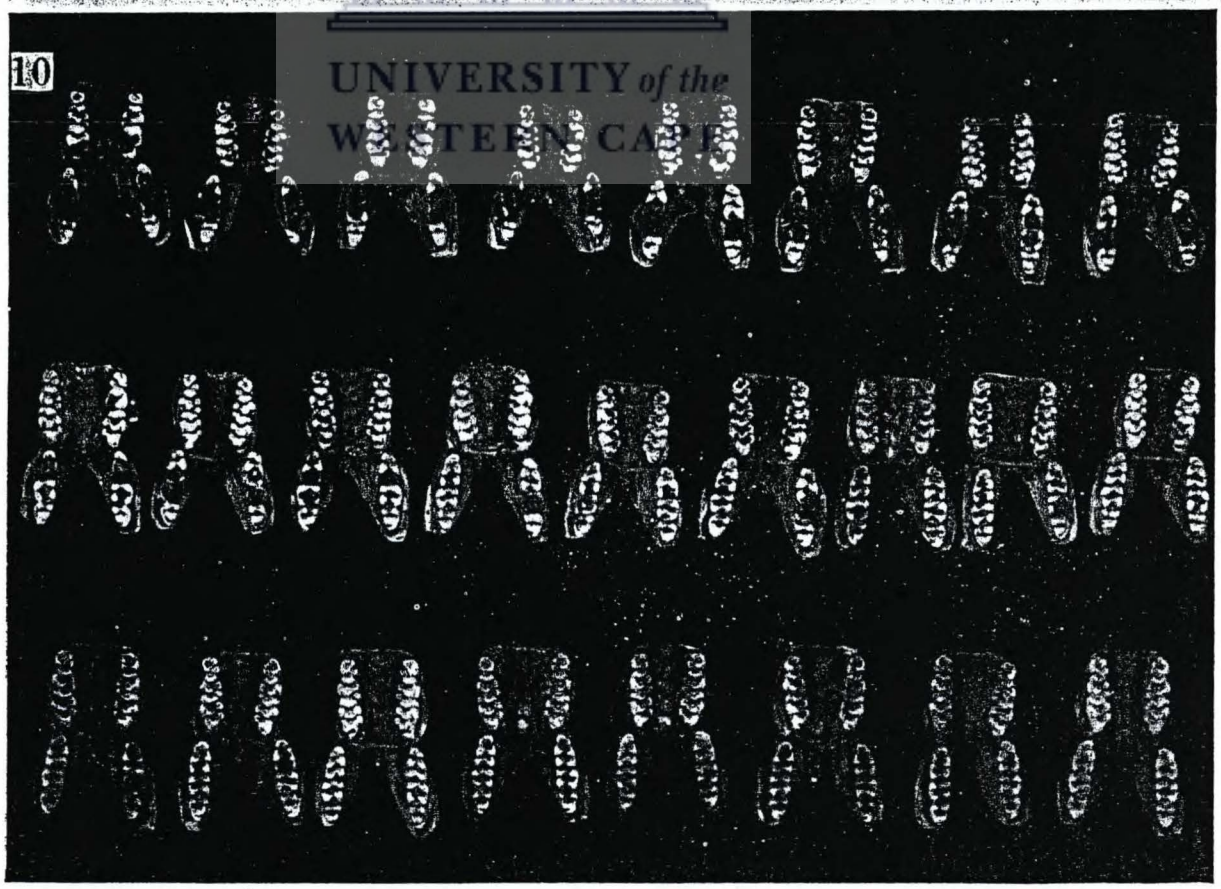


Fig. XVII. Molars of rats in group IV showing extensive caries in lower molars and in some upper molars.

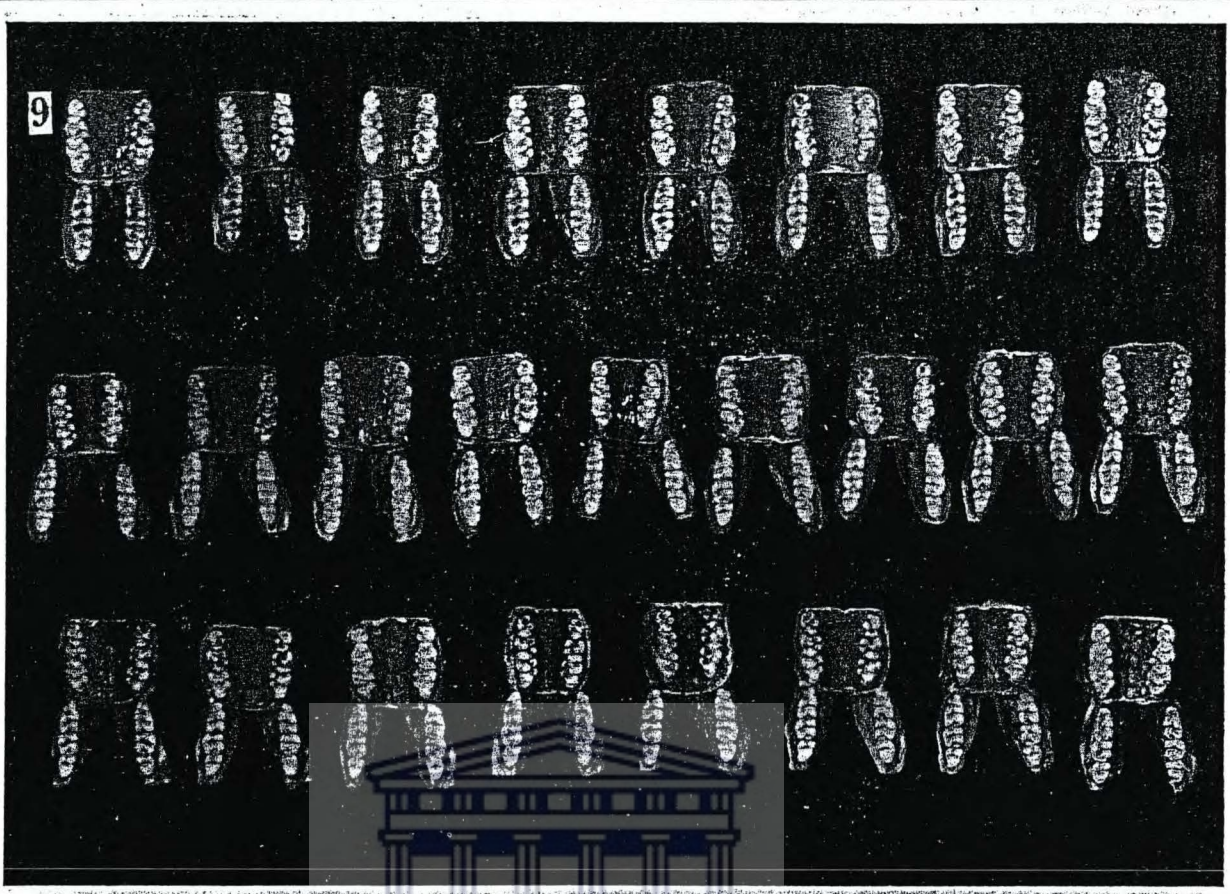


Fig. XVIII. Molars of rats in group V showing slight caries in lower molars.

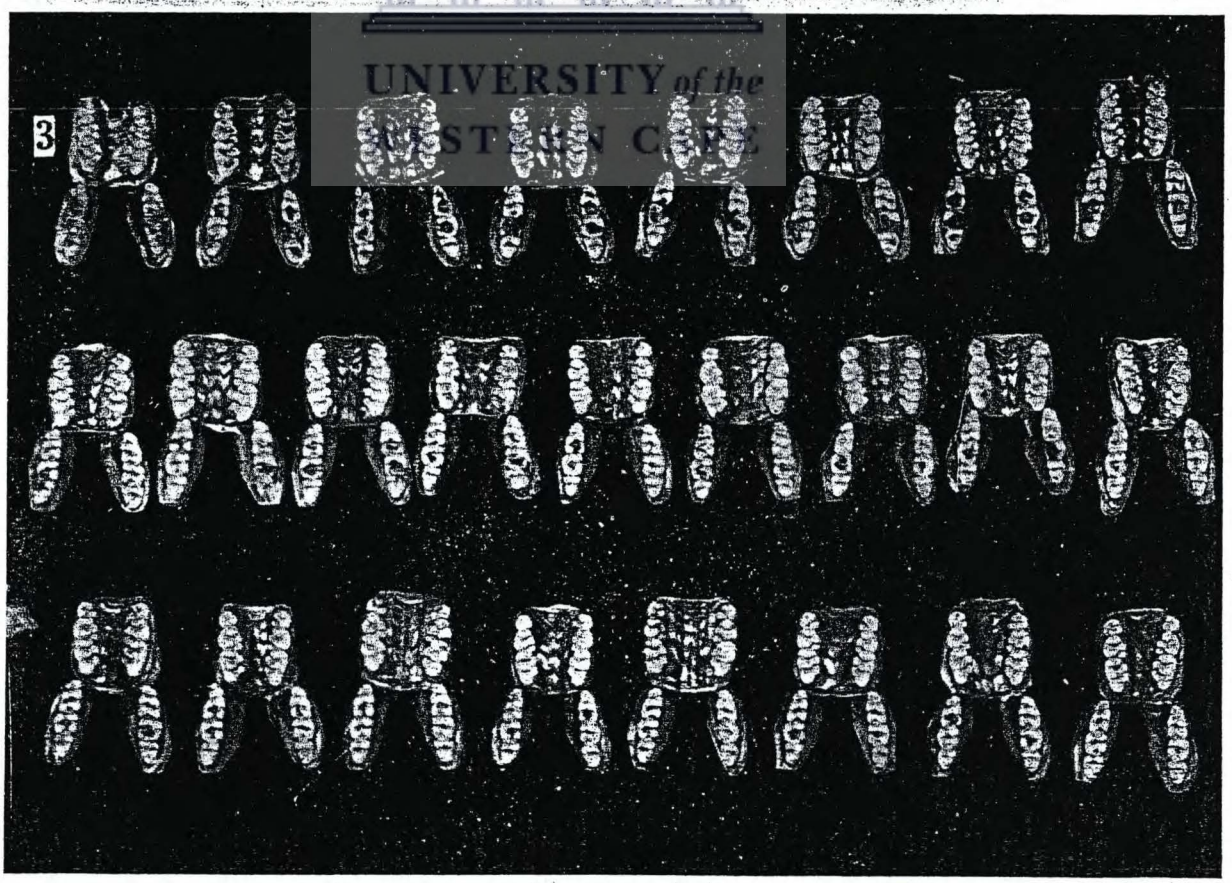


Fig. XIX. Molars of rats in group VI showing extensive caries in lower molars.

Fig. XX. Molars of rats in group VII showing extensive destruction of enamel but no caries.

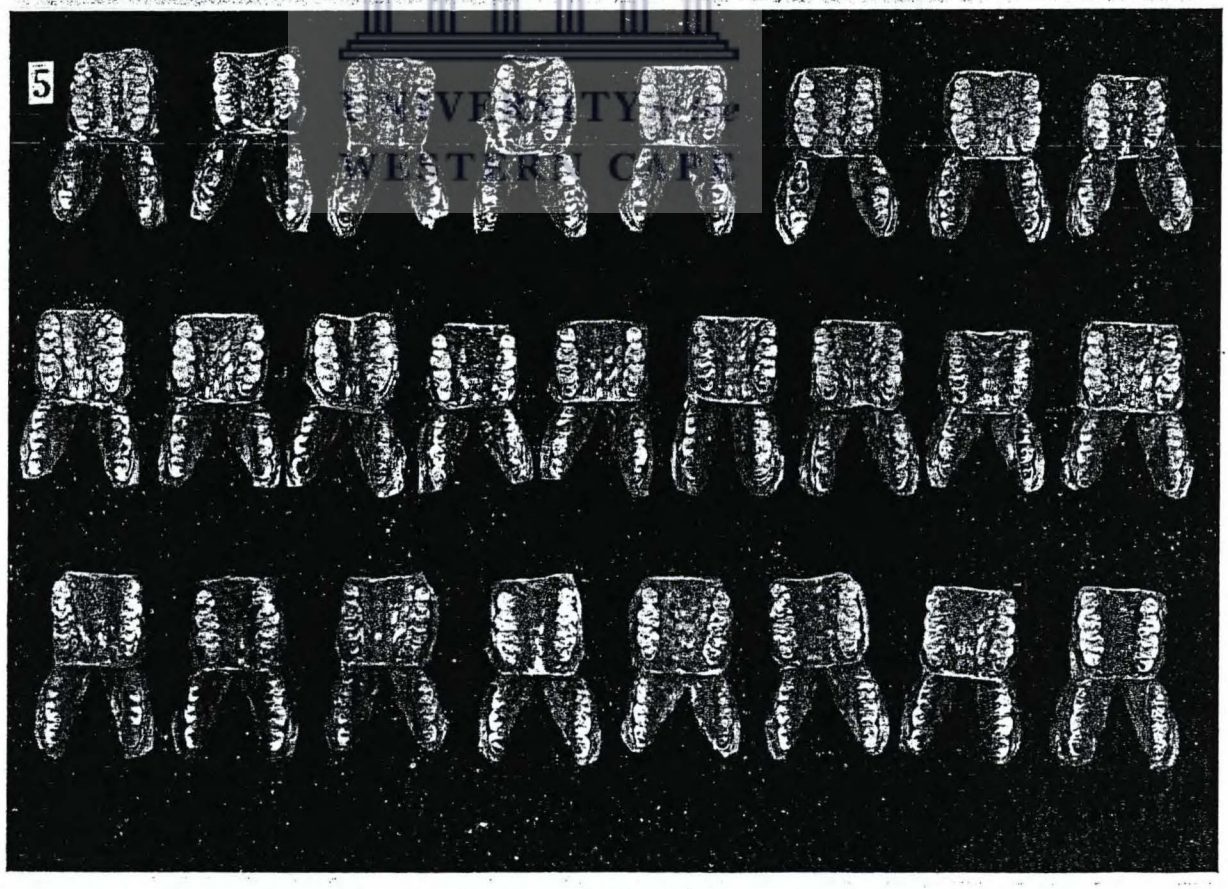


Fig. XXI. Molars of rats in group VIII showing extensive destruction of enamel and caries in lower molars.

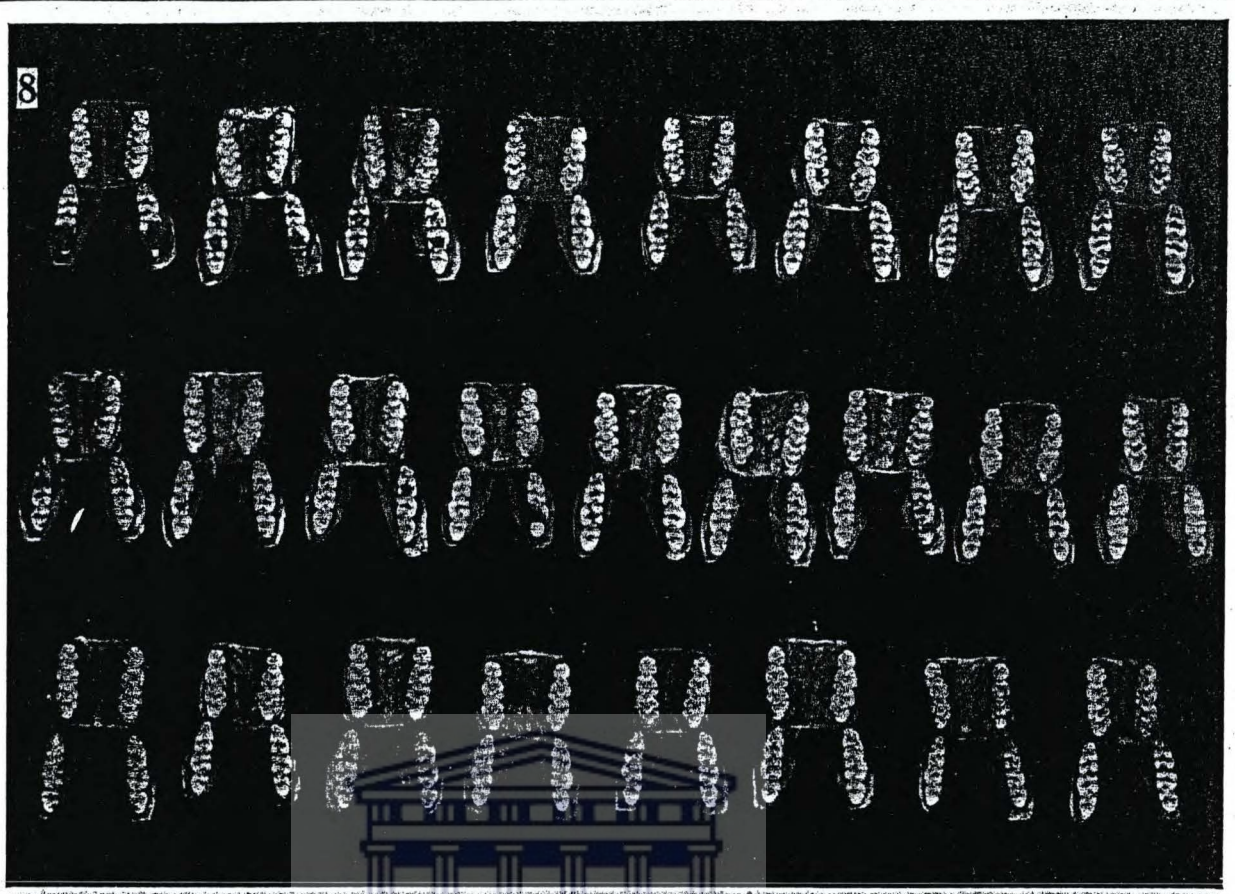


Fig. XXII. Molars of rats in group IX showing caries in lower molars and in some upper molars.

Fig. XXIII. Molars of rats in group X showing caries in lower molars.

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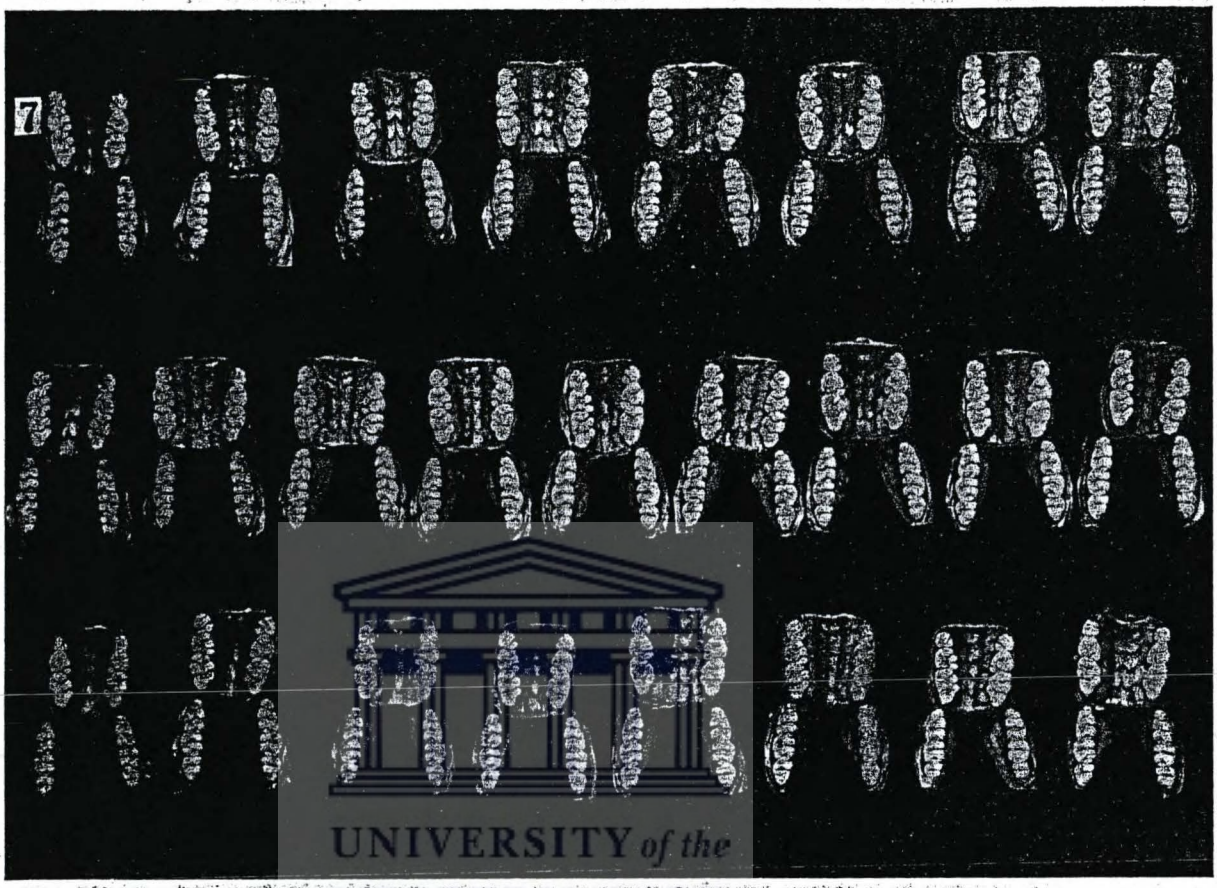

Fig. XXIV. Molars of rats in group XI showing slight caries in two lower molars.

(*b*) PRE-NATAL INVESTIGATIONS

The following table (table XV) shows the effect of the caries-producing diets on molars of rats, whose mothers were fed fluorine, a high-carbohydrate low-protein diet and low-carbohydrate high-protein diet during pregnancy and lactation:—

TABLE XV

Group.	Diet of Mothers during Pregnancy and Lactation.	No. of Rats with Caries.	No. of Molars with Caries.	No. of Upper Molars with Caries.	No. of Lower Molars with Caries.	Caries Index.
XII	Caries-producing and 100 p.p.m. F.....	20	53	None	53	2·8
XIII	High-carbohydrate, low-protein.....	25	101	None	101	8·8
XIV	Low-carbohydrate, high-protein.....	20	38	None	38	3·0

Figs. XXV to XXVII are the photographs of the upper and lower molars of the rats in these three groups.

Fig. XXV. Molars of rats in group XII showing caries in lower molars.

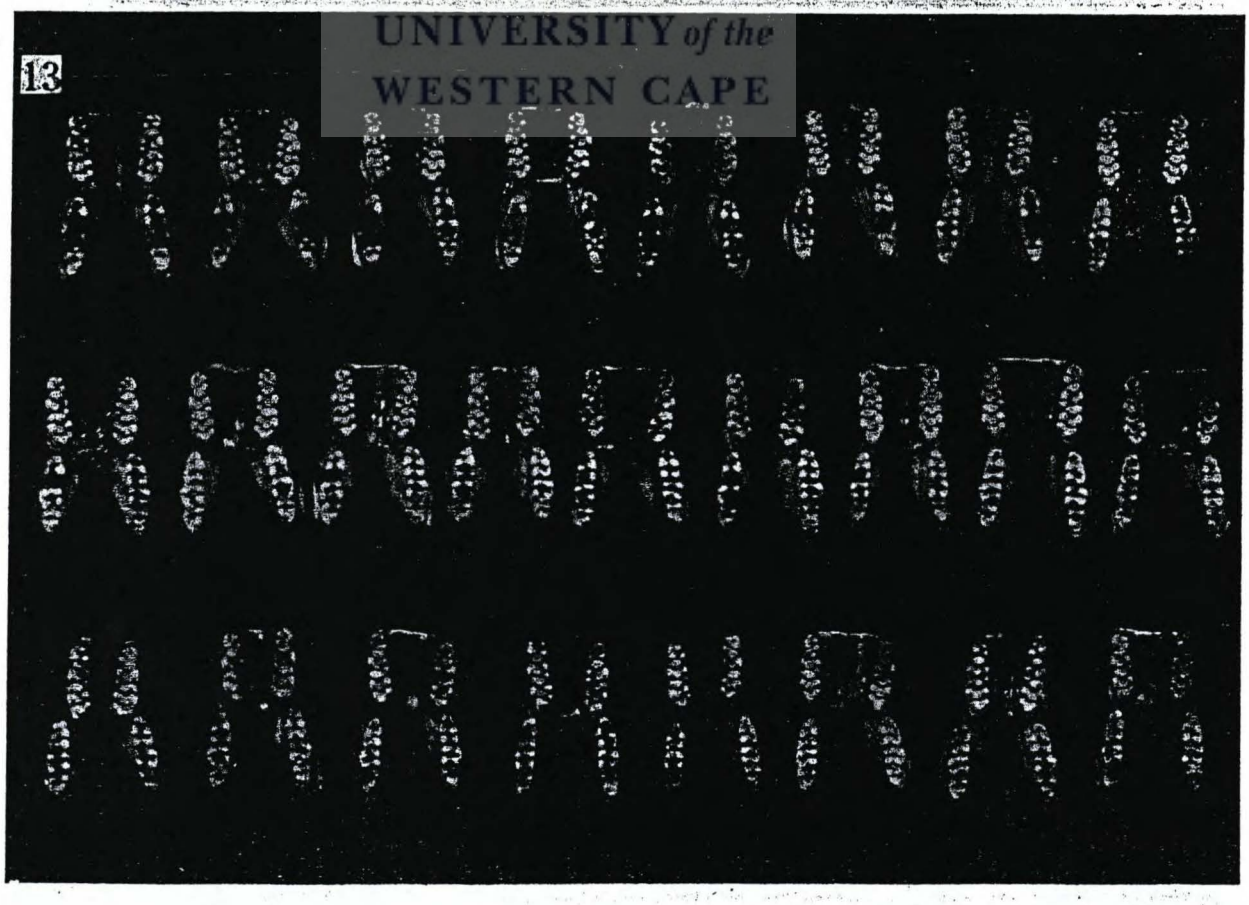

Fig. XXVI. Molars of rats in group XIII showing extensive caries in lower molars.

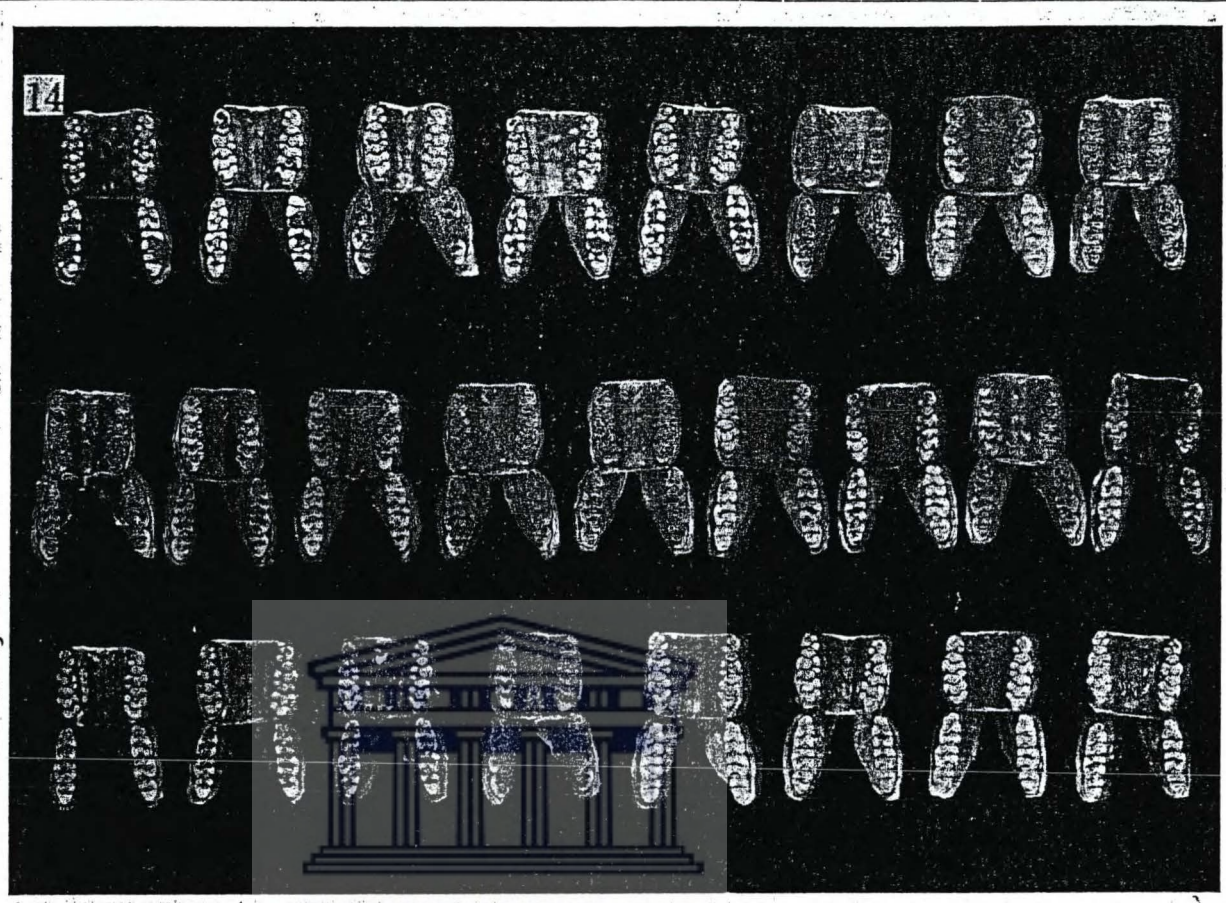

Fig. XXVII. Molars of rats in group XIV showing caries in lower molars.

(c) Heredity and Pre-natal Investigations

The following table XVI shows the effect of different diets on molars of rats, whose mothers were closely inbred, and placed on a high-carbohydrate low-protein diet and low-carbohydrate high-protein diet with and without fluorine during pregnancy and lactation:—

Table XVI

Group.	Diet of Mothers during Pregnancy and Lactation.	Diet of Offspring during 80-day Experimental Period.	No. of Rats with Caries.	No. of Molars with Caries.	No. of Upper Molars with Caries.	No. of Lower Molars with Caries.	Caries Index.
XV	Caries producing.....	Caries producing.....	25	88	None	88	9·0
XVI	High carbohydrate, low protein	Non-caries producing with sweet potatoes	8	22	None	22	1·0
XVII	High carbohydrate, low protein	Non-caries producing with sweets	15	40	None	40	2·4
XVIII	High carbohydrate, low protein	Caries producing.....	24	96	8	88	11·0
XIX	Low carbohydrate, high protein	Caries producing.....	20	52	None	52	3·4
XX	High carbohydrate, low protein plus 100 p.p.m. F	Caries producing.....	19	56	None	56	3·9
XXI	Low carbohydrate, high protein plus 100 p.p.m. F	Caries producing.....	15	21	None	21	1·3

Figs. XXVIII to XXXIV are the photographs of the upper and lower molars of the rats in these groups.

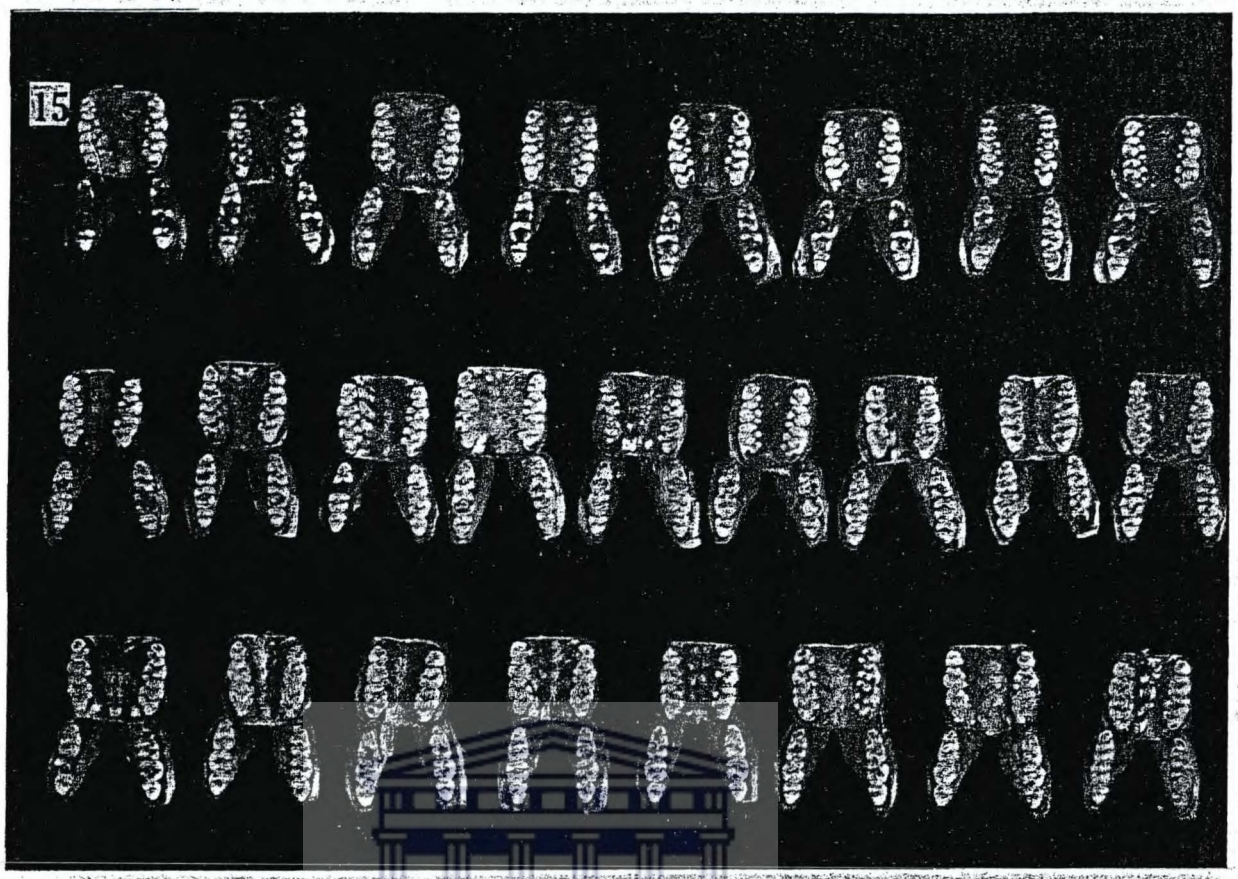

Fig. XXVIII. Molars of rats in group XV showing extensive caries in lower molars.

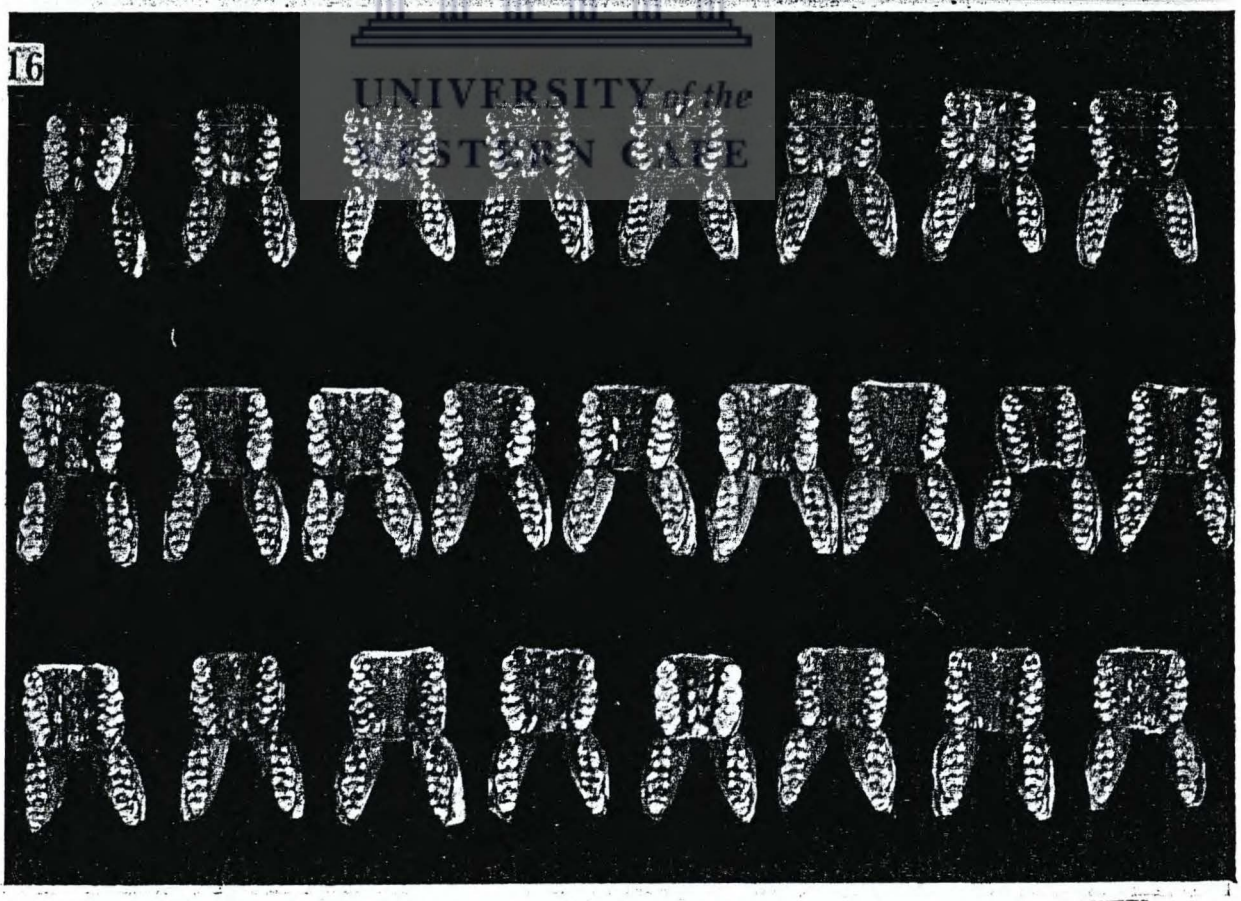

Fig. XXIX. Molars of rats in group XVI showing slight caries in some lower molars.

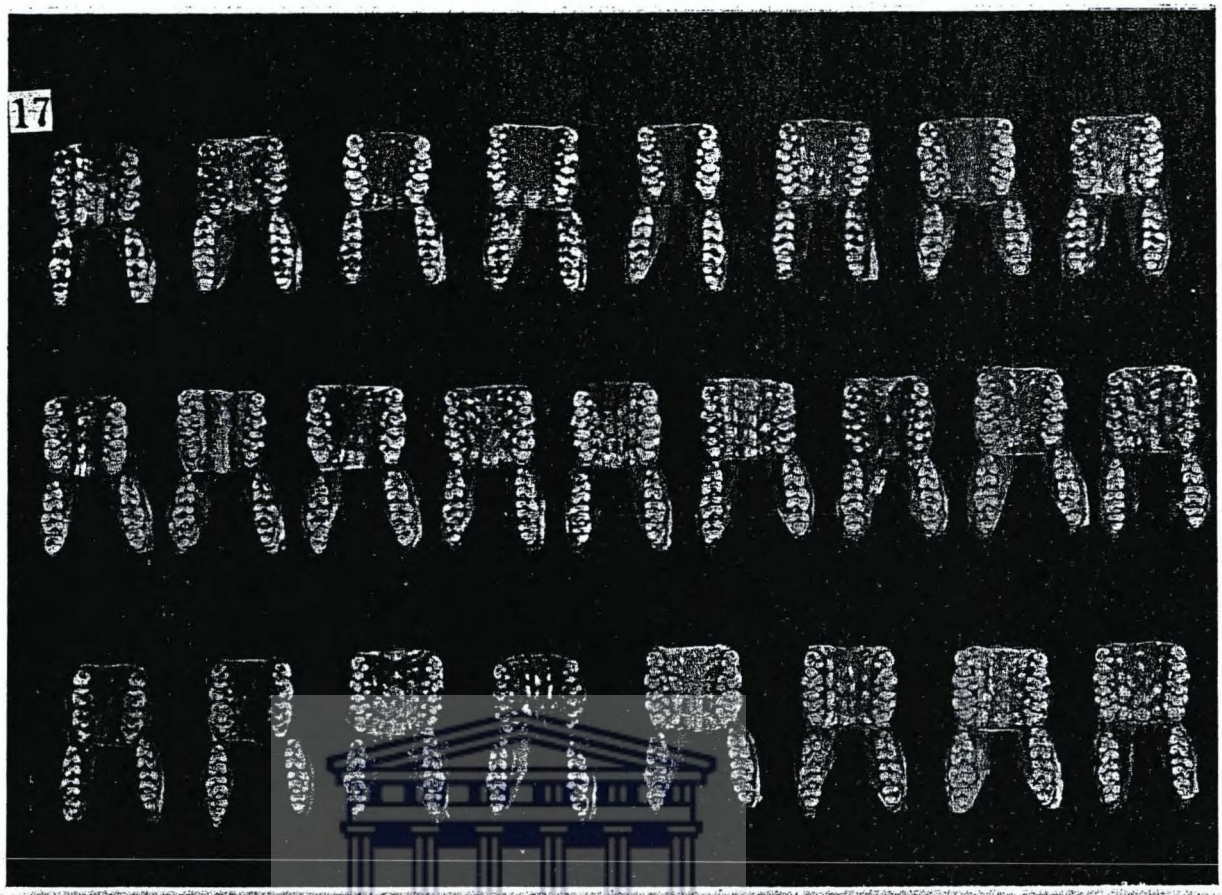

Fig. XXX. Molars of rats in group XVII showing caries in lower molars.

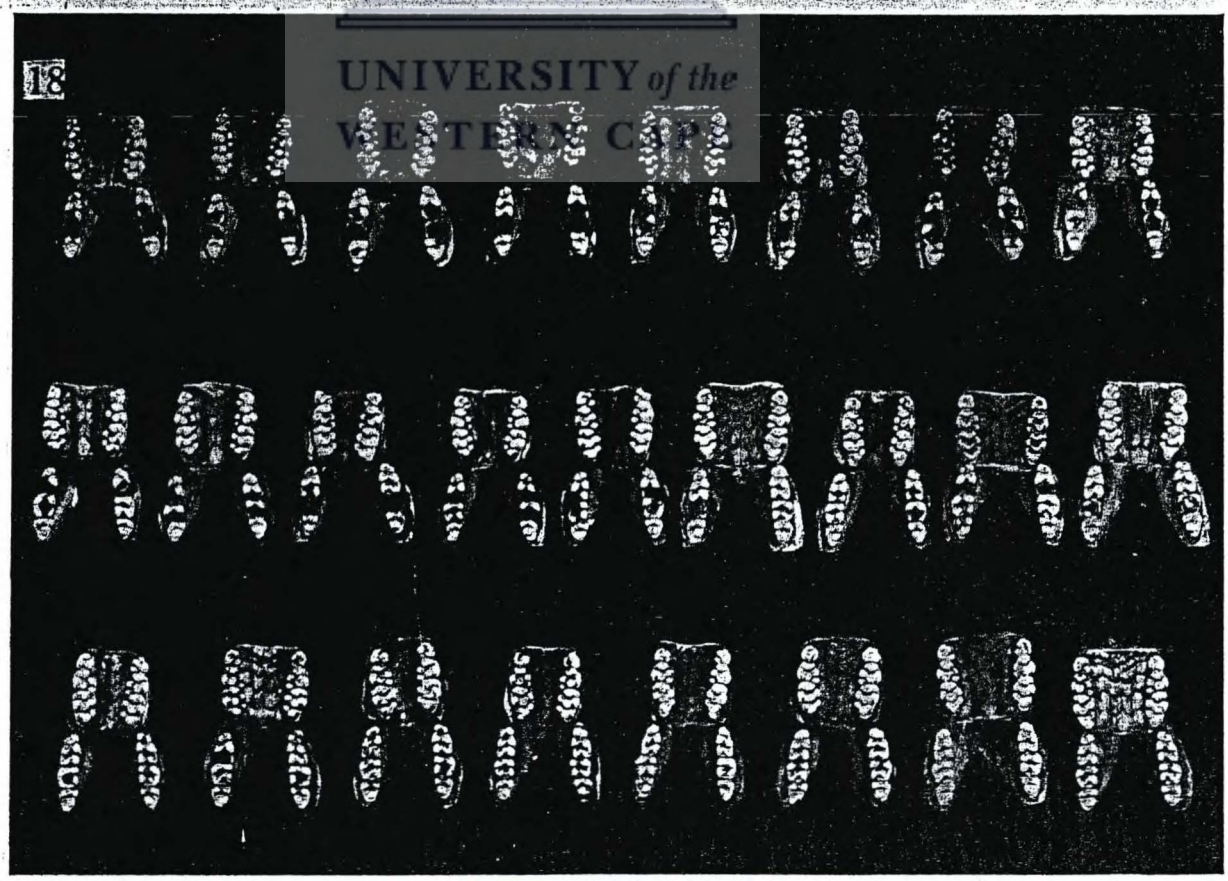

Fig. XXXI. Molars of rats in group XVIII showing extensive caries in lower molars and in some upper molars.

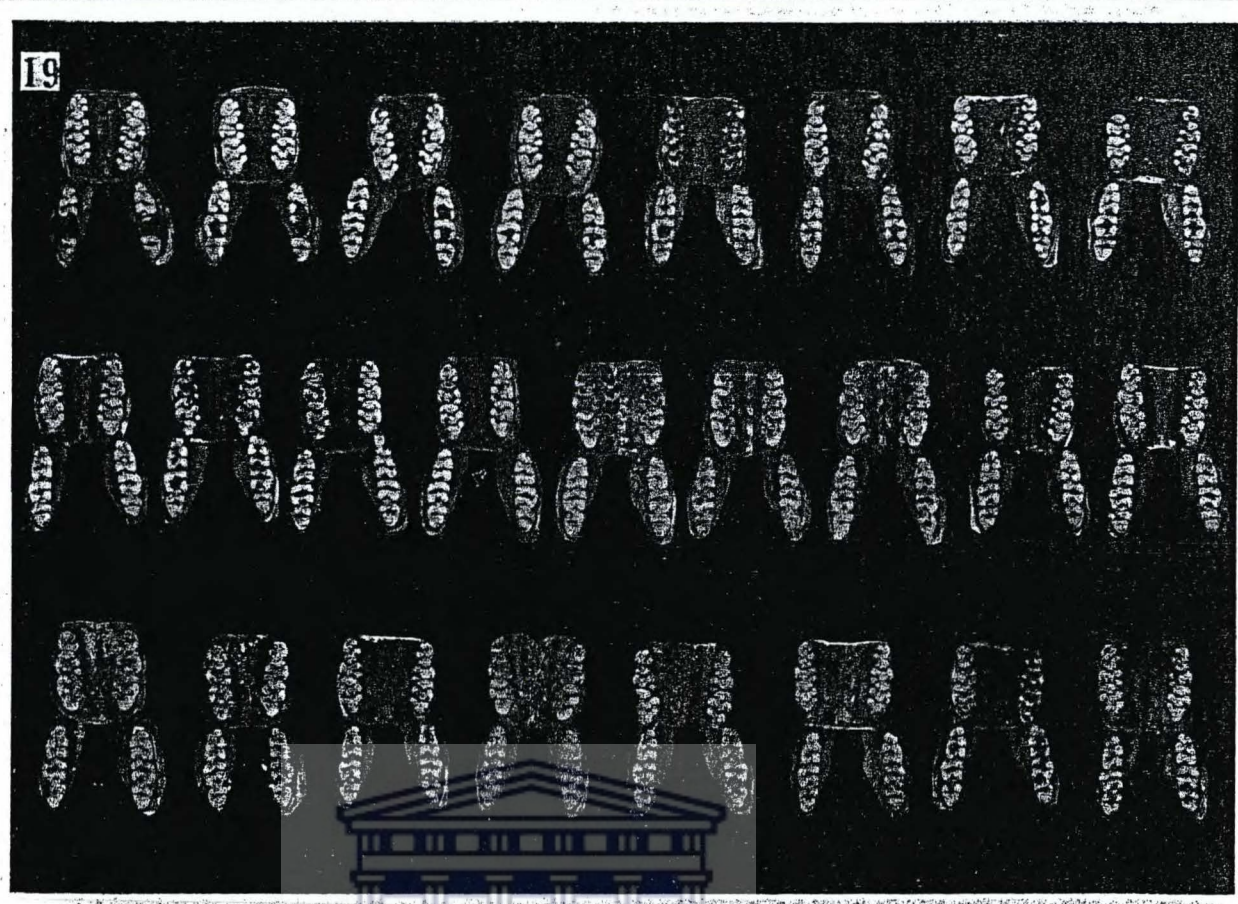

Fig. XXXII. Molars of rats in group XIX showing caries in lower molars.

Fig. XXXIII. Molars of rats in group XX showing caries in lower molars.

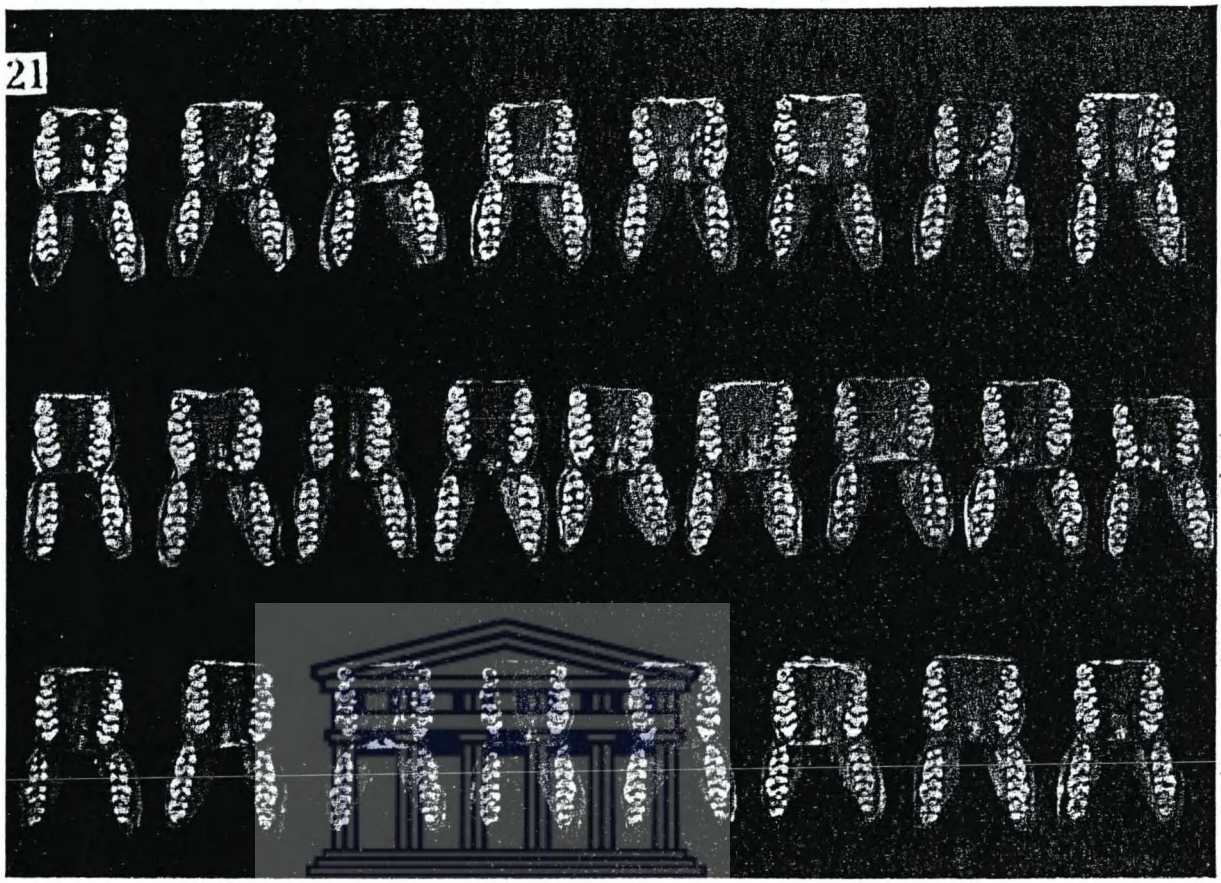

Fig. XXXIV. Molars of rats in group XXI showing slight caries in lower molars.

IV. DISCUSSION.

1. CLINICAL SURVEY

(*a*) Caries Incidence Rate

The caries incidence rate of European schoolchildren in South Africa is lower (85 per cent.) than the caries incidence rate of 95 per cent. found in most civilized countries. The caries incidence rate in most cities, towns and districts in South Africa, however, is 95 per cent. and more. The very low caries incidence rate of 20 per cent. in the Kenhardt district, and the high caries incidence rate of Knysna is unique, and offered an excellent opportunity to investigate some of the contributory causal factors experimentally in the rat.

The Caries Incidence Rate in Urban and Rural Areas.—The variation in the caries rate in the urban and rural areas appears to be associated with excess carbohydrates, and will be discussed under the dietary investigation.

Caries in Age Groups.—The variation of the caries incidence rates in the age groups, as shown in table VIII, is difficult to explain. It is possible, but not yet proved, that the deciduous teeth are more susceptible to dental caries than the permanent teeth owing to changing dietary habits. This may be a reason for the large number of children affected in the earlier age groups in this series, but the fact that the deciduous teeth are exposed for a longer period to the risk of attack by caries than the permanent teeth in these age groups may be an even greater factor.

More boys in the 6 to 8 and 9 to 11 age groups are affected by caries than girls, but in the 12 to 14 age group more girls than boys are affected. These findings correspond to those of Stoughton and Meaker (1932), who found that a greater proportion of boys among the younger children and of girls among the older children had

decayed, missing or filled teeth. Sloman (1941) also found that more girls between the ages of 12 to 14 years are affected by dental caries than boys of the same ages. The percentage of boys and girls between 6 and 14 years affected by dental caries is identical, namely, 84 per cent.

(*b*) Mottled Enamel, Fluorine and Dental Caries

This survey shows that chronic endemic dental fluorosis is widespread in South Africa. It also proves conclusively that mottled enamel is associated with a lower caries incidence rate, and that the presence of fluorine in drinking water has an inhibitory effect on dental caries. This corresponds to the findings of Dean *et al.* (1939), Silva *et al.* (1940), Wilson (1941), etc. Only 38 children with mottling of the deciduous teeth were seen, which seems to indicate that very little fluorine passes through the placenta during calcification of the deciduous teeth, and that probably only drinking water with an extremely high fluorine content causes mottling of these teeth. A significant difference of mottled enamel was found among boys and girls—55 per cent. boys and 45 per cent. girls. This may be due to the fact that boys being more active than girls, drink more water in the hot climate of South Africa, and therefore ingest more fluorine. It was not possible to correlate the degree of mottling with the amount of fluorine in drinking water, as the fluorine content in the same water may vary considerably from time to time, owing to climatic influences.

(*c*) The Chemical Composition of Teeth

The results of the analysis of the chemical composition of the teeth correspond to the findings of Armstrong and Brekhus (1937) and Bowes and Murray (1935, 1936*a*) as the following table (XVII) will indicate:—

Table XVII

Investigator.	Carious Enamel.			Sound Enamel.		
	Ca.	Mg.	P.	Ca.	Mg.	P.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Armstrong and Brekhus	35·64	0·32	17·21	35·41	0·30	17·45
Bowes and Murray	—	—	—	37·07	0·464	17·22
Division of Chemical Services	36·15	0·426	17·72	35·54	0·426	17·42
	Carious Dentine.			Sound Dentine.		
	Ca.	Mg.	P.	Ca.	Mg.	P.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Armstrong and Brekhus	—	—	—	26·18	0·83	12·74
Bowes and Murray	27·98	1·26	13·87	27·79	0·835	13·81
Division of Chemical Services	27·45	0·90	13·39	27·33	0·96	13·03

Although the calcium, magnesium and phosphorus content of drinking water and soils vary considerably in different parts of South Africa, they do not appear to affect the calcium, magnesium and phosphorus content of the enamel and dentine of people living in these areas. The fluorine content, however, varies considerably It is very much higher in the low caries incidence areas than in the high incidence areas, and probably has its origin in the drinking water.

Other investigators have also found that mottled enamel and dentine contain more fluorine than normal enamel and dentine, as the following table will show:—

TABLE XVIII

Investigator.	Enamel.		Dentine.	
	Normal.	Mottled.	Normal.	Mottled.
	Per cent.	Per cent.	Per cent.	Per cent.
Bowes and Murray (1936)	0·025	0·035	0·0246	0·07
Armstrong and Brekhus (1938)	0·0111	—	0·0169	0·0504
Sognnaes and Armstrong (1941)	0·04	—	0·027	—
Roholm (1937)	0·044 0·057	0·29	0·31 0·30	0·83

The fluorine is probably taken up by the teeth through the odontoblasts and ameloblasts, from the blood during calcification, and by adsorption when the teeth come in contact with drinking water containing excess amounts of fluorine.

(*d*) THE pH AND MINERAL CONTENT OF WATER AND DENTAL CARIES

It is doubtful whether pH values of drinking waters will affect the caries incidence rate, but as an acid media favours the growth and activities of the oral bacteria (mainly *Lacto-bacillus acidophilus*) causing dental decay, the possibility of acid drinking waters being a contributory causal factor of dental caries merits further investigation.

The cause of the acidity of the coffee-colourd waters in the southern Cape coastal areas seems not quite clear. Bosman (1941) and others are of the opinion that it is due to the decomposition of vegetable mould, whereby organic acids, mostly humic, ulmic or geic, crenic and apocrenic are produced. Bond (1943) has proved that the acidity is due to so-called " humic acids ", which are now supposed to be in reality organic colloids with acidic properties. Others maintain that the acidity is due to CO_2 in solution. The drinking waters in the low-caries incidence areas, on the other hand, are alkaline.

The Effect of Hardness of Drinking Water on Dental Caries.—Hardness of water can be temporary, permanent or both. Temporary hardness is due to the bicarbonates of calcium and magnesium and can be removed by boiling the water: the calcium and magnesium being precipitated as carbonates in the form of scale. Permanent hardness is due to the sulphates and chlorides of calcium and magnesium and sometimes to nitrates and cannot be removed by boiling. Total hardness is temporary and permanent hardness combined, and is usually expressed as calcium carbonate in parts per million.

Very little information is available concerning the effect of hardness of drinking water on dental caries. East (1941*a*, 1942) found that children who have been raised in communities where the water supplies are " hard " have lower caries rates than those using " soft " water. Cook (1914) is of the opinion that an association exists between excessive softness of water and an increased amount of dental caries among school children and obversely.

As calcium plays the most important role in the calcification of the teeth, it appears probable that the amount present in drinking water may affect the caries incidence rate. The mean calcium content of 50 samples of drinking water in the low-caries area was found to be 400 p.p.m. The daily amount of calcium obtained from this source alone (based on $4\frac{1}{2}$ pints intake) would be approximately one gram or, according to Sherman, the normal daily requirement (0·65 gram for an adult and 1 gram for a child). The mean calcium content of 16 samples of drinking water in the high-caries areas, on the other hand, was found to be 6 p.p.m., and the daily intake would be only 0·015 gram.

It has been proved that by supplementing the diet with calcium after the calcification of the teeth does not inhibit the progress of dental caries (Jones, 1935; Schour, 1938*a*; McCall and Krasnow, 1938; Malan and Ockerse, 1941). The high amount of calcium available in the drinking water in the low-caries areas may, however,

assist the calcification of the teeth both in utero and after birth up to eight years, making them more caries-resistant. The very low calcium content in drinking water in the high-caries areas may be responsible for a calcium deficiency during calcification of the teeth, and may be an important contributory causal factor of the high caries-incidence rate.

Dreyer (1935, 1939) made some interesting discoveries concerning teeth from skulls of prehistoric tribes unearthed in the Knysna area. He found that the teeth of the pre-historic Bushmen found near the top of the Mossel Bay layer and from burials in the Wilton-without-pottery layer show no signs of dental caries. The teeth of the Strandlopers found in the next layer of sea-shells (mostly Mytilus), on the other hand show extensive caries. In the next layer of ashes and bedding teeth of the modern Bushmen were found which showed slight caries. These prehistoric tribes drank water (very low in calcium) from the same sources used by people living in those areas to-day, a fact that would point against the importance of low calcium in drinking water. A diagram showing the different layers, etc., in which the teeth of these prehistoric tribes were found, is given on page 63, in appendix B. The caries incidence rate among European schoolchildren in the Umtata district was found to be 99 per cent., whilst among the Native schoolchildren it was 33 per cent. They all drink water from the same source with a very low calcium content.

(*e*) Dietary Investigations and Caries

From the clinical survey of the incidence of dental caries it appears that the diet is the most important contributory causal factor. The low caries incidence rate in the north-west Cape, Karroo and the northern Transvaal districts is associated with a low carbohydrate and high protein diet, while the high caries incidence rate in the southern Cape coastal areas is associated with a high carbohydrate and low protein diet. Nizel and Bibby (1944) have made similar observations in the United States and found that the caries incidence rate among soldiers coming from the ranching states of Texas, New Mexico, Wyoming and Montana was less than half the incidence rate of soldiers coming from Maine, Rhode Island, New York and New Jersey. More proteins and less carbohydrates are probably eaten in the beef-producing states than in the northern states mentioned. This also corresponds to the findings of Jones (1930) in regard to the islanders of Lewis, who have little caries and whose diet is high in proteins and low in carbohydrates. Schwartz (1946) also found the lowest caries incidence rate among the meat-eating Masai tribe. The Eskimos, before they adopted the white man's dietary habits, had no caries, and their diet consisted mainly of proteins. Sprawson (1934) has reported that caries is rampant among the islanders of Pitcairn, whose diet consists chiefly of carbohydrates in the form of yams, sweet potatoes and fruit, and practically no meat. In certain counties in Ireland, where the staple diet is also mostly carbohydrates in the form of potatoes and little proteins, dental caries is rampant. The diet of the prehistoric and modern Bushmen, previously referred to, consisted chiefly of meat. They were hunters, as evidenced from the bones and teeth of game found in the middens. It is very difficult to explain the extensive caries of the Strandlopers. Their diet consisted mostly of shellfish, according to the excavations.

The low caries incidence rate in the north-west Cape, Karroo and northern Transvaal and the high caries incidence rate of the southern Cape coastal areas are, however, not associated with the carbohydrate and protein intake only. It must be remembered that fluorine is usually present in variable amounts in drinking water in the low caries areas while, in the high caries areas, the fluorine content is very low or absent. From these observations it therefore appears that the fluorine in the drinking water, the low carbohydrate and high protein diet are responsible for the low caries incidence rate in the north-west Cape, Karroo and northern Transvaal, while the low fluorine content of the drinking water, high carbohydrate and low protein diet are responsible for the rampant caries in the southern Cape coastal areas.

(*f*) The Effect of Climate, Geology and Soils on the Incidence of Dental Caries

The caries incidence rate is considerably lower in the areas with a low rainfall and high amount of sunshine, than in areas with a high rainfall and a lower amount of sunshine. East (1939), and East and Kaiser (1940), also found that the greater the amount of sunshine available at a given locality the lower is the caries incidence rate, and East (1941) is of the opinion that the amount of solar ultra-violet energy is related to the amount of dental decay. The higher degree of solar intensity may be responsible for the formation of extra vitamin D in the body.

In the more arid areas there is less leaching of the minerals in the soils and underlying geological formations, and the fluorine content and total hardness of the drinking water in these dry areas are usually high. The majority of the endemic fluorosis areas in South Africa occur in those regions where the higher amounts of sunshine prevail. (See fluorosis map at the end of the text.)

The low caries incidence rate areas of the north-western Cape and northern Transvaal are mostly underlain by Old Granite, an igneous rock containing comparatively high amounts of fluorine. The rainfall is low in these areas and the fluorine content and the total hardness of the drinking water, mostly from underground sources, very high.

The high caries incidence rate areas of the southern Cape and Natal coastal areas are mostly underlain by the Table Mountain Series of the Cape System, built up of sedimentary rocks which are low in fluorine. The drinking water in these areas is mostly of surface origin, and the fluorine content and total hardness very low.

It has not been established whether a deficiency of minerals such as Ca, Mg and P_2O_5 in the soils will reduce the mineral content of cereals, vegetables and fruit grown on these soils, and whether such a deficiency will materially affect the human Ca, Mg and P_2O_5 intake from these sources. The highest caries incidence rate was found in the forest-covered areas, which are in the high rainfall areas. Trees grow better in humid than arid areas, and there is probably no relationship between vegetation and high caries incidence rate.

2. EXPERIMENTAL WORK

(*a*) Post-natal Investigations—Eruption of Rat Molars

The eruption data of the rats' molars, obtained from the preliminary studies, correspond more or less to the findings of other investigators, as the following table will indicate:—

Table XIX

Investigator.	First Molar.		Second Molar.		Third Molar.	
	Eruption Begins.	Eruption Complete.	Eruption Begins.	Eruption Complete.	Eruption Begins.	Eruption Complete.
	Days.	Days.	Days.	Days.	Days.	Days.
Addison and Appleton (1921)	19	—	21	—	—	35
Mellanby (1939)	20–21	21–22	20–21	21–22	29	35
Hoffman and Schour (1940)	19	23	21–22	25–28	35	39–40
Cheyne (1942a)	19	—	21–22	—	35	—
Ockerse	19	25	21	27	31	35

In the case of the beginning of the eruption of the third molar, Mellanby's findings approximate the figure obtained in the course of this preliminary study. The other investigators found that it erupted much later. In regard to the completion of the eruption of the third molar the figures are similar, except those of Hoffman and Schour, who found that the completion is much later.

Diet and Caries in the Rat.—The rats in group I, on the non-caries-producing diet, developed no caries (Fig. XIV). The fineness of the corn-meal probably prevents impaction and retention of the fermentable carbohydrates in the fissures. Nineteen rats in group II, on the caries-producing diet, developed caries in the lower molars only, with a caries index of 5·7 (Fig. XV). Caries is probably produced by the lodgement and impaction of the coarse corn particles in the diet, whereby the fermentable carbohydrates are retained long enough in the fissures to initiate caries. The addition of carbohydrates in the form of boiled sweet potatoes and acid drinking water to the non-caries-producing diet in group III (Fig. XVI) did not initiate caries, but the addition of these to the caries-producing diet in group IV (Fig. XVII), resulted in twice as much caries as in group II on the control caries-producing diet (11·0 as against 5·7). Eight upper molars were affected.

The addition of sweets to the non-caries-producing diet in group V initiated caries in 10 rats (Fig. XVIII) with a caries index of 1·2, while the sweets added to the caries-producing diet in group VI produced nearly twice as much caries as in the rats on the control caries-producing diet (9·6 as against 5·7). Lilly and Grace (1932), Rosebury and Karshan (1935, 1939), King (1935), Day *et al.* (1935) and Cox (1944) found that excessive intake of carbohydrates does not initiate caries in the rat, but rather increases the rate of decay already present. Rosebury (1939) could not induce caries in rats by adding sugar to a non-caries-producing diet. McClure (1945), on the other hand, observed that rats developed a significant incidence of microscopic caries when fed synthetic diets containing excessive quantities of sucrose and glucose. It appears that carbohydrates, in the form of boiled sweet-potatoes, added to a non-caries-producing diet does not initiate caries, but, in the form of sweets, it can. The sticky sweets are probably retained in the fissures long enough to initiate caries, whereas refined sugar is perhaps more readily washed out. In group VII, the orange juice with the non-caries-producing diet did not initiate caries, but destroyed the enamel on the lingual surfaces of all the lower molars and of some of the upper molars, as will be seen in Fig. XX. Similar results were obtained with acid beverages by McClure (1943*a*), Restarski *et al.* (1945) and McClure and Ruziska (1946). The orange juice added to the caries-producing diet, in group VIII (Fig. XXI), not only destroyed the enamel to the same extent as in group VII but increased the rate of decay significantly. The caries index for this group (VIII) was 8·0, as compared with 5·7 for group II on the control caries-producing diet. The addition of meat to the caries-producing diet in group IX did not affect the caries incidence rate to any significant extent, but initiated caries in five upper molars. It is possible that the fermentable carbohydrates in the diet were impacted in some of the upper fissures long enough by the meat to initiate caries.

The effect of 5 p.p.m. fluorine in the drinking water in group X did not appear to reduce the caries incidence rate (Fig. XXIII). In this group the number of rats that developed caries was greater than the number of rats in the group on the control caries-producing diet—24 as against 19—and 77 molars were affected as against 76. The extent of the caries, however, was slightly less, the caries index being 4·3 as against 5·7. The addition of 100 p.p.m. fluorine to the drinking water in group XI had a very marked inhibitory effect on caries. Only two rats developed caries and only two molars were affected (Fig. XIV). The caries index was 0·1. Miller (1938), Hodge and Finn (1939), Cheyne (1940*a*), McClure (1941), Norvold and Armstrong (1943), etc., have obtained similar results. This proves conclusively that when teeth are exposed to high fluorine concentrations, such concentrations have a definite inhibitory effect on the incidence of caries.

These investigations have shown that caries occurred mostly in the lower first and second molars. In only two groups (IV and IX) were the upper molars affected. Rosebury *et al.* (1933), King (1935), Cox (1944), etc., also found that only the lower molars were affected, but Finn and Hodge (1941) observed that in their rat-caries experiments the upper molars developed more caries than the lower molars. It appears that, owing to the difference in the morphological structure of the fissures of the upper and lower molars, the coarse corn particles are impacted and retained more readily in the lower molars than in the upper. It is difficult to explain why the upper molars of the rats in group IV, on the caries-producing diet with sweet-potatoes and an acid drinking water, were attacked by caries. From observations made of the numerous cavities which developed in the lower molars, it appears that the loss of the subdistal cusp in the lower first and second molars is the result of the decay which develops in the sub-distal groove. Cox (1944) is of the same opinion.

Rosebury *et al.* (1933), Bibby and Sedwick (1933), and King (1935), on the other hand, suggest that these cavities originate as fractures of the subdistal cusps.

(*b*) Pre-natal Investigations

The caries index of 2·8 of the rats in group XII, whose mothers were given 100 p.p.m. fluorine in the drinking water during pregnancy and lactation, is considerably lower than the caries index (5·7) of the control rats in group II, whose mothers received no fluorine during pregnancy and lactation. Cox *et al.* (1939*b*) obtained similar results. How the ingestion of fluorine during pregnancy and lactation inhibits caries in the offspring is not clear. It appears that some of the fluorine is probably deposited in the bones of the foetus, and optimum

amounts are later withdrawn during calcification of the teeth, making them more resistant to caries. Calcification of rat molars commences at birth. Very little fluorine was transmitted through the milk, as none of the offspring showed mottling.

The rats in group XIII, whose mothers were fed a high-carbohydrate low-protein diet during pregnancy and lactation, had a caries index of 8·8, which is significantly higher than that of the control rats in group II (5·7), whose mothers were fed the standard caries ration.

The rats in group XIV, whose mothers were fed a low-carbohydrate high-protein diet during pregnancy and lactation, developed considerably less caries, the index being 3·0 as compared with 5·7 of the controls. Similar results were obtained by Cox and Levin (1942). A high protein diet during pregnancy and lactation may reduce the caries susceptibility in the offspring in several ways:—

(i) It may result in better calcified teeth, improving them structurally, making them more resistant to caries. Proteins affect growth, and a sub-optimal intake during pregnancy may stunt the growth of the foetus and probably also affect calcification of the teeth. About half the serum calcium is bound to serum proteins, but this part of the blood calcium differs from the ionized portion, which is regulated by the parathyroids. Fluctuations in plasma proteins result in concomitant reduction of blood calcium and possibly of calcium deposition. Assuming augmented protein intake results in increased absorption of calcium from the intestine, then, at a constant level of calcium intake, the percentage of calcium absorption will appear to be proportional to the percentage of protein intake. If the supply of calcium to the ameloblasts and odontoblasts during calcification is affected, a defective structure of the enamel and dentine may result. Amounts of protein which fall short of the optimal amount may also affect the normal function of the ameloblasts and odontoblasts during the calcification process, thus causing a defective structure of the enamel and dentine. As the organic matrix of the enamel and dentine consists mostly of protein, it may be possible that insufficient protein may result in a defective enamel and dentine matrix.

(ii) A high protein intake during lactation results in a good milk production, in quantity and possibly also in quality, and may thus benefit the calcification of the teeth of the young.

(iii) A high protein intake during pregnancy increases the amino acids in the foetus and may later increase the amino acids in the saliva of the offspring. How these amino acids in the saliva may exert an inhibitory action on caries will be discussed later.

(*c*) Heredity and Pre-natal Investigations

The results of the experimental investigations to ascertain whether heredity can influence rat caries, show that a highly caries-susceptible strain of rats can be bred by selection and close inbreeding. The caries index of the 25 rats in group XV offspring of the inbred fourth generation was 9·0, compared with 5·7 of the control rats in group II, not inbred. These findings correspond to those of Hunt *et al.* (1944), and Hunt and Hoppert (1944). The rats in group XVI, whose mothers were fed a high-carbohydrate low-protein diet during pregnancy and lactation, developed caries when placed on a non-caries-producing diet with sweet-potatoes and an acid drinking water. The rats in group XI not inbred, and whose mothers were placed on the standard caries ration, did not develop caries when placed on a non-caries-producing diet with sweet-potatoes and an acid drinking water. This would indicate that the heredity factor and a high-carbohydrate, low-protein diet during pregnancy and lactation influenced caries-susceptibility in the offspring. The same applies to the rats in group XVII with a caries index of 2·4, which is much higher than the caries index (1·2) of the non-inbred rats of group IX, who were also placed on a non-caries-producing diet with sweets, and whose mothers were fed a standard caries ration during pregnancy and lactation. This substantiates that caries can be initiated in rats when fed a non-caries-producing diet with added sweets and with soft cooked starch.

The rats in group XVIII, whose mothers were fed a high-carbohydrate, low-protein diet during pregnancy and lactation, developed extensive caries when fed a caries-producing diet, the caries index being 11·0, which is twice as high as the index (5·7) of the non-inbred control rats in group II. Heredity and the high-carbohydrate,

low-protein diet during pregnancy and lactation appear to influence the caries susceptibility. The rats in group XIX, whose mothers were fed a low-carbohydrate, high-protein diet during pregnancy and lactation, developed considerably less caries, the index being 3·4. This corresponds to the findings observed in the non-inbred groups XIII and XIV and shows the influence of heredity on caries susceptibility.

The pre-natal effect of fluorine on calcification of rats' teeth was again clearly demonstrated by the results in groups XX and XXI. The mothers of the rats in group XX were fed a high-carbohydrate, low-protein diet with 100 p.p.m. fluorine in the drinking water during pregnancy and lactation. The caries index of this group was 3·9 and considerably less than that of group XVIII (11·0) whose mothers were fed the same diet without fluorine. The lowest caries index obtained in this experimental investigation was found among the inbred rats in group XXI, whose mothers were placed on a low-carbohydrate, high-protein diet with 100 p.p.m. fluorine in the drinking water during pregnancy and lactation. The caries index of this group was 3·9 and considerably less than that of group XVIII (11·0) whose mothers were fed the same diet without fluorine. The lowest caries index obtained in this experimental investigation was found among the inbred rats in group XXI, whose mothers were placed on a low-carbohydrate, high-protein diet with 100 p.p.m. fluorine in the drinking water during pregnancy and lactation. The caries index was 1·3 and and none of the rats developed extensive caries, most of the cavities being small fissure cavities. Fifteen rats were affected and they had 22 cavities in 21 lower molars, with a total score of 32. The rats in groups XX and XXI had no fluorine after they were weaned. This remarkable low caries incidence rate in the latter group appears to indicate that these rats developed highly caries-resistant teeth, and that proteins with fluorine play an important role in calcification and caries prevention.

(*d*) Interpretation of the Clinical Findings in the Light of the Results of the Experimental Investigations

(i) *Carbohydrates and Dental Caries*

The findings of the clinical survey indicate that a high caries incidence rate is associated with a high carbohydrate intake. The kind of carbohydrate appears to play an important role in caries. Some probably initiate caries, while others only accelerate the progress of decay once it has started. In areas where children have easy access to refined carbohydrates such as sugar, sweets, cakes, etc., the caries incidence is high. Schour and Massler (1945) found that although the carbohydrate intake of Italians is very high, the caries incidence rate is much lower than in the United States. They are of the opinion that this is due to the higher intake of refined sugar in the United States. Similarly, although the diet of the primitive Native in South Africa consists largely of carbohydrates in the form of mealie porridge, the caries incidence rate is low. When these Natives adopt the white man's dietary habits, their teeth are soon attacked by caries.

In the experimental investigation it was found that the addition of sweets to a non-caries-producing diet initiated caries in the rat, and when added to the caries-producing diet they greatly accelerated the process of decay. The carbohydrate, in the form of boiled sweet-potatoes, added to the non-caries-producing diet, did not initiate caries in the non-inbred rats, but accelerated the process of decay when added to the caries-producing diet. In the inbred rats, the sweet-potatoes with the non-caries-producing diet initiated comparatively little caries.

From the clinical survey and the results of the experimental investigations it would appear that excessive intake of refined sugar and some starches is probably largely responsible for the high caries incidence rate among city children and in the high caries areas in South Africa. Other carbohydrates accelerate the progress of decay once it has started. These refined sugars and other carbohydrates probably increase the number of acid-producing organisms in the mouth, resulting in higher acid production. The excessive intake of refined sugars or other carbohydrates during pregnancy and lactation appear to affect the structure of the teeth of the child, making them more caries susceptible.

(ii) *Proteins and Dental Caries*

From the clinical survey it was found that in areas where the protein intake is high, the caries incidence rate is much lower than in areas where the protein intake is low. A high protein intake may lower the caries incidence rate as follows: (*a*) By increasing the protein intake at the expense of carbohydrates, less carbohydrate degradation

takes place, resulting in less caries. (*b*) A high-protein intake during calcification of the teeth, i.e., from the fifth month in utero until eight years after birth, may result in better calcified teeth, which are structurally strong and more caries-resistant. It was found that the teeth of the children in the low caries areas are structurally sounder than the teeth of the children in the high caries areas. (*c*) A high-protein intake during lactation may result in a good milk production, in quantity and possibly also in quality. This may benefit the calcification of the teeth of the child in so far as the protein as well as the calcium content is concerned. (*d*) A high-protein intake increases the amino acids in the body and also the amino acids in the saliva (Turner and Crowell, 1947). According to these investigators the ingestion of crystalline di-tryptophane changes the saliva of individuals with carious teeth to such an extent that it has chemical and enzymic characteristics like that of caries-free persons. In the saliva of caries-free persons a high content of tryptophane or similar substance could be demonstrated with a slower redox poise drop, fast reduction of preformed starch blue with a slow dextrinizing time. Since the addition of tryptophane to alpha amylase working on starch, they postulate that tryptophane acts as an inhibitor with this enzyme.

It is noteworthy, however, that Kirsch *et al.* (1947) discovered variations in the concentrations of 16 amino acids in human saliva, as determined by the microbiological method, independent of caries activity. Also Calandra and Fosdick (1947), working on the acid formation in saliva-glucose-enamel mixtures, could find no satisfactory evidence that amino acids in saliva may be responsible for the decreased rate of acid formation in caries-immune saliva.

The experimental investigations have shown that rats whose mothers were fed a low-carbohydrate, high-protein diet during pregnancy and lactation developed considerably less caries than rats whose mothers were fed a high-carbohydrate, low-protein diet during pregnancy and lactation. From the clinical survey and the experimental investigations it would appear that the low caries incidence rate among the children in the low-caries areas is associated with a low-carbohydrate, high-protein diet of their mothers during pregnancy and lactation, and the low-carbohydrate, high-protein consumption of these children.

The experimental studies have shown that the addition of meat to the caries-producing diet did not affect the caries incidence rate significantly in the rat. Unless the detergent action of chewing meat differs in man and rat, it is doubtful whether the chewing of meat in the low caries areas can reduce the caries incidence rate after the teeth are calcified.

(iii) *Fluorine and Dental Caries*

From the clinical survey it was found that a lower caries incidence rate is associated with fluorine in the drinking water. The fluorine content of the drinking water in the low caries areas varies considerably, but the majority are under five parts per million. The results of the experimental investigations show that 5 p.p.m. fluorine in the drinking water did not affect the caries incidence rate in rats fed a caries-producing diet. It would appear, then, that these amounts of fluorine are too small to have any inhibitory effect on caries after the teeth are fully calcified. Bellinger (1947) is of the same opinion. Such small concentrations can only affect caries when present during calcification.

In the pre-natal experimental investigation it was found that the caries incidence rate of rats, whose mothers were given drinking water containing 100 p.p.m. fluorine during pregnancy and lactation, was low. Very little of this fluorine is transmitted in the milk, as the young had no mottled teeth. This seems to indicate that non-toxic amounts of fluorine ingested during pregnancy and lactation, and amounts between one and two parts per million, which will not cause mottling, ingested post-natally during calcification of the teeth, will reduce caries-susceptibility. These optimal amounts of fluorine probably result in more caries-resistant teeth. Considering the above data it would appear that the lack of fluorine during calcification of the teeth is an important causal factor of the high caries incidence rate in certain areas in South Africa, especially in the south coastal regions. It has been demonstrated that the topical application of high fluorine concentration reduces the caries incidence rate in man. In the experimental investigations, rats developed much less caries when placed on a caries-producing diet with 100 p.p.m. fluorine in the drinking water. This would indicate that only when teeth are exposed to high fluorine concentrations, do they become less susceptible to caries.

(iv) *Proteins, Fluorine and Dental Caries*

From the clinical survey it was found that in areas where the protein intake is high and where the drinking water contains fluorine, the caries incidence rate was the lowest. From the experimental investigations it was found that the rats, whose mothers were fed a high-protein diet with 100 p.p.m. fluorine in the drinking water during pregnancy and lactation, developed practically no caries when fed a caries-producing diet without fluorine.

This would indicate that a high-protein intake with fluorine during the formative period of the teeth affects calcification and structure, resulting in less caries-susceptibility.

(v) *Acid Beverages and Dental Caries*

The clinical survey showed that, in the citrus growing areas, the caries incidence rate among children is very high.

The experimental investigations have demonstrated that orange juice does not initiate caries in the rat when given with a non-caries-producing diet, but greatly accelerates the progress of decay when given with the caries-producing diet. It is possible that excessive drinking of orange juice is a contributory causal factor of the high caries incidence rate found in the citrus-growing areas.

(vi) *Heredity and Dental Caries*

When the clinical survey was conducted it was noted that, in certain areas, especially in the Hoeree Valley in the Coega Mountains, Langkloof, and Knysna areas where considerable intermarriage takes place, the caries incidence rate among children is very high, often 100 per cent.

The experimental investigations showed that the caries index of the inbred rats of the fourth generation was twice as high as the index of the control rats not inbred. It is possible that caries-susceptibility may be transmitted in man, which may be a contributory factor in aggravating the progress.

It must not be forgotten, however, that in the above-mentioned areas, the protein intake is low and the fluorine content of the drinking water usually below 1 p.p.m. or even absent. Heredity in this case cannot be the sole factor.

V. SUMMARY AND RECOMMENDATIONS.

1. SUMMARY OF CLINICAL SURVEY

A brief description of the situation, topography, climatology, hours of sunshine, temperature, rainfall, geology and the European population of South Africa is given.

Of 78,563 European schoolchildren examined in different parts of South Africa it was found that 67,063 (85 per cent.) suffer or have suffered from dental caries.

The highest caries incidence rate and the highest DMF teeth per child were found among the children in the George, Humansdorp and Knysna districts.

The lowest caries incidence rate and the lowest DMF teeth per child were found in the north-western Cape and northern Transvaal districts. The incidence of dental caries was found to be higher among urban children (93 per cent.) than among rural children (79 per cent.).

Caries data of 51,734 children was analysed in relation to sex and age groups of 6 to 8, 9 to 11, and 12 to 14 years. It was found that more boys in the 6 to 8 and 9 to 11 age groups are affected by caries than girls but, in the 12 to 14 group, more girls are affected. The percentage of boys and girls between 8 and 14 years affected by dental caries is identical.

Chronic endemic dental fluorosis is widespread in South Africa, 805 endemic areas were mapped. Fluorine in drinking water is associated with a lower caries incidence rate. Of 3,067 children with mottled enamel, only 873 had caries, or 28 per cent., while of the 9,806 children with no mottling examined in the endemic areas 6,749, or 69 per cent., had caries.

Thirty-eight cases of mottling of the deciduous teeth were seen.

No significant difference was found in the percentage calcium, magnesium and phosphorus content of carious and non-carious teeth from children in different areas, but the fluorine content was very much higher in the teeth of children living in the low caries incidence areas than of those living in the high caries incidence areas.

The reaction, fluorine content and total hardness of drinking water in different areas are given, and their effect upon the incidence of dental caries is discussed.

The possible influence of climate, geology, soils and vegetation on dental caries is dealt with.

The diet appears to be the most important contributory causal factor of dental caries. High-carbohydrate (especially sugar, cakes, etc.) low-protein diets are associated with a high caries incidence rate, while low-carbohydrate, high-protein diets are associated with a low caries rate.

It was found that the caries incidence rate is very high among children living in the citrus-growing areas.

2. SUMMARY OF EXPERIMENTAL WORK

(*a*) Post-natal Investigations

Rats fed a fine particle corn-meal diet did not develop caries, but those fed a coarse corn diet developed extensive caries. Added carbohydrates in the form of sweet-potatoes to the fine corn particle diet did not initiate caries but, in the form of sweets, caries developed. These carbohydrates added to the coarse corn diet greatly increased the rate of decay already present. Likewise orange juice given with the fine corn diet did not initiate caries, but greatly accelerated the progress of decay when given with a coarse corn diet.

The addition of meat to the coarse corn diet did not affect caries. Five p.p.m. fluorine in the drinking water did not reduce the caries incidence rate, but 100 p.p.m. had a marked inhibitory effect.

Caries developed mostly in the lower molars. A few rats fed a coarse corn diet, supplemented with sweet-potatoes and with meat, developed caries in both the upper and lower jaws.

(*b*) Pre-natal Investigations

Rats whose mothers were fed a high-carbohydrate, low-protein diet during pregnancy and lactation developed more caries than rats whose mothers were fed a low-carbohydrate, high-protein diet during pregnancy and lactation. Rats whose mothers were given 100 p.p.m. fluorine in the drinking water during pregnancy and lactation also developed considerably less caries than the rats whose mothers were not given fluorine.

(*c*) Heredity and Pre-natal Investigations

Highly caries-susceptible rats were bed by close in-breeding. When these rats were fed high-carbohydrate low-protein, low-carbohydrate high-protein diets with and without fluorine, during pregnancy and lactation, similar results were obtained in their offspring as in the pre-natal experiments.

The rats whose mothers were fed a low-carbohydrate, high-protein diet with 100 p.p.m. fluorine in the drinking water during pregnancy and lactation developed very little caries.

Interpretation of the clinical findings in the light of these experimental investigations are discussed.

3. RECOMMENDATIONS

The following measures for the control and prevention of dental caries are recommended:—

(*a*) That the carbohydrate consumption, especially sweets, cakes, biscuits, etc., be reduced to a minimum, and that more proteins in the form of meat, eggs and cheese be eaten. To develop strong, healthy and caries-resistant teeth in children adequate amounts of protein should be eaten during pregnancy and lactation, and also by the children themselves during the post-natal calcification period, i.e., from birth to eight years. The poorer section of the population, especially those living in the high caries areas and who are physically below normal, should be assisted to improve their diet in regard to more meat, eggs, butter, milk and cheese. People should be encouraged, and assisted where necessary, to produce and to eat more of these agricultural products and not to barter them for rice, flour, sweets, etc. Excessive drinking of fruit juices and other acid beverages should be avoided. The improvement of the diet of the population as a whole, especially in regard to a higher protein intake, will not only reduce dental decay, but will also improve the health of the population, making it more resistant to other diseases.

(*b*) That a high-protein diet, which should include non-toxic quantities of fluorine in the drinking water, should be taken during pregnancy and lactation. Fluorine not exceeding 1 p.p.m., preferably in the drinking water, should be given to children during the post-natal calcification period.

(*c*) That more attention should be paid to oral hygiene. The teeth should be brushed regularly, especially after meals, and the mouth should be rinsed with an antiseptic mouthwash to reduce the number of acid-producing organisms.

(*d*) That regular visits should be paid to the dentist, to have decayed teeth filled or extracted, to prevent decay from spreading.

(*e*) That the value of these preventive dietetic measures and of oral hygiene should be stressed by means of dental health propaganda.

It is further recommended that the following investigations be carried out:—

(*a*) The effect of a low-carbohydrate high-protein diet during pregnancy and lactation on caries-susceptibility of the children.

(*b*) The effect of non-toxic amounts of fluorine taken during pregnancy and lactation on caries-susceptibility of the children.

(*c*) The effect of a low-carbohydrate, high-protein diet with non-toxic amounts of fluorine during pregnancy and lactation on caries-susceptibility of the children. These investigations could be conducted in ante-natal clinics.

(*d*) The effect of varying amounts of fluorine, topically applied, to a number of children in the high caries areas, to ascertain what amount and how many applications will be most beneficial to caries inhibition.

(*e*) The effect of different antiseptic mouthwashes on caries in the high caries areas.

(*f*) To determine the amino acids in saliva of caries-free children in the low caries areas and of saliva of children with rampant caries in the high caries areas.

APPENDICES

(*a*) Table giving a brief description of the geology of South Africa.

(*b*) Diagram showing the layers, etc., of the Matjes River Shelter.

(*c*) Table giving the details of the chemical analysis of teeth.

(*d*) Table giving the percentage caries, incidence pH values, fluorine content and total hardness of drinking water in a number of cities, towns, places and districts in South Africa.

(*e*) Statistical analysis of caries data.

APPENDIX (A)

The subjoined table gives a brief description of the systems and subordinate groups of rocks and their distribution in South Africa.

The table deals with the systems in sequence, commencing with the oldest formations. The Primitive System, divided into the Swaziland System and Pongola System, has been held to include all sedimentary and volcanic rocks believed to be older than the Witwatersrand System.

Vast areas of Namaqualand, Swaziland, Prieska, Kenhardt, Gordonia, North and Eastern Transvaal are covered by ancient granite and gneiss of this system.

System.	Subordinate Groups.	Type of Rocks.	Distribution.
MITIVE:			
Swaziland..	Jamestown Series.....	Basic schists, altered sediments and volcanics	Barberton.
	Tugela Series.....	Basic schists, altered sediments and volcanics	Natal.
	Onverwacht Series.....	Volcanic beds, cherts and basic schists.....	Komati Valley.
	Nondweni Series and Mfongosi Series	Volcanic beds, cherty quartzites, phyllites, ironstones and limestones	Northern Natal.
	Fig Tree Series.....	Variegated shales, banded ironstones, greywackes and grits	Barberton.
	Moodies Series.....	Quartzites, shales, lavas and conglomerates..	Barberton, Murchison Range, Mount Mare.
	Nkandhla Series...-.....	Schists and Quartzites.....	Zululand.
	Kheis Series:		
	(1) Marydale beds.....	Sedimentary and volcanic.....	Prieska, Kenhardt, Gordonia.
	(2) Kaaien beds.....	Quartzites and schists.....	
	(3) Wilgenhoutdrift beds	Sedimentary and volcanic.....	
(*b*) Pongola....	Lower Pongola Series.....	Quartzites, phyllites and lavas.....	Piet Retief, Northern Zululand, Wakkerstroom.
	Upper Pongola Series.....	Shales, quartzites, conglomerates and ferruginous beds	
WITWATERSRAND	LOWER:		
	(1) Dominion Reef Series	Lavas with basal arkose, quartzites and conglomerates	Southern Transvaal and adjoining Free State.
	(2) Hospital Hill Series..	Quartzites, shales, arkoses and ferruginous beds	Southern Transvaal and adjoining Free State.
	(3) Government Reef Series	Quartzites, shales, conglomerates.....	
	(4) Jeppestown Series....	Quartzites, shales and lavas.....	
	UPPER:		
	(1) Main-Bird Series.....	Quartzites, shales, lavas and conglomerates	Southern Transvaal and adjoining Free State.
	(2) Kimberley-Elsburg Series	Quartzites, shales and conglomerates.....	
VENTERSDORP....	Koras Series.....	Lavas, sandstones and conglomerates.....	Kenhardt and Gordonia.
	Zoetlief Series.....	Lavas, shales, quartzites and conglomerates..	Vryburg, Hopetown, Prieska, Klerksdorp and other areas in the Transvaal.
	Kuip Series.....	Lavas, sandstones, limestones, chert and arkose	Prieska.
	Pniel Series.....	Lavas, quartzites, shales and conglomerates	Southern Transvaal, Northern Free State, Prieska.
TRANSVAAL.....	Black Reef Series.....	Quartzites, shales and conglomerates.....	Large areas in Transvaal and Niewerust, Cape.
	Cango beds.....	Conglomerates, slates, quartzites and limestones	Oudtshoorn.
	Malmesbury beds.....	Quartzites, shales and limestones.....	Malmesbury.
	Dolomite or Campbell-Rand Series	Dolomite and shales.....	Transvaal and Northern Cape.
	Pretoria Series.....	Quartzites and shales.....	Transvaal and Griqualand West.
	Rooiberg Series.....	Quartzites, shales and felsitic lavas.....	Transvaal.
	INTRUSION OF BUSHVELD IGNEOUS COMPLEX, CENTRAL AND NORTHERN TRANSVAAL		
WATERBERG.....	Gamagara Series.....	Quartzites, conglomerates and shales.....	Postmasburg.
	Klipheuwel beds.....	Quartzites, conglomerates and shales.....	Western Province.
	Matsap Series.....	Conglomerates, quartzites, lavas and tuffs....	Griqualand West and Prieska.
	Waterberg Series.....	Sandstones, shales and conglomerates.....	Waterberg, Zoutpansberg, Pretoria and Middelburg.

System.	Subordinate Groups.	Type of Rocks.	Distribution.
CAPE.....	Table Mountain Series....	Quartzitic sandstones and shales.....	Cape Peninsula ranges, flanking Karroo, south and west.
	Bokkeveld Series.....	Shales and sandstones.....	Ceres, north and south of Zwartberg range.
	Witteberg Series.....	Quartzites and shales.....	Between Touws River and Matjesfontein.
KARROO.....	Dwyka Series.....	Tillite and shales.....	Central Cape, most of the Free State, S.E. Transvaal and western portion of Natal.
	Ecca Series.....	Sandstones and shales.....	
	Beaufort Series.....	Sandstones and shales.....	
	Stormberg Series.....	Sandstones and shales and volcanics.....	
INTRUSION OF BASIC ROCKS (MOSTLY DOLERITE) THROUGHOUT THE UNION			
CRETACEOUS.....	Uitenhage Series.....	Conglomerates, sandstones, shales, limestones, sand, clays	South and east coasts.
TERTIARY.....	Marine inshore beds of Alexandria, Addo, Bredasdorp, Zululand, West Coast and lignites of Knysna	Sand, gravels, clays.....	Alexandria, Addo, Bredasdorp, Zululand, Knysna and West Coast.
RECENT.....	Recent and subrecent coastal and inland sands, alluvium and limestones	Ironstones, gravels, sands, surface limestones, tufas and surface quartzites	Many areas in South Africa.

APPENDIX (B)

LAYERS, ETC., OF THE MATJES RIVER SHELTER

Natural Deposits.	Cave Deposits.	By whom Inhabited.	Caries.
Black Sand	Ashes and bedding.....	Modern Bushmen, Hunters (diet: meat).....	Slight.
	Principally Mytilus *shells*.....	Bushmen hybrids, "Strandlopers", Fishermen (diet: mostly shellfish)	Extensive caries.
	Wilton Layer, *black soil* (with little or no shells)	Primitive North African or Southern European race.....	No caries.
	Ashes.....	Hybrid race with cremation ceremony.....	No caries.
	Hard ashes.....	Prehistoric Bushmen, hunters (diet: meat).....	No caries.
	Mossel Bay layer.		
	Mossel Bay period.		
	Mossel Bay culture.		
	Some shells; mostly Domax.		

CORRELATION IN TIME BY MEANS OF IMPLEMENTS

Layer of hard red sand:—Stellenbosch culture

Lime deposits

APPENDIX (C)

DETAILS OF CHEMICAL ANALYSIS OF TEETH

District.	Carious Enamel.					Non-carious Enamel.				
	No. of Sample.	CaO %.	MgO %.	P_2O_5 %.	F p.p.m.	No. of Sample.	CaO %.	MgO %.	P_2O_5 %.	F p.p.m.
Calvinia	—	—	—	—	—	1	51·2	0·72	40·1	250
Carnarvon	—	—	—	—	—	2	48·9	0·66	40·5	700
	—	—	—	—	—	3	48·5	0·73	39·0	620
Humansdorp	4	51·86	0·79	N.D.	200	5	53·33	0·64	N.D.	180
	6	50·12	0·64	N.D.	60	7	49·09	0·86	N.D.	165
Kenhardt	—	—	—	—	—	8	49·27	0·77	N.D.	750
Knysna	9	52·15	0·76	N.D.	180	16	50·46	0·72	N.D.	130
	10	49·94	0·83	N.D.	100	—	—	—	—	—
	11	51·3	0·68	41·1	70	—	—	—	—	—
	12	51·5	0·58	41·0	160	—	—	—	—	—
	13	49·5	0·68	40·8	230	—	—	—	—	—
	14	50·3	0·66	41·2	70	—	—	—	—	—
	15	48·8	0·76	39·0	310	—	—	—	—	—
Potchefstroom	17	50·2	0·68	39·6	100	19	50·2	0·7	40·7	70
	18	48·3	0·85	39·3	140	—	—	—	—	—
Sutherland	—	—	—	—	—	20	50·7	0·81	39·9	200
	—	—	—	—	—	21	50·0	0·56	39·9	150
Waterberg	22	51·0	0·60	40·7	200	24	50.0	0·75	40·8	200
	23	48·2	0·80	38·8	200	25	49·4	0·93	40·0	100
	—	—	—	—	—	26	50·3	0·68	41·1	140
	—	—	—	—	—	27	49·7	0·5	35·4	1,104

District.	Carious Dentine.					Non-carious Dentine.				
	No. of Sample.	CaO %.	MgO %.	P_2O_5 %.	F p.p.m.	No. of Sample.	CaO %.	MgO %.	P_2O_5 %.	F p.p.m.
Calvinia	—	—	—	—	—	28	37·5	2·2	30·1	500
	—	—	—	—	—	29	38·0	2·16	28·3	500
Carnarvon	—	—	—	—	—	30	37·9	1·67	31·2	900
	—	—	—	—	—	31	37·3	1·88	28·9	980
	—	—	—	—	—	32	39·7	1·37	31·7	980
Humansdorp	33	37·2	1·48	29·8	200	37	38·5	1·28	30·0	200
	34	38·13	1·73	N.D.	150	38	39·25	1·71	N.D.	300
	35	37·85	2·14	N.D.	340	39	39·95	2·08	N.D.	320
	36	37·73	0·86	N.D.	200	40	37·62	1·3	N.D.	200
Kenhardt	—	—	—	—	—	41	39·35	1·3	N.D.	1,700
Knysna	42	39·87	1·72	N.D.	370	47	38·1	1·5	N.D.	320
	43	37·75	1·57	N.D.	250	—	—	—	—	—
	44	37·8	1·52	29·6	140	—	—	—	—	—
	45	38·9	1·61	31·2	200	—	—	—	—	—
	46	39·4	1·14	31·9	520	—	—	—	—	—
Potchefstroom	48	36·6	1·42	29·9	200	51	38·6	1·5	29·8	330
	49	37·6	1·41	28·9	250	52	35·9	1·34	28·7	400
	50	40·0	1·25	30·9	200	—	—	—	—	—
Sutherland	—	—	—	—	—	53	38·3	1·0	30·0	130
	—	—	—	—	—	54	38·0	1·22	28·8	350
Waterberg	55	38·2	1·35	30·9	90	57	38·7	1·52	30·8	230
	56	40·1	1·45	30·0	320	58	39·0	1·34	30·3	250
	—	—	—	—	—	59	36·5	2·1	27·4	2,347

N.D.—Not Determined.

APPENDIX (D)

PERCENTAGE CARIES, ETC. OF PLACES IN SOUTH AFRICA

Place.	Per cent. Caries.	pH.	F p.p.m.	Total Hardness as $CaCO_3$.
Van Wyksvlei	26	7·4	1·9	1,460
Loeriesfontein	27	7·6	1·26	2,118
Nieuwerust	30	7·4	2·95	1,105
Brandvlei	32	7·6	1·76	890
Rietbron	35	7·6	3·31	61
Calvinia	56	7·2	1·6	120
Richmond (Cape)	57	7·7	1·1	300
Murraysburg	59	7·8	0·29	535
Hanover	60	7·8	0.48	340
Nieuwoudtville	60	7·4	0·05	1,055
Carnarvon	62	7·7	0·99	200
Shannon	65	8·7	4·54	63
Williston	67	7·6	0·42	200
Dewetsdorp	69	8·0	4·8	30
Edenville	70	7·8	1·0	33
Sutherland	71	7·5	2·7	244
Ventersburg	71	7·1	0·63	293
Van Rhynsdorp	72	7·7	0·10	640
Delareyville	73	7·4	0·29	435
Bloemhof	75	7·6	0·14	380
Victoria West	75	7·7	0·08	750
Venterstad	77	7·6	0·21	256
De Aar	78	7·4	0·15	1,334
Philippolis	79	7·8	0·29	440
Trompsburg	79	8·6	0·52	150
Cradock	80	8·2	0·65	256
Bethulie	81	7·6	0·2	233
Marquard	81	7·5	0·23	180
Beaufort West	82	7·6	0·15	396
Klipplaat	82	7·6	0·61	530
Pietersburg	82	7·6	0·56	295
Rouxville	82	9·0	0·47	23
Warden	82	6·9	1·25	256
Zeerust	82	8·0	0·19	498
Burgersdorp	84	7·5	0·32	260
Messina	84	7·6	0·19	80
Steynsburg	84	7·6	0·11	240
Sterkstroom	84	8·2	0·8	255
Graaff-Reinet	85	7·9	0·55	160
Heilbron	85	7·4	0·4	355
Ladybrand	85	8·6	0·16	49
Smithfield	85	7·4	0·19	261
Laingsburg	86	7·0	0·57	355
Vryburg	86	7·5	Nil	320
Christiana	87	7·4	0·19	530
Hopetown	87	7·5	0·19	365
Ottoshoop	87	8·5	0·24	115
Schweizer Reneke	87	7·6	0·24	515
Theunissen	87	7·1	0·38	145
Winburg	87	7·3	0·32	285
Bloemfontein	88	7·5	0·58	90
Hobhouse	88	7·7	0·42	23
Wepener	88	7·35	0·19	185
Vredefort	89	7·2	0·29	185
Potchefstroom	90	7·6	0·23	379
Klerksdorp	91	7·4	0·19	175
Kuruman	91	7·3	0·07	182
Potgietersrust	91	7·4	Nil	336
Rustenburg	91	7·7	0·1	513
Fauresmith	92	7·9	0·49	300
Weenen	92	8·0	0·22	412
Zastron	92	8·8	0·13	123
Koster	93	7·4	0·05	113
Malmesbury	93	7·4	0·53	27
Reitz	93	8·7	0·31	215
Vereeniging	93	7·1	0·8	110
Kimberley	94	8·7	0·11	123

APPENDIX (D)—continued.

Place.	Per cent. Caries.	pH.	F p.p.m.	Total Hardness as $CaCO_3$.
Molteno	94	7·8	0·7	434
Porterville	94	7·3	0·4	25
Clanwilliam	95	7·0	0·57	16
Lichtenburg	95	6·8	0·57	345
Nelspruit	95	6·6	0·29	58
Nylstroom	95	7·8	0·04	40
Vrede	95	7·5	0·38	183
Vryheid	95	7·3	0·08	13
Bulwer	96	6·9	0·08	18
Louis Trichardt	96	7·0	0·07	70
Lydenburg	96	7·7	0·19	35
Paarl	96	6·5	Nil	28
Pretoria	96	7·9	0·38	130
Richmond (Natal)	96	7·2	0·17	10
Standerton	96	7·1	0·8	110
Underberg	96	7·4	Nil	8
Weza	96	8·6	0·17	10
East London	97	6·7	0·5	120
Empangeni	97	7·7	0·08	78
Eshowe	97	6·8	0·04	20
Harrismith	97	6·6	0·53	25
Johannesburg	97	7·1	0·8	110
Pietermaritzburg	97	7·3	0·03	19
Cape Town	98	5·4	0·76	15
Durban	98	6·0	0·57	52
Mossel Bay	98	6·1	0·3	40
Oudtshoorn	98	7·6	0·23	15
White River	98	6·8	0·23	18
Worcester	98	6·0	Nil	15
Bredasdorp	99	7·1	0·3	10
Knysna	99	5·6	0·3	98
Port Elizabeth	99	7·2	0·21	43
Riversdale	99	7·1	0·4	12
Swellendam	99	7·0	0·38	10
Uitenhage	99	8·1	0·32	40
Umtata	99	6·9	0·5	30
Caledon	100	7·1	0·38	25
George	100	5·9	0·4	30
Herbertsdale	100	7·3	0·08	36
Humansdorp	100	6·9	0·27	73
Kingwilliamstown	100	7·3	0·5	30
DISTRICTS.				
Kenhardt	20	7·4	3·25	1,086
Namaqualand	25	7·3	2·15	1,891
Gordonia	28	7·5	2·92	914
Carnarvon	38	7·2	1·12	537
Williston	42	7·5	1·21	314
Calvinia	43	7·3	1·3	1,116
Hanover	53	7·8	0·48	340
Murraysburg	53	7·8	0·53	482
Richmond (Cape)	54	7·7	0·92	318
Sutherland	54	7·5	2·7	244
Prieska	59	7·5	0·59	587
Colesberg	60	7·7	0·79	331
Aberdeen	61	8·0	0·91	445
Phillipstown	62	7·8	0·56	514
Hopetown	64	7·5	0·39	671
Beaufort West	65	7·6	0·6	593
Zoutpansberg	66	7·1	0·8	370
Jansenville	67	7·5	0·69	565
Potgietersrust	67	7·5	0·8	393
Rustenburg	69	7·3	0·81	286
Dewetsdorp	70	8·0	3·9	91
Bloemfontein	70	7·9	1·12	252
Kuruman	70	7·4	0·21	517
Venterstad	71	7·6	0·28	482
Bloemhof	72	7·6	0·14	380
Prince Albert	72	7·4	0·86	334

APPENDIX (D)—continued.

Place.	Per cent. Caries.	pH.	F p.p.m.	Total Hardness as $CaCO_3$.
Rouxville	72	8·9	0·6	105
Maraisburg	73	7·8	0·38	661
Edenburg	74	8·5	1·35	193
Laingsburg	74	7·0	0·6	426
Philippolis	74	8·0	0·63	345
Victoria West	74	7·5	1·0	593
Cradock	75	8·2	0·82	303
De Aar	76	7·4	0·33	339
Pietersburg	76	7·7	0·41	346
Waterberg	77	7·7	0·8	207
Bothaville	78	7·4	0·8	148
Vryburg	78	7·6	0·28	373
Smithfield	79	7·5	0·19	261
Albert	80	7·6	0·39	213
Bethulie	80	7·5	0·23	363
Pretoria	80	7·3	0·8	229
Sterkstroom	80	8·2	0·8	225
Steynsburg	80	7·6	0·29	263
Marico	81	8·1	0·31	336
Senekal	81	7·5	0·23	215
Trompsburg	82	8·6	0·9	150
Fauresmith	83	7·5	0·5	312
Lichtenburg	84	7·3	0·36	316
Schweizer Reneke	84	7·6	0·24	359
Thaba 'Nchu	84	7·7	0·5	117
Winburg	84	8·1	0·3	205
Ladybrand	85	8·7	1·1	80
Wepener	85	7·4	0·19	185
Zastron	85	8·7	0·17	152
Christiana	86	7·4	0·32	466
Heilbron	86	7·7	0·58	198
Molteno	88	7·8	0·7	389
Potchefstroom	88	7·6	0·26	379
Vredefort	88	7·2	0·34	182
Malmesbury	90	7·3	0·31	39
Klerksdorp	91	7·4	0·19	194
Alfred	92	8·6	0·17	10
Reitz	92	8·7	0·4	201
Harrismith	93	6·6	0·38	25
Oudtshoorn	93	7·2	0·31	15
Vrede	93	7·5	0·38	183
Vryheid	93	7·3	0·3	68
Lydenburg	94	7·7	0·27	60
Polela	94	6·9	0·29	18
George	96	6·0	0·4	36
Pietermaritzburg	97	7·3	0·03	19
Lower Umfolozi	97	7·7	0·08	80
Uitenhage	97	8·1	0·3	40
Underberg	97	7·4	Nil	18
Eshowe	98	6·8	0·4	20
Humansdorp	98	6·6	0·17	44
Umtata	98	6·9	0·5	30
Bredasdorp	99	7·1	0·3	10
Kingwilliamstown	99	7·3	0·5	30
Knysna	99	5·6	0·3	28
Mossel Bay	99	6·5	0·19	29
Richmond (Natal)	99	7·2	0·17	10
Riversdale	99	7·1	0·4	12
Swellendam	99	7·0	0·38	10
Caledon	100	7·1	0·38	25

APPENDIX (E)

STATISTICAL ANALYSIS OF CARIES DATA

(1) The object of this analysis is to express, in terms of correlation co-efficients, the relationship between the incidence of caries and the fluorine content, total hardness as $CaCO_3$ and pH value of drinking water. The variables employed are denoted by the following symbols:—

x_1 = per cent. Caries.
x_2 = Fluorine.
x_3 = $CaCO_3$.
x_4 = pH.

Small letters are employed to denote deviations from the respective means.

The theory of correlation can be found in a number of text-books, but for ready reference and for the sake of refreshing the memory, we select the following well-known equations as being directly applicable to the case under consideration:—

$$r^2_{12} = \frac{(x_1 x_2)^2}{x_1^2 x_2^2} \quad (1)$$

$$r^2_{12\cdot3} = \frac{(r_{12} - r_{13} r_{33})^2}{(1-r^2_{13})(1-r^2_{23})} \quad (2)$$

$$r^2_{12\cdot34} = \frac{(r_{12\cdot4} - r_{13\cdot4} r_{23\cdot4})^2}{(1-r_{13\cdot4})(1-r_{23\cdot4})} \quad (3)$$

$$1 - r^2_{1\cdot234} = (1-r^2_{12})(1-r^2_{13\cdot2})(1-r^2_{14\cdot23}) \quad (4)$$

In the above expressions a symbol such as r_{12} denotes a *total* correlation coefficient and measures the amount of correlation between the variables x_1 (caries) and x_2 (fluorine), disregarding possible effects of the remaining variables. It is calculated from the original data. If positive it can vary from 0 to +1 and then indicates that both variables increase (or decrease) simultaneously; if negative it ranges from −1 to 0 and then indicates inverse relationship.

The symbol $r_{12\cdot3}$ denotes a *partial correlation* and measures the relationship between x_1 (caries) and x_2 (fluorine) when taking the simultaneous variation of x_3 ($CaCO_3$) into account. Similarly $r_{12\cdot34}$ is also a partial correlation between x_1 and x_2 when taking into account the simultaneous variation of both x_3 and of x_4. Any partial correlation coefficient can also vary between −1 and +1.

A symbol such as $r_{1\cdot234}$ denotes a multiple correlation coefficient, and measures the relationship between the actual value of x_1 and the estimated value of x_1 based upon the variation of x_2, x_3 and x_4, and its possible values lie between 0 and +1 only.

It will readily be seen that once the total correlation coefficients (six of them in our case) have been determined from the original data, equations (2) to (4) may be used to calculate all the required partial and multiple correlation coefficients.

(2) TOTAL CORRELATION COEFFICIENTS

	Places.	Districts.
r_{12}	−0·570*	−0·662*
r_{13}	−0·678*	−0·811*
r_{14}	−0·237*	−0·191
r_{23}	−0·132	0·457*
r_{24}	0·123	0·132
r_{34}	0·153	0·683*

* Highly significant.

It is notable that the chemical variables (2, 3, 4) for *places* show a negligible correlation, whereas r_{23} and r_{34} are highly significant for the *districts*. Whether this must be ascribed to the fact that in some cities and towns the drinking water is treated whilst, in the districts, the water is obtained directly from boreholes is not quite clear.

(3) Partial Correlation Coefficients of the Second Order

	Places.	Districts.
$r_{12 \cdot 34}$	−0·604**	−0·936**
$r_{13 \cdot 24}$	−0·735**	−0·905**
$r_{14 \cdot 23}$	−0·201*	+0·913**

* Significant. ** Highly significant.

This table is the principal aim of the analysis and shows that when due allowance is made for the interaction of the chemical factors considered, the per cent. caries depends in the case of *places* mainly on fluorine and the hardness of the water, but in the case of *districts* it also depends to a very marked extent upon the pH concentration.

(4) Multiple Correlation Coefficients

	Places.	Districts.
$r_{1 \cdot 23}$	0·833**	0·874**
$r_{1 \cdot 234}$	0·841**	0·981**

** Highly significant.

In the case of *places* the inclusion of pH as one of the variables gives only a slight improvement, from 0·833 to 0·841, in the value of the multiple correlation coefficient and confirms the previous evidence that it is not of great importance. In the case of *districts*, however, the inclusion of pH increases the value of the multiple correlation coefficient from 0·874 to the remarkably high value of 0·981. The highly significant values obtained must serve as ample justification for the attempt to explain the variation of caries in terms of the considered chemical factors, the only point needing further elucidation being the discrepancy of the influence of pH as between places and districts.

(5) Partial Correlations of the First Order

For the sake of completeness the following table is appended, which confirms the previous evidence that the interdependence of the chemical factors is significant for districts but not for places.

	Places.	Districts.
$r_{12 \cdot 3}$	−0·659**	−0·559**
$r_{12 \cdot 4}$	−0·561**	−0·654**
$r_{13 \cdot 2}$	−0·74**	−0·762**
$r_{13 \cdot 4}$	−0·669**	−0·949**
$r_{14 \cdot 2}$	−0·237*	−0·139
$r_{14 \cdot 3}$	−0·22	0·849**
$r_{23 \cdot 4}$	0·115	0·507**
$r_{24 \cdot 3}$	0·105	−0·277*
$r_{34 \cdot 2}$	0·14	0·706**

* Significant. ** Highly significant.

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MAP
showing
ENDEMIC FLUOROSIS AREAS
and areas in which Fluorides occur in
WATER - in SOUTH AFRICA

LEGEND

Less than	1 p.p.m	Fluorine ◎
1 to 2	"	" o
2 to 3	"	" ×
3 to 6	"	" □
6 and more	"	" +
Fluorine not determined		•

Place names — Pella

TRANSVAAL

ORANGE FREE STATE

NATAL

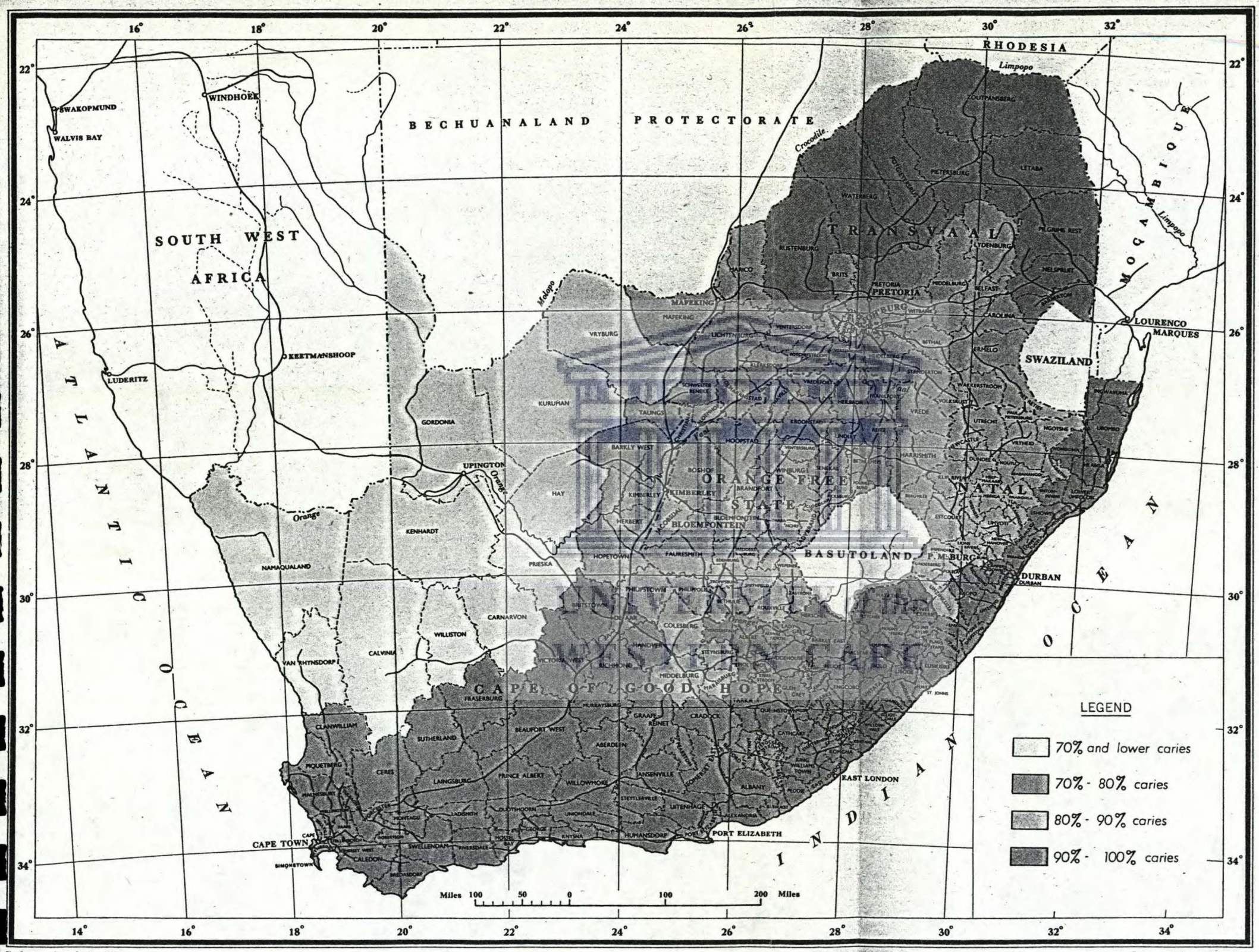
BASUTOLAND

CAPE OF GOOD HOPE

Pietersburg
Warmbaths
Pilansberg
Pretoria
Johannesburg
Theunissen
Bloemfontein
Trompsburg
Durban
Upington
Pella
Kenhardt
Springbok
Van Wyksvlei
Brandvlei
Garies
Williston
Van Rhynsdorp
Cradock
Graaff-Reinet
Jansenville
East London
Piquetberg
Port Elizabeth
Cape Town

Scale in Miles
100 50 0 100 200 300 400

UNION OF SOUTH AFRICA



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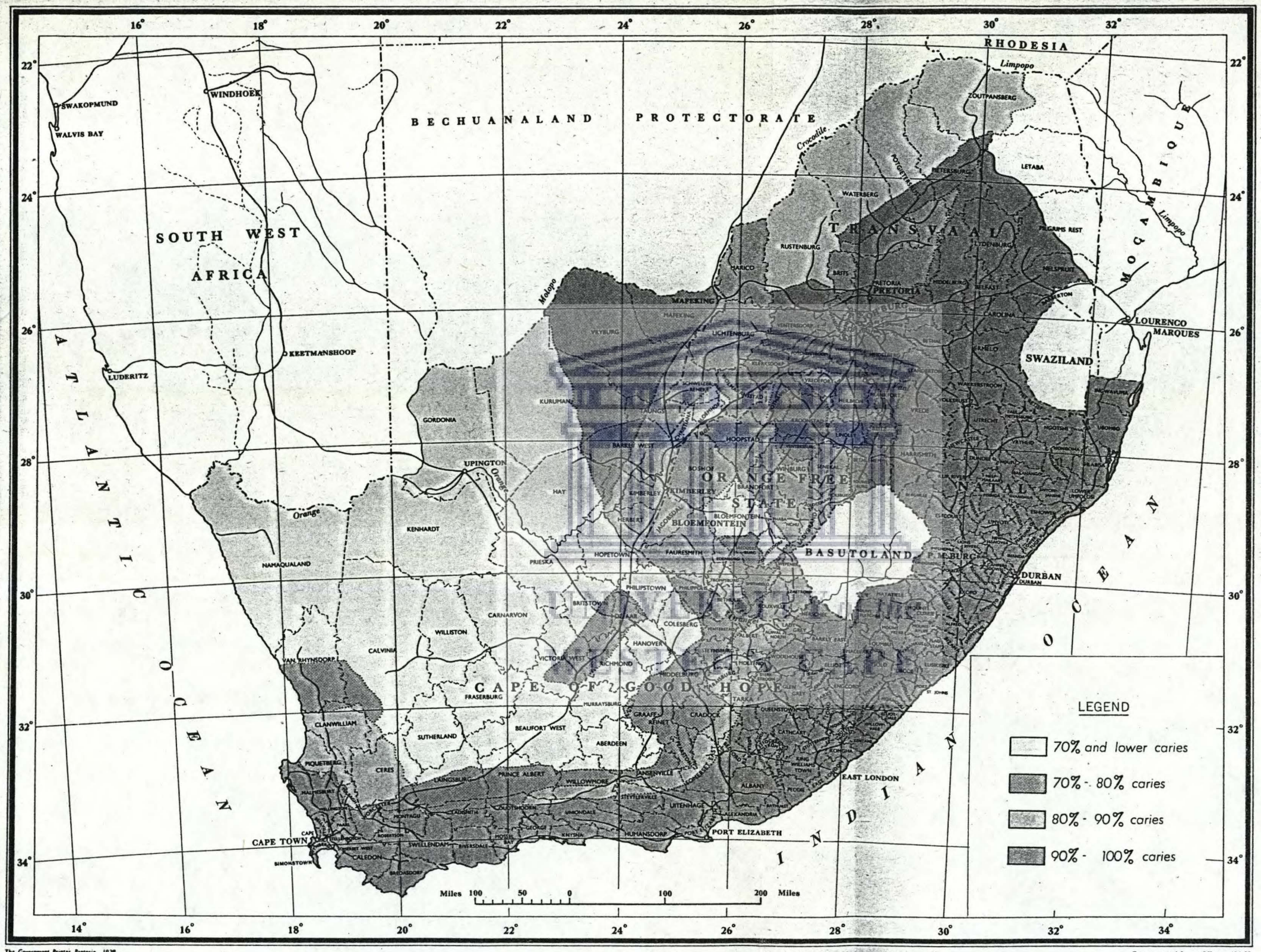
UNION OF SOUTH AFRICA



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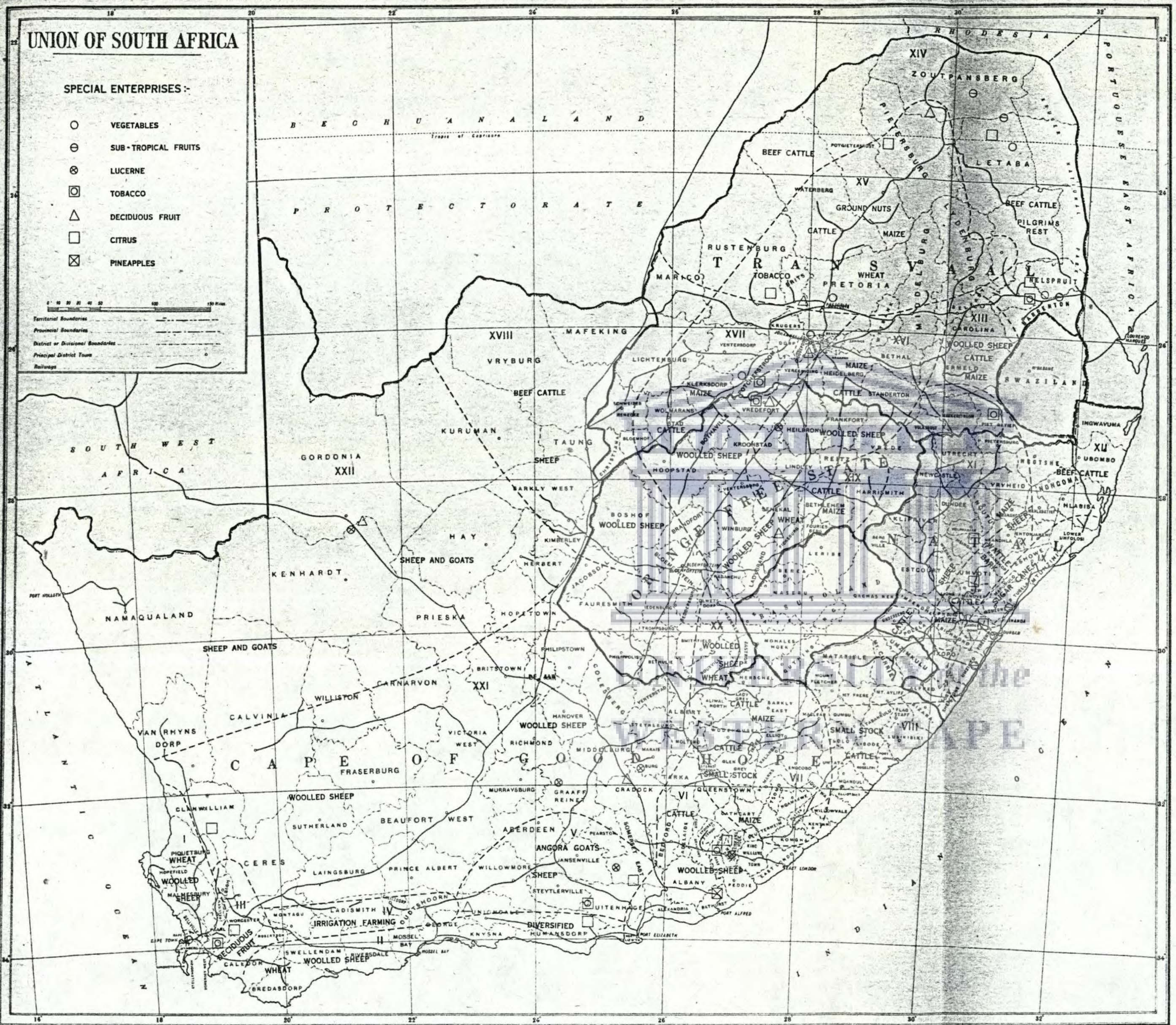
THE TYPICAL AGRICULTURAL REGIONS OF THE UNION.



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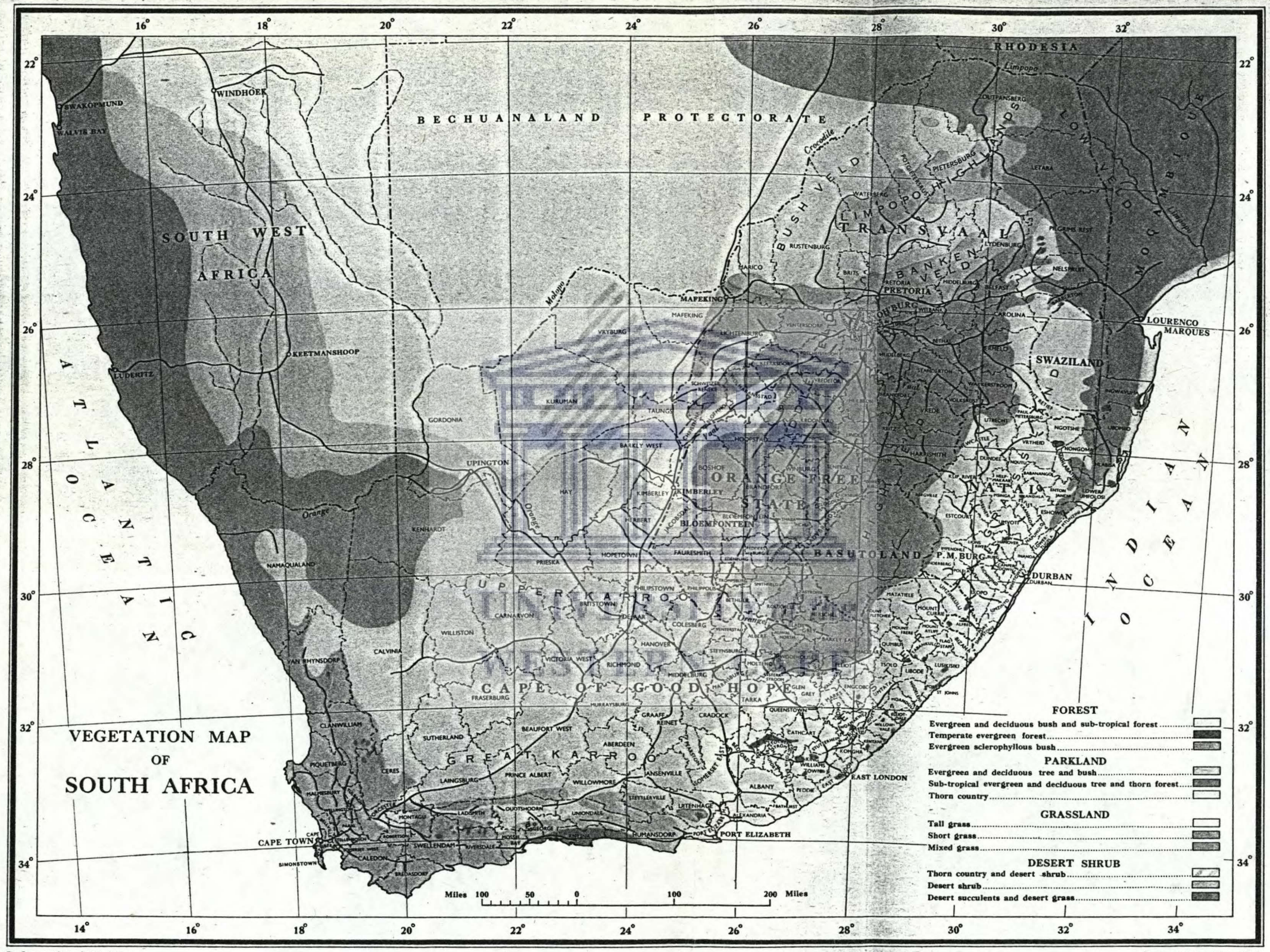
VEGETATION MAP OF SOUTH AFRICA



FOREST

- Evergreen and deciduous bush and sub-tropical forest
- Temperate evergreen forest
- Evergreen sclerophyllous bush

PARKLAND

- Evergreen and deciduous tree and bush
- Sub-tropical evergreen and deciduous tree and thorn forest
- Thorn country

GRASSLAND

- Tall grass
- Short grass
- Mixed grass

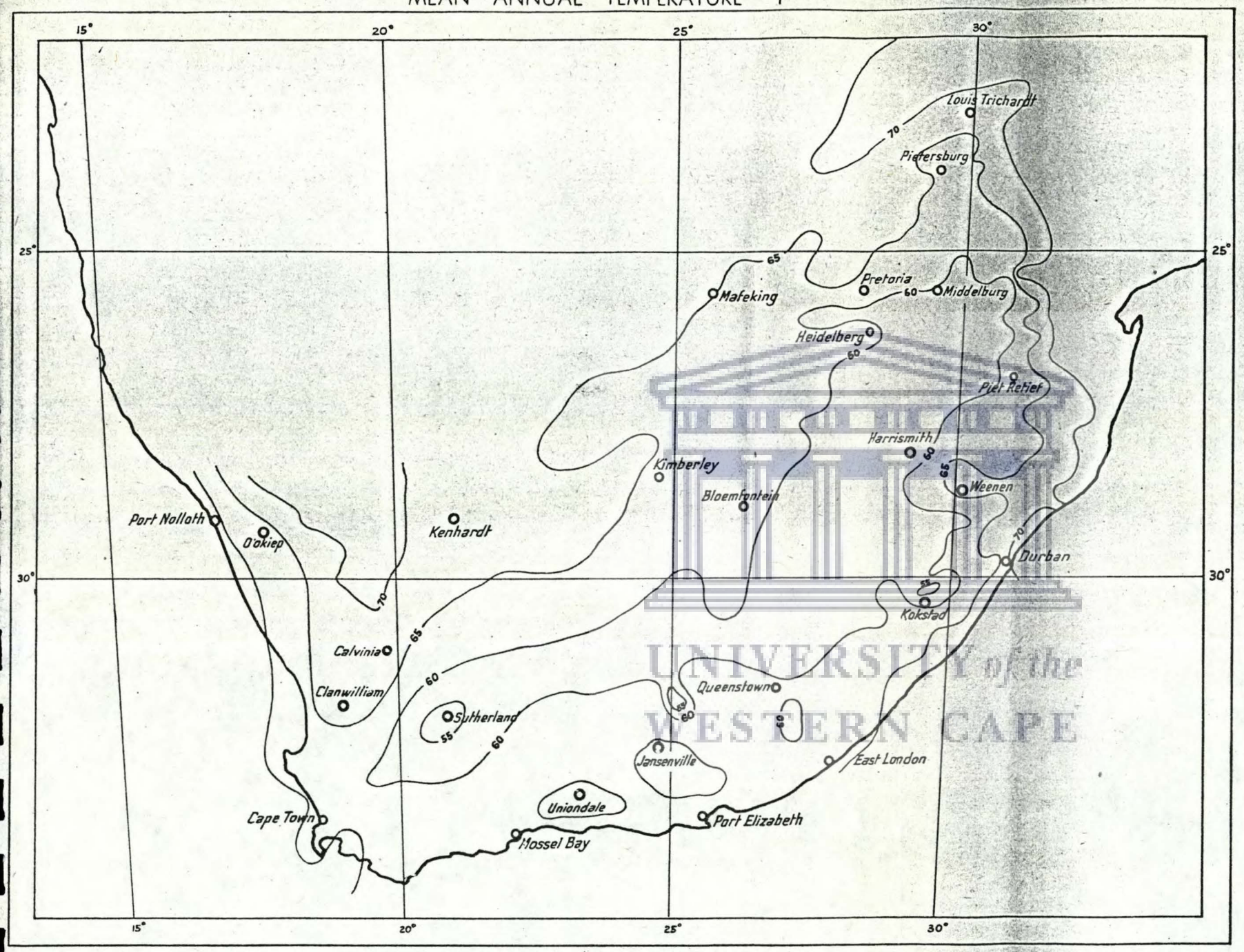
DESERT SHRUB

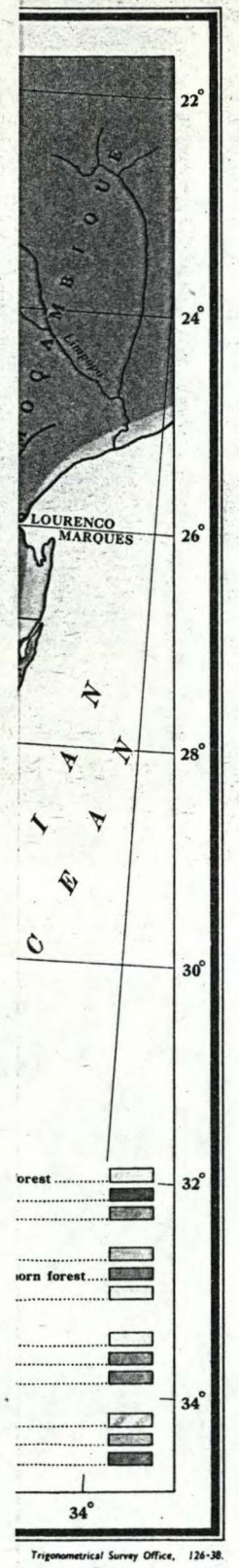
- Thorn country and desert shrub
- Desert shrub
- Desert succulents and desert grass

The Government Printer, Pretoria. 1938.

Trigonometrical Survey Office, 126-38.

MEAN ANNUAL TEMPERATURE °F





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PHYSICAL RELIEF MAP OF THE UNION OF SOUTH AFRICA

A.D.LEWIS. M.A., M.Inst.C.E.
Director of Irrigation

REFERENCE

10,000 FEET
8,000 ..
6,000 ..
5,000 ..
4,000 ..
3,000 ..
2,000 ..
1,000 ..
SEA LEVEL
100 FATHOMS

SCALE OF STATUTE MILES
100 50 0 50 100 150 200

BECHUANALAND PROTECTORATE
SOUTH WEST AFRICA
S. RHODESIA
MOCAMBIQUE
SWAZILAND
BASUTOLAND
ORANGE FREE STATE
NATAL
ATLANTIC OCEAN
INDIAN OCEAN
CAPE OF GOOD HOPE

WINDHOEK, WALVIS BAY, GOBABIS, SEROWE, MESSINA, LOUIS TRICHARDT, TSANE, MOLEPOLOLE, PIETERSBURG, NYLSTROOM, GIBEON, MAFEKING, PRETORIA, MIDDELBURG, LOURENÇO MARQUES, KEETMANSHOOP, LÜDERITZ BAY, KURUMAN, KROONSTAD, CHRISTIANA, KIMBERLEY, UPINGTON, ORANGE, BLOEMFONTEIN, PRIESKA, SPRINGBOK, BETHULIE, DURBAN, CARNARVON, CALVINIA, QUEENSTOWN, BEAUFORT WEST, SOMERSET EAST, EAST LONDON, CAPE TOWN, PORT ELIZABETH, MOSSEL BAY

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