

In developing a method for categorizing colonies of Streptococcus spp. on the basis of morphology, it was considered that a system that could be reproduced by the personnel concerned with the work was preferable to adopting, in detail, criteria used by others. However, descriptions of streptococcal colony morphology and, where available, experimental conditions used by Clarke,⁽¹⁹⁴⁾ Krasse,⁽¹⁹⁵⁾ Fitzgerald et alii.,⁽¹⁹⁶⁾ de Stoppelaar et alii.,⁽¹⁹⁷⁾ Zinner and Jablon,⁽¹⁹⁸⁾ Shklair et alii.,⁽¹⁹⁹⁾ (200) Jordan,⁽²⁰¹⁾ Jordan et alii.,⁽¹⁶⁵⁾ Littleton et alii.,⁽¹⁶⁹⁾ and Edwardsson,⁽¹⁷⁴⁾ were carefully reviewed together with criteria used in a previous study in New South Wales by ourselves.⁽²⁰⁾ Typical strains isolated in the course of the latter study and trial specimens of plaque were processed under the proposed experimental conditions and the colonies were examined in detail and classified. These procedures, together with painstaking examination of the initial New Guinea cultures led to the development of 12 principal colony categories, to one of which most of the colonies were assigned. Infrequently occurring colony forms which were proven to be streptococci but could not be classed into any of the 12 categories were placed into a 'miscellaneous' group.

Colonies representative of the 12 categories are illustrated in Figure 22, pages 178 - 182.

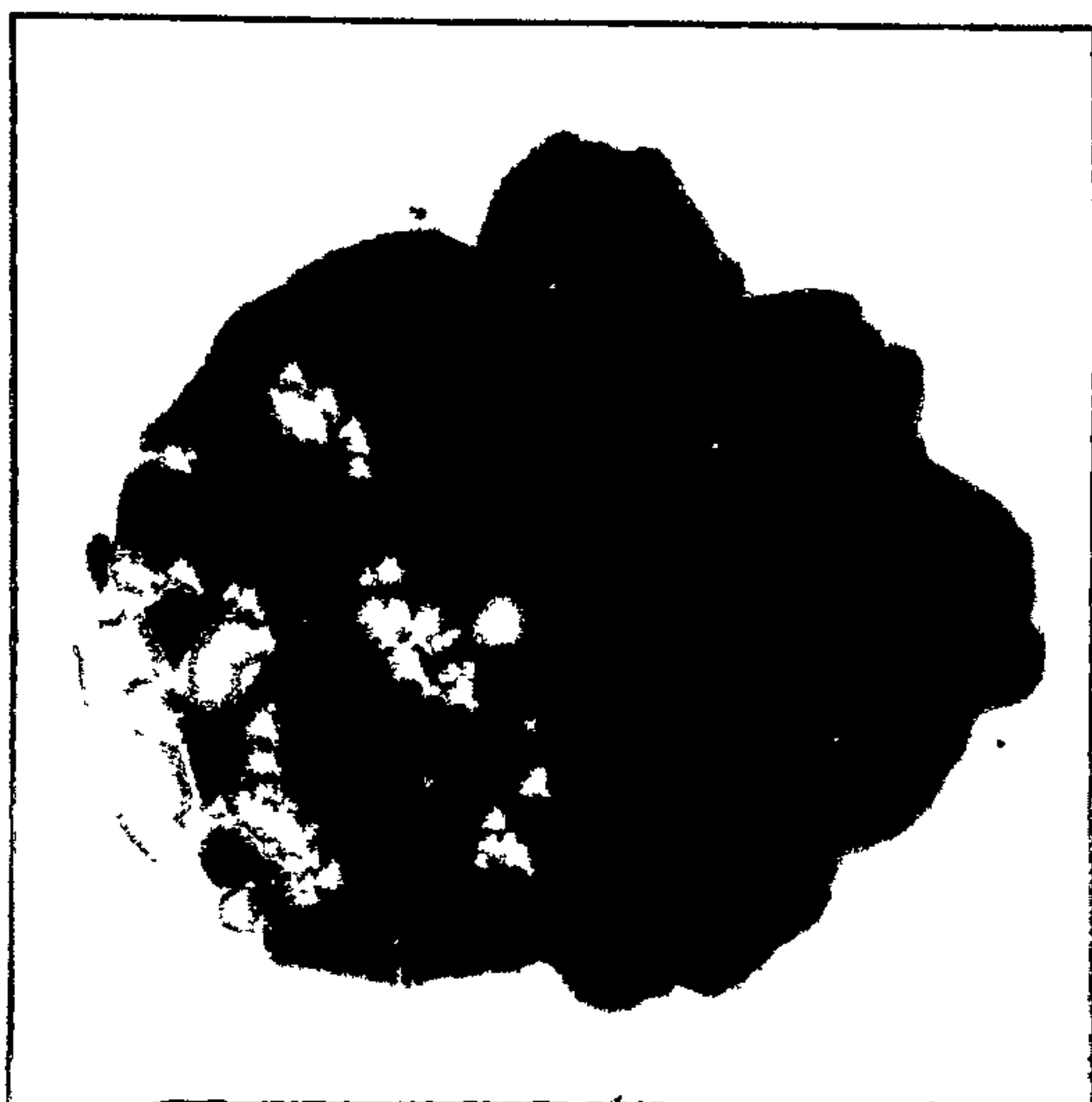
Summaries of the main morphological characteristics of the 12 major streptococcal colony types, most of which include sub-varieties not detailed here, are given below:

Colony type 1.

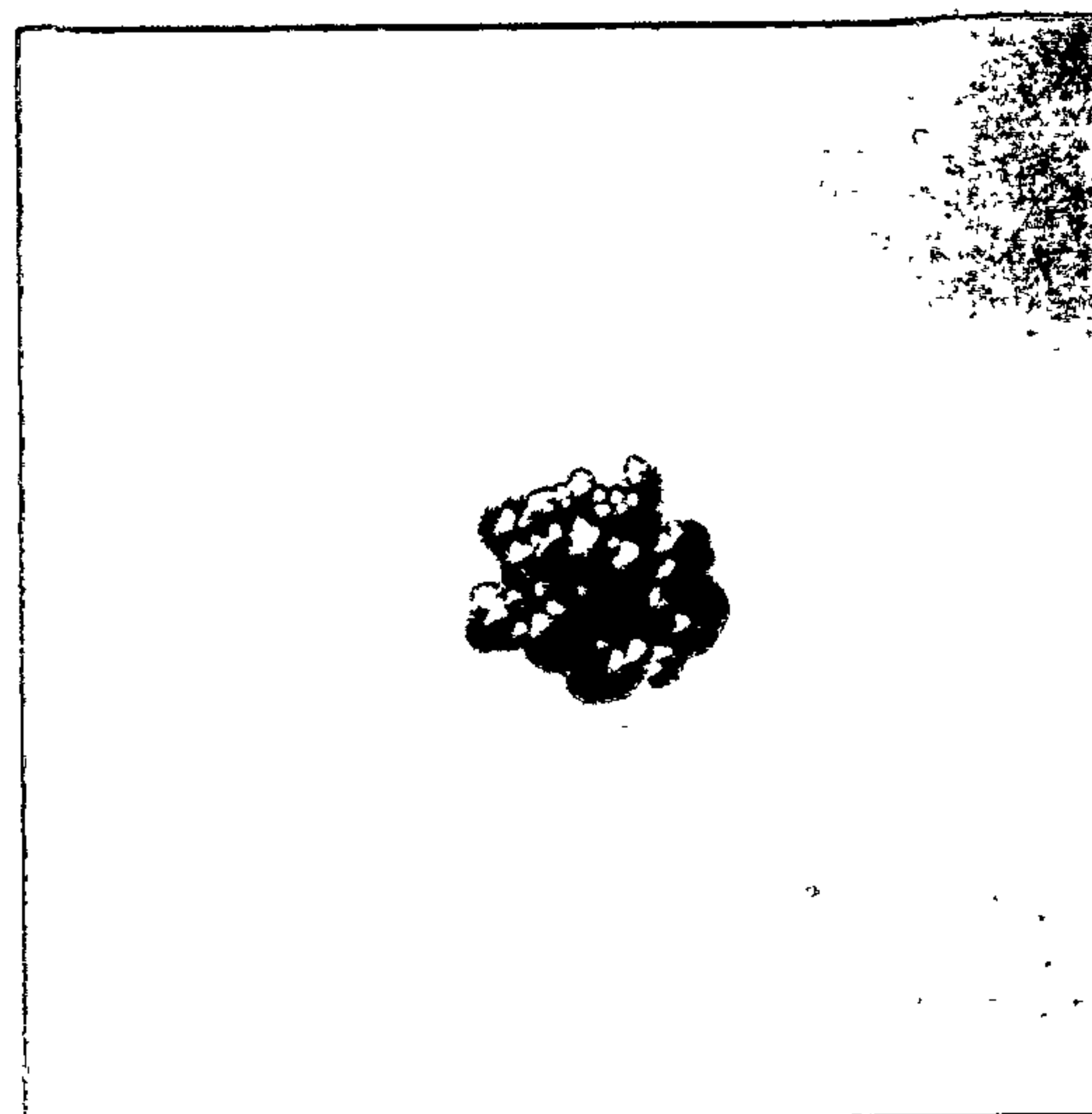
Colony form: Mostly circular colony outline with slightly undulating margin. Resembles a mulberry.

FIGURE 22

STREPTOCOCCAL COLONY TYPES



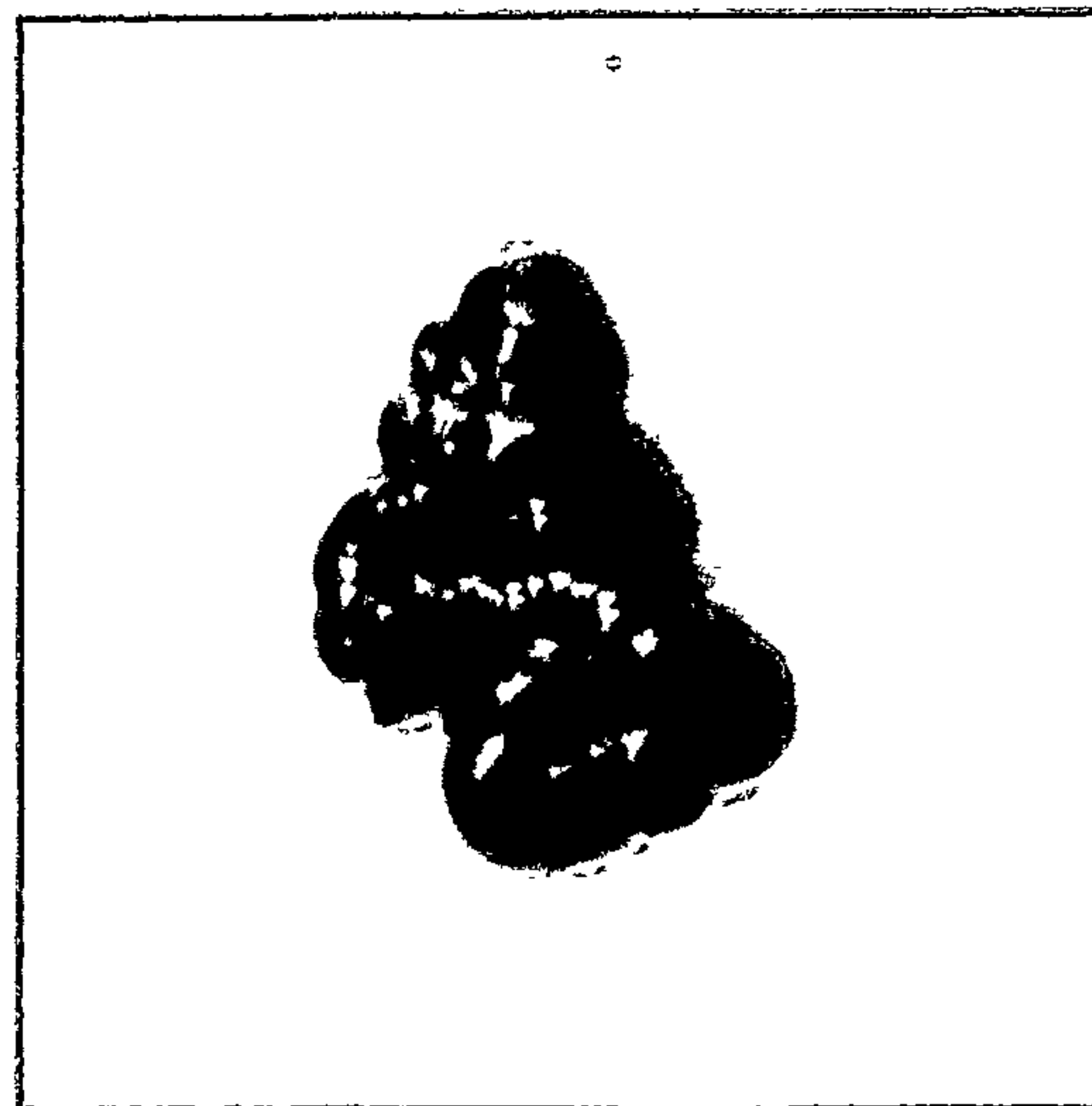
Colony type 1: Magnification 120x



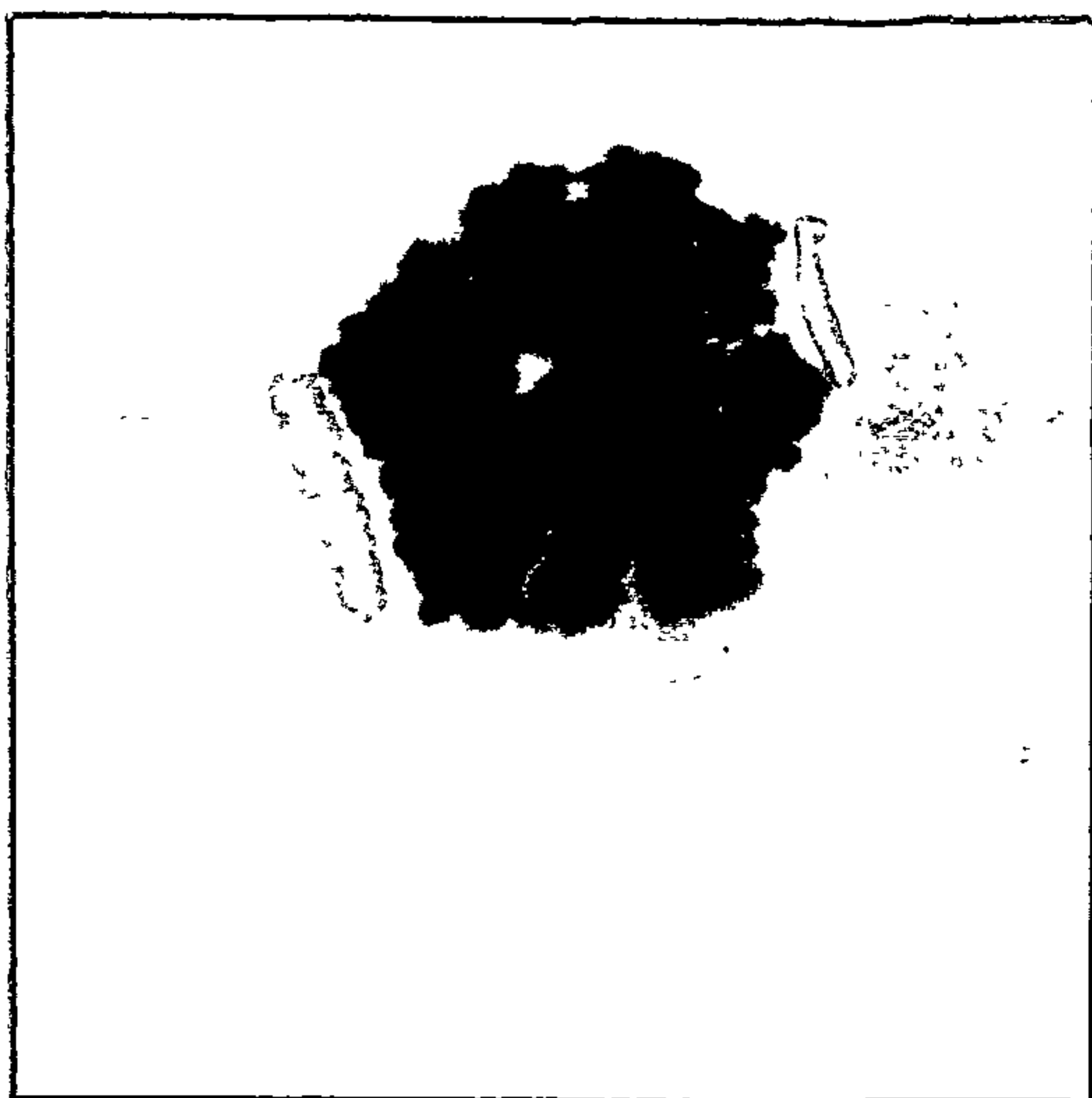
Colony type 2: Magnification 30x



Colony type 2: Magnification 70x



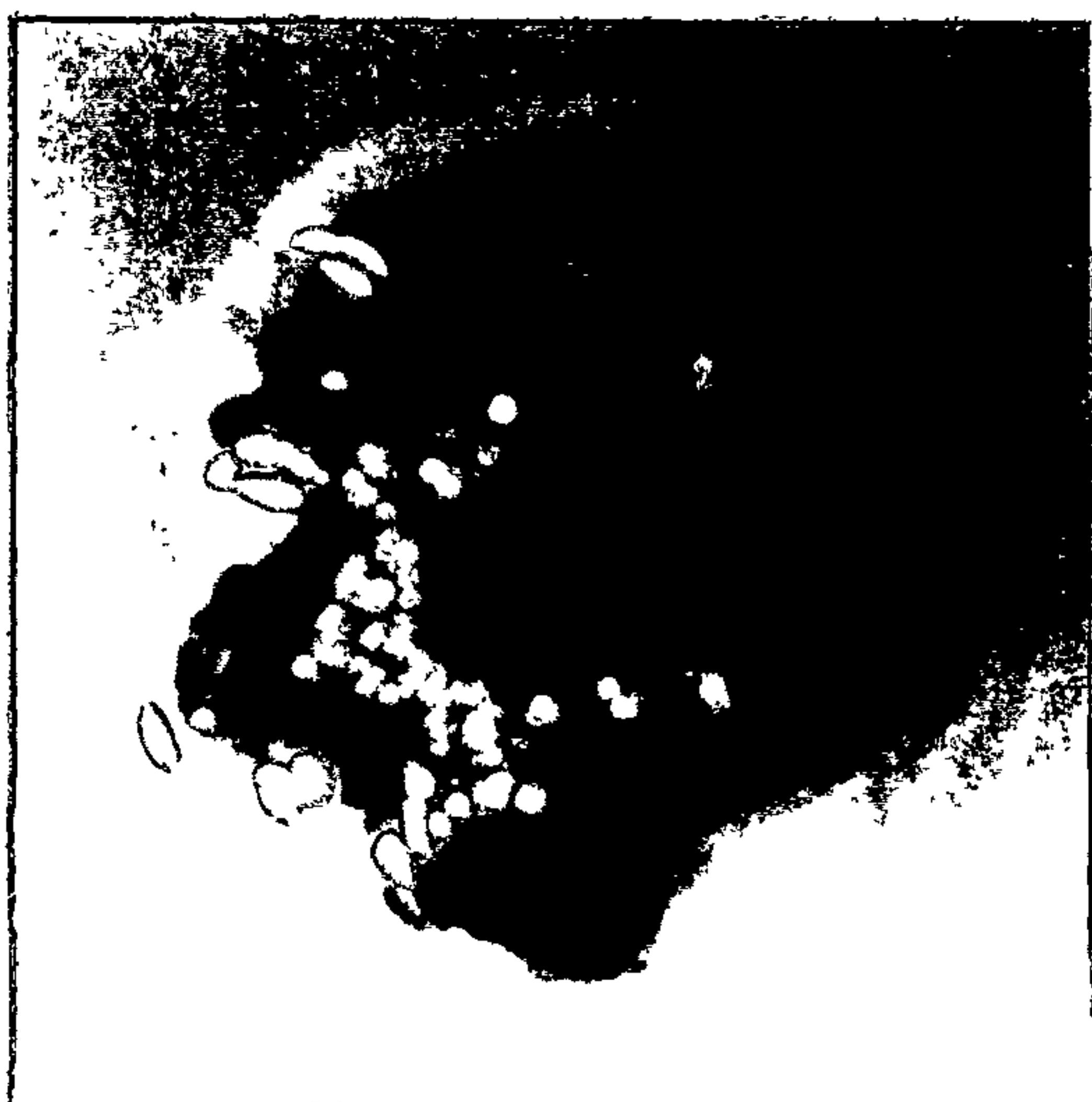
Colony type 3: Magnification 40x



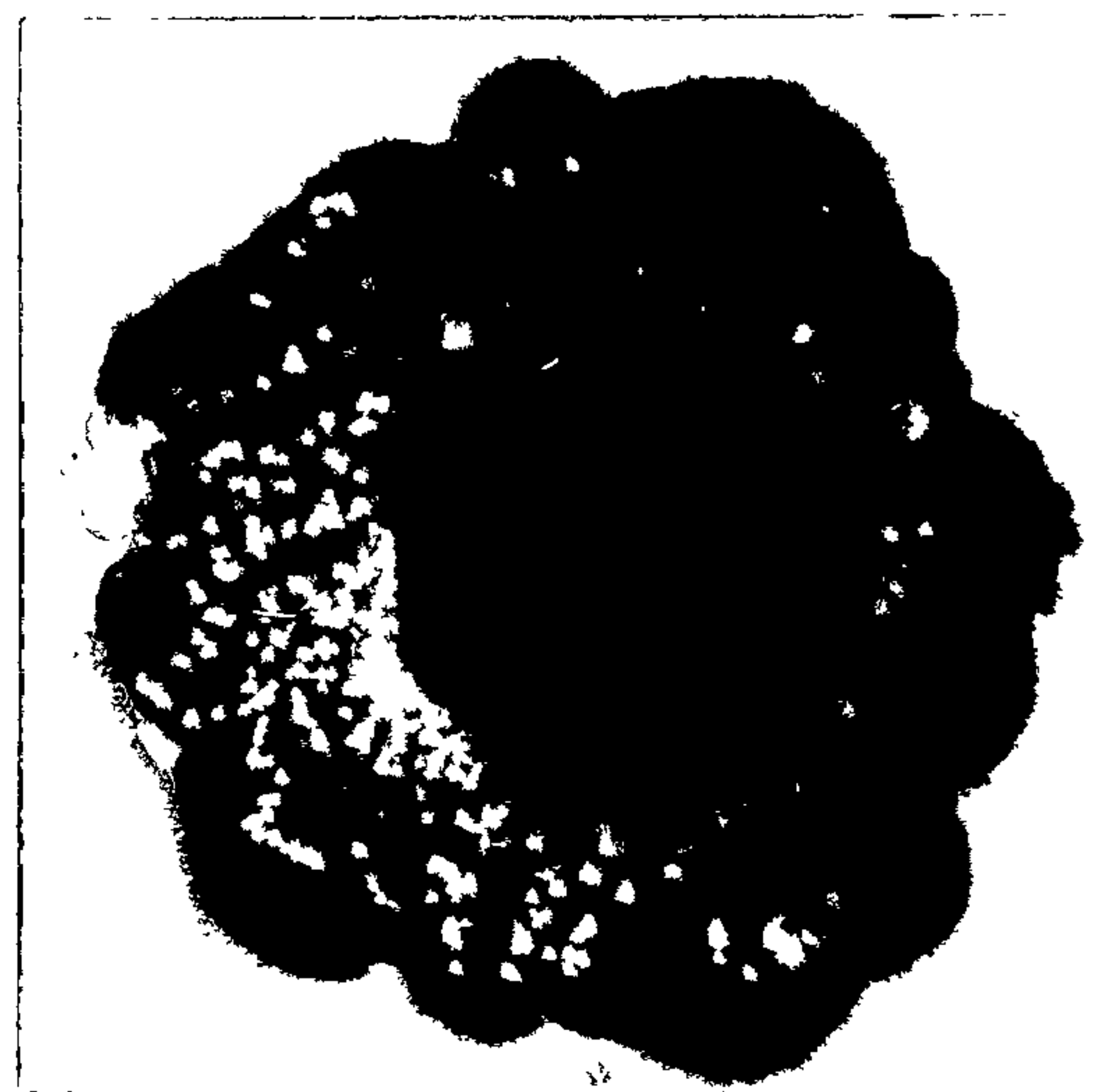
Colony type 4: Magnification 30x



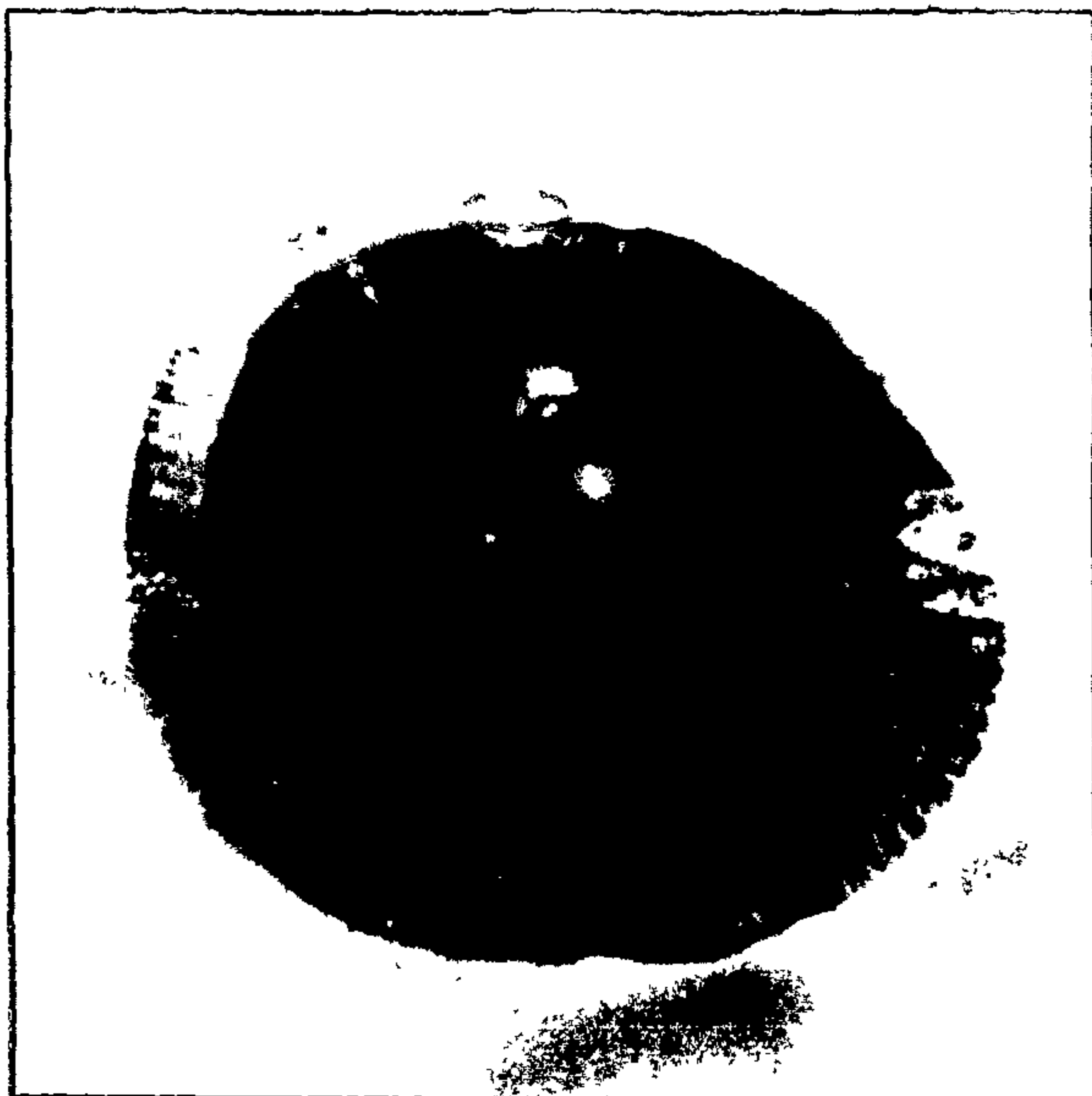
Colony type 5: Magnification 40x



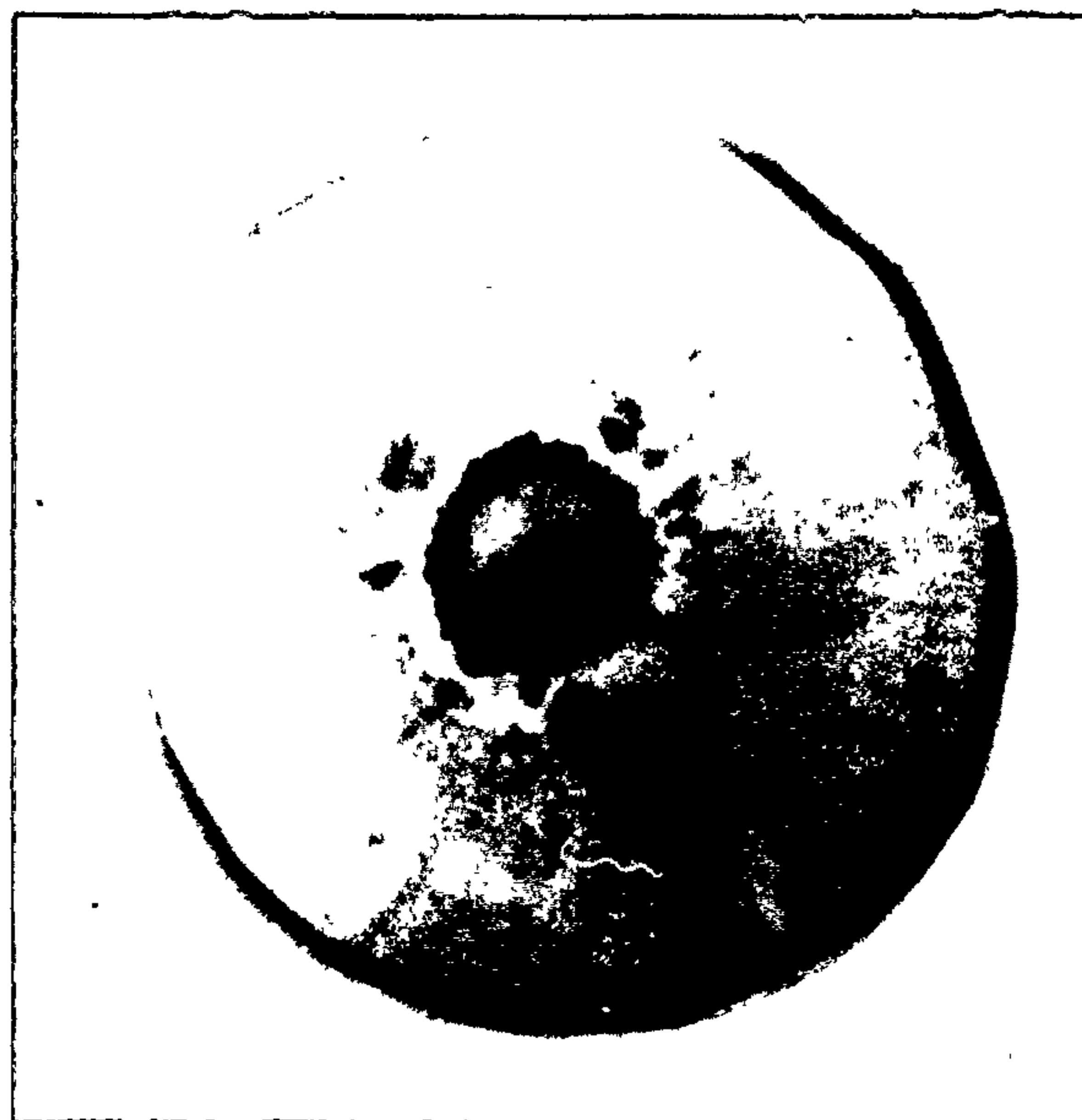
Colony type 5: Magnification 50x



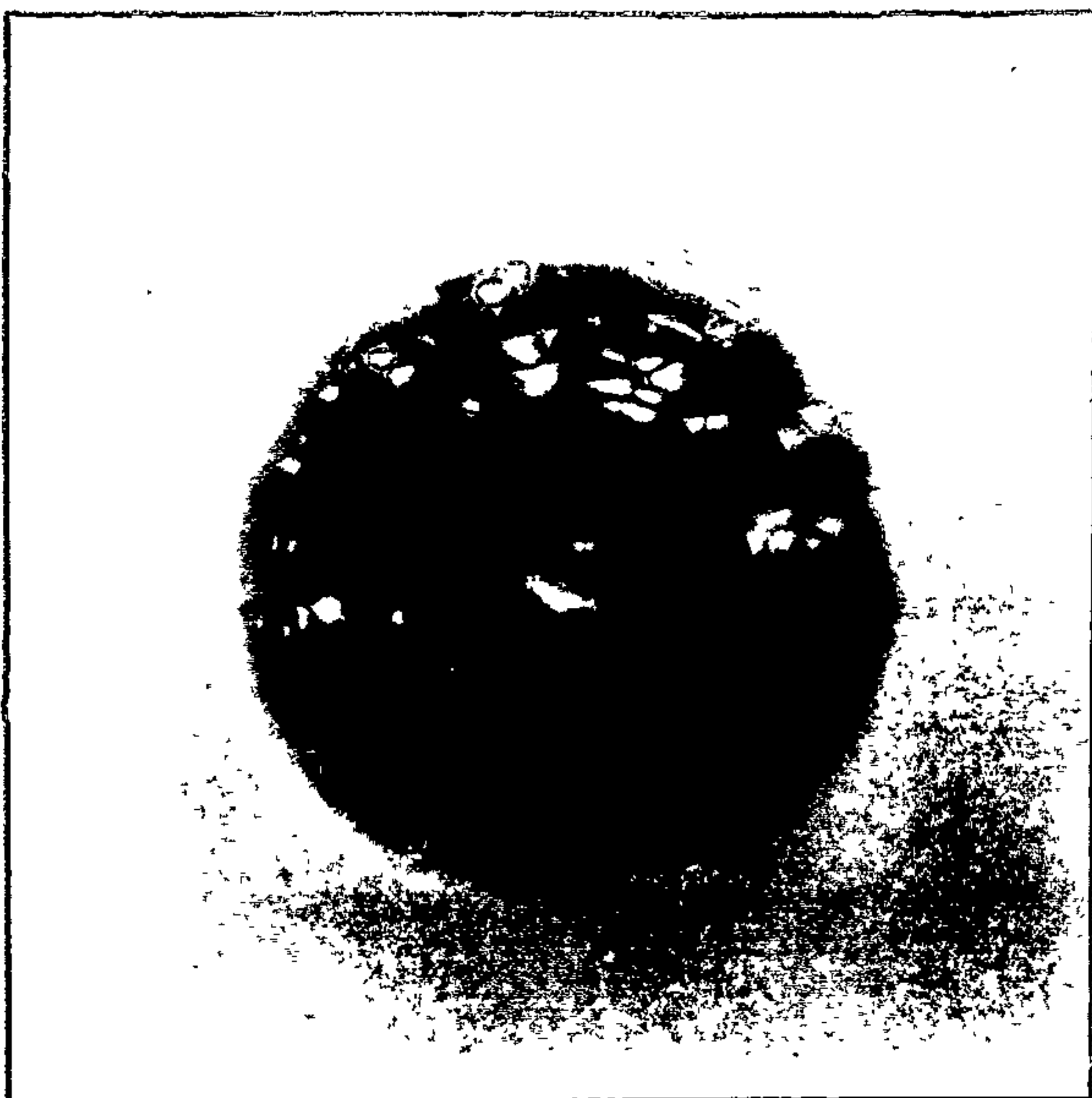
Colony type 5: Magnification 50x

FIGURE 22 (Ctd.)

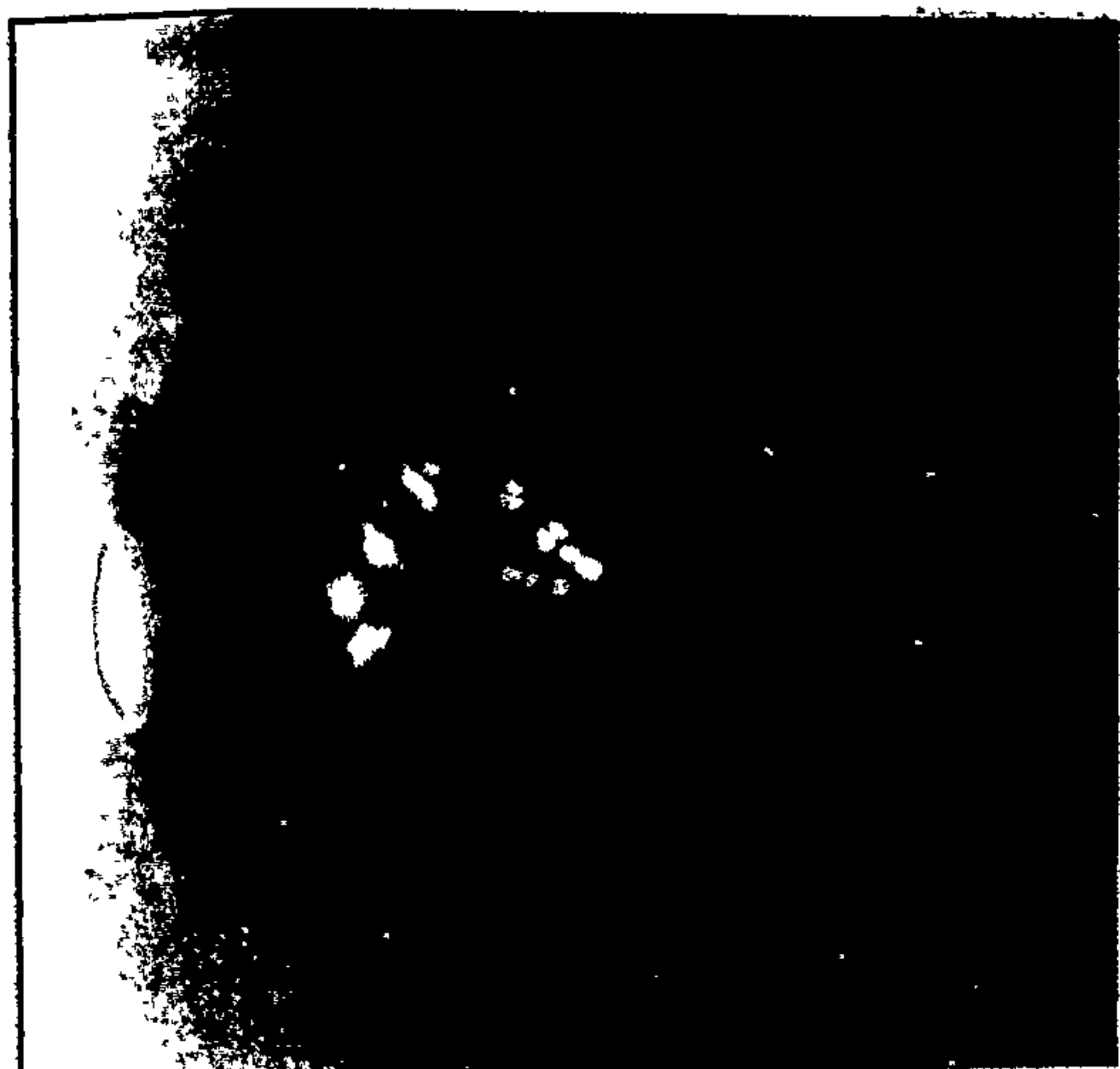
Colony type 6: Magnification 30x



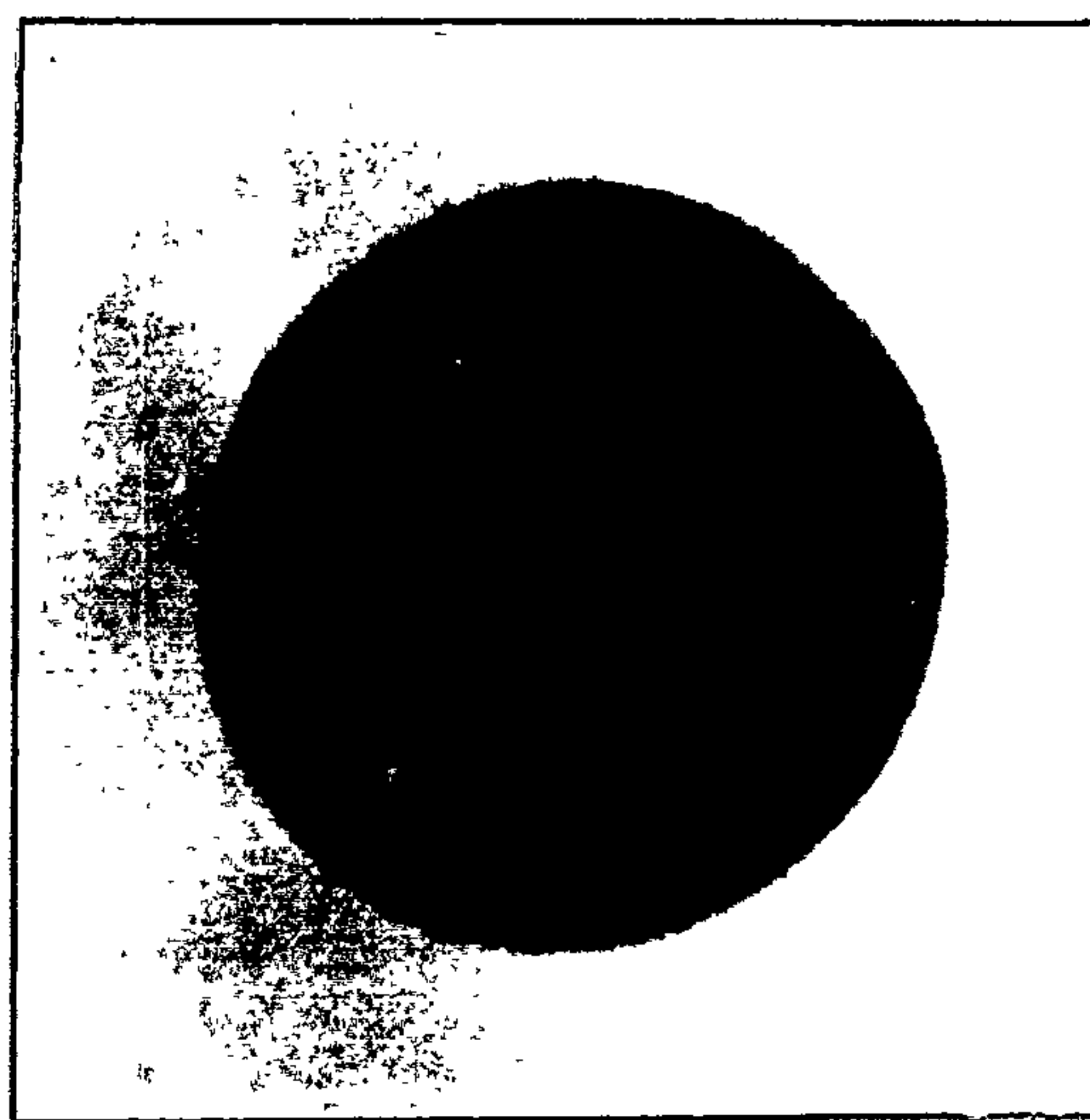
Colony type 6: Magnification 30x



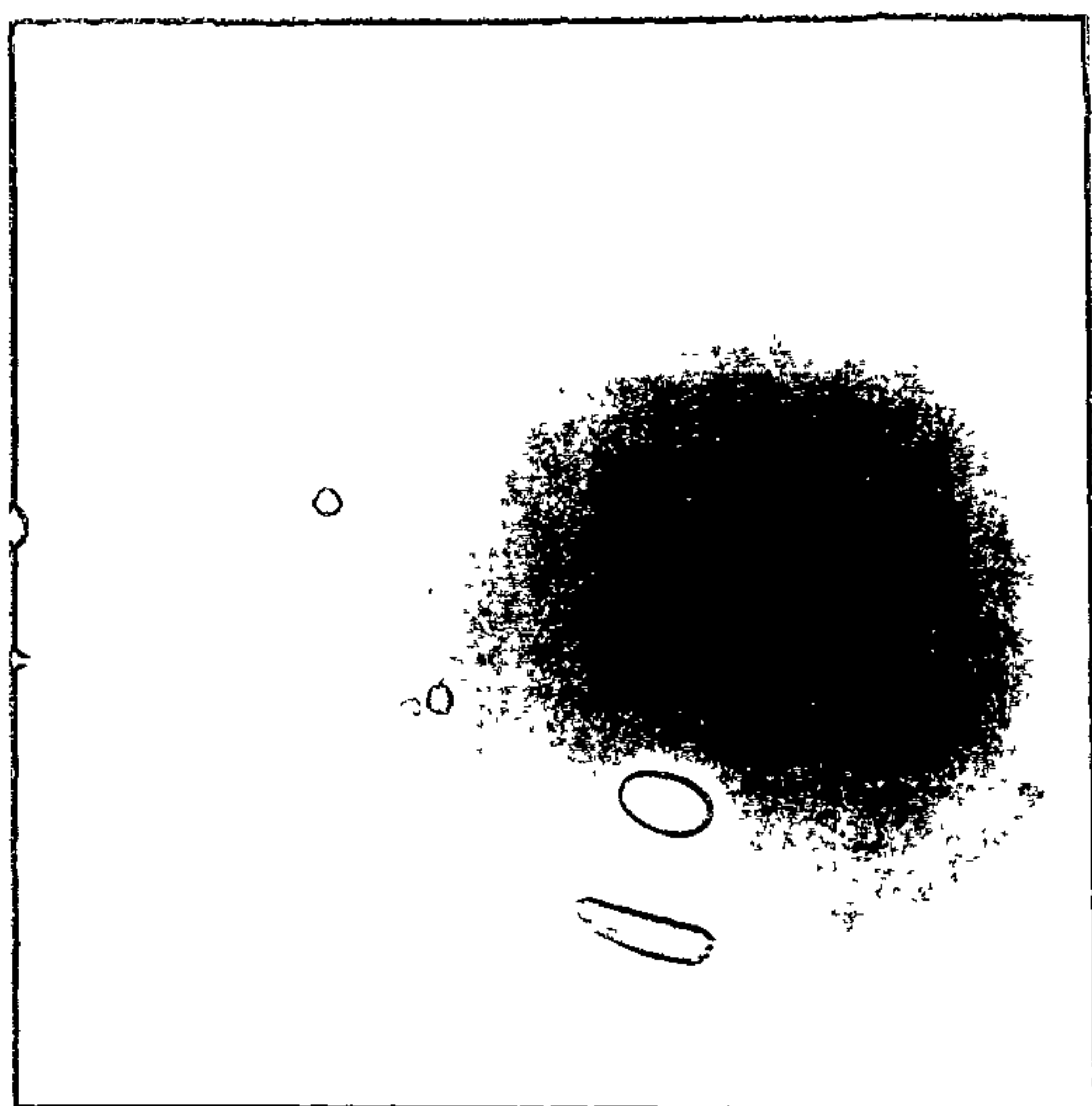
Colony type 7: Magnification 15x

FIGURE 22 (Ctd.)

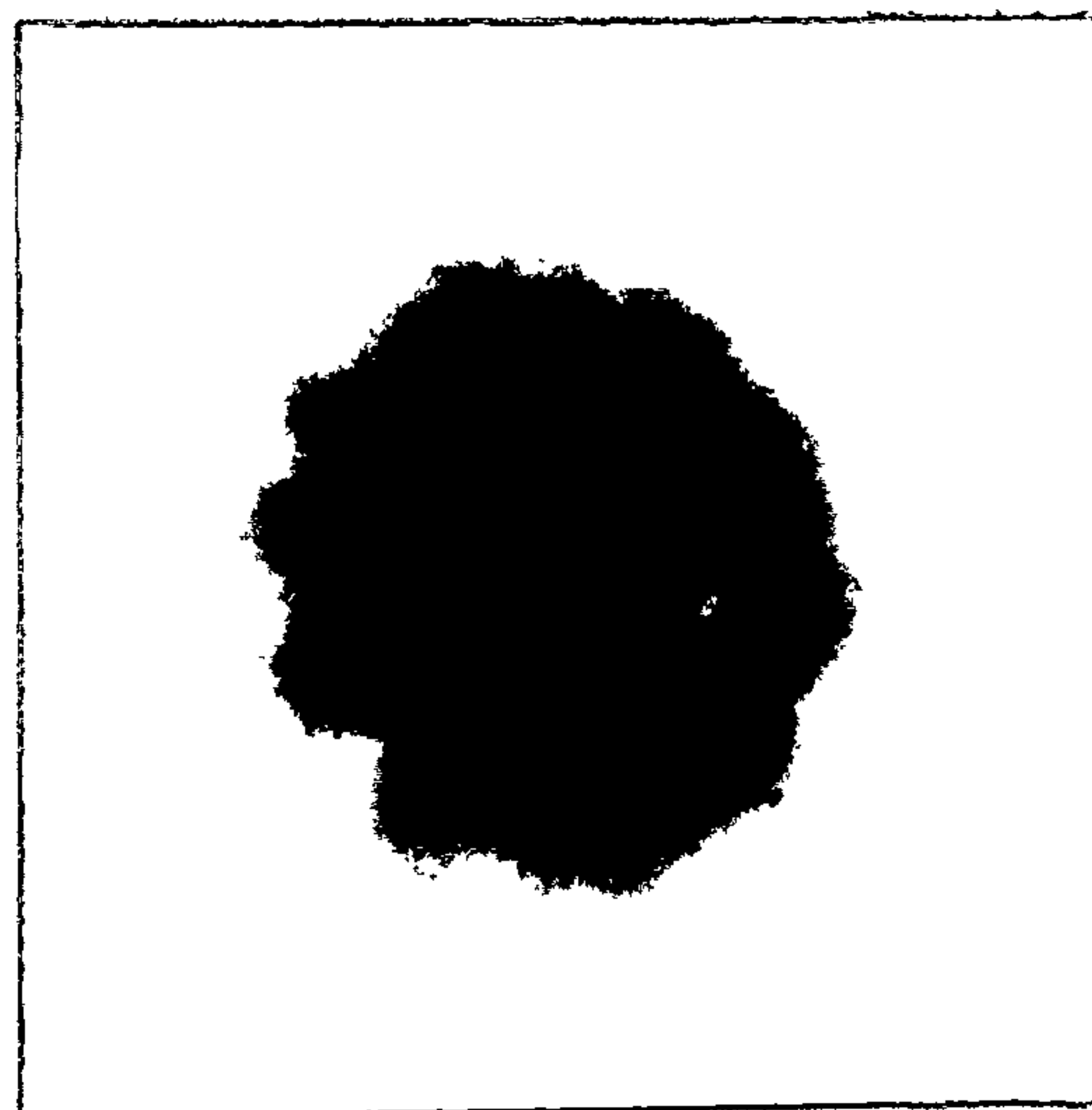
Colony type 8: Magnification 50x



Colony type 8: Magnification 45x



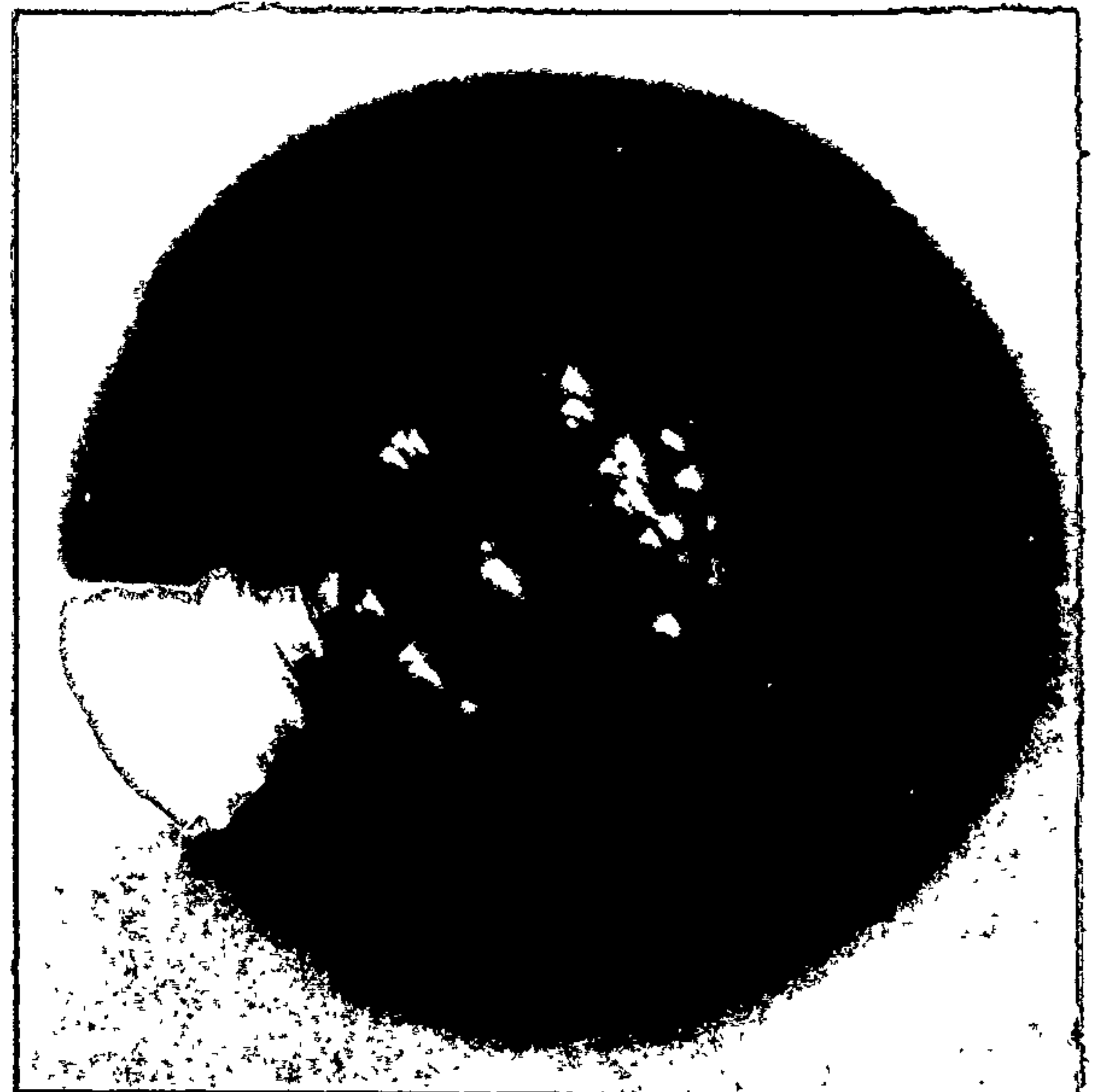
Colony type 9: Magnification 15x



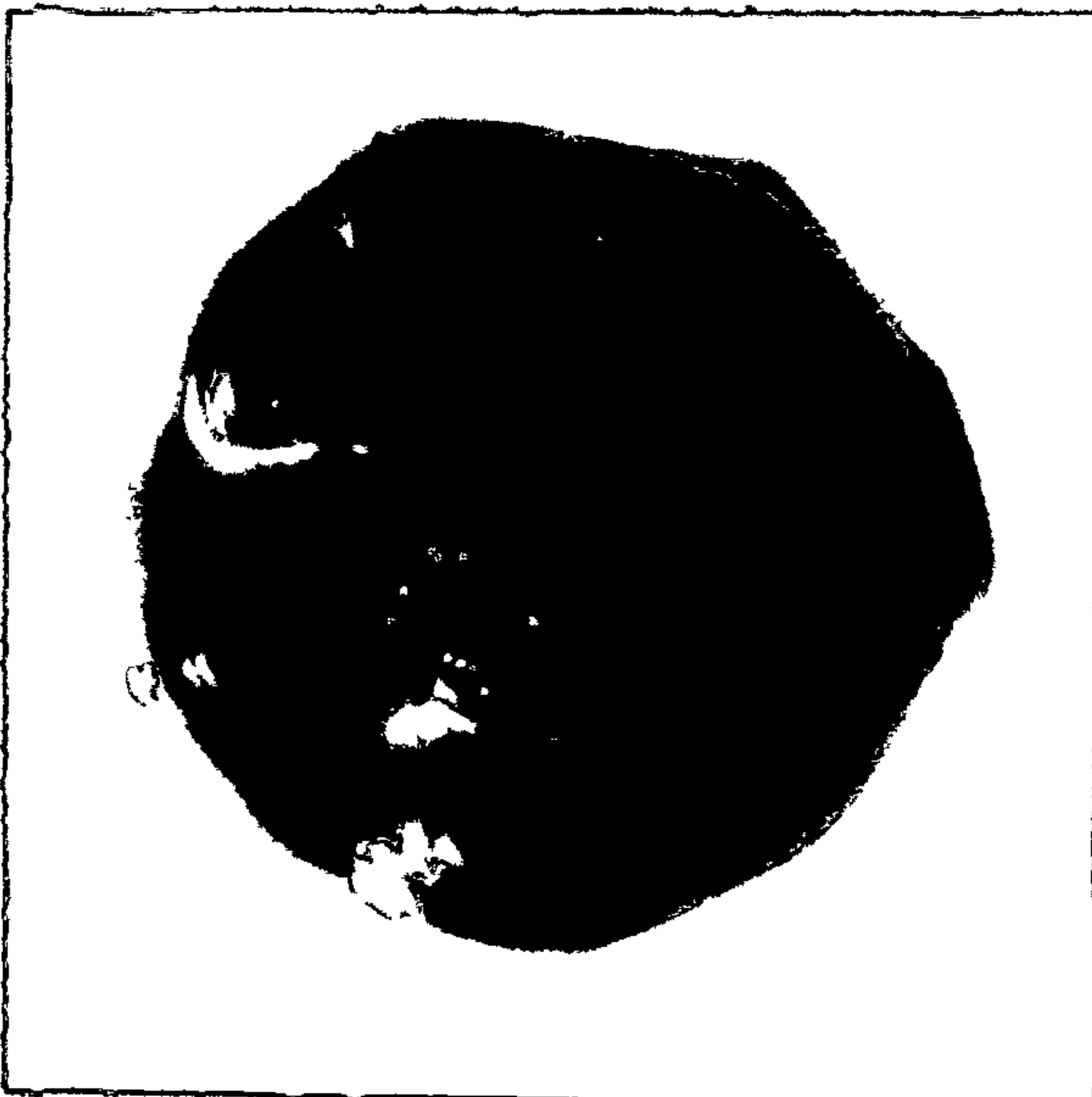
Colony type 10: Magnification 45x



Colony type 11: Magnification 40x



Colony type 11: Magnification 45x



Colony type 12: Magnification 40x

Size: * 0.5 mm in diameter.

Elevation: High convex, frequently heaped-up.

Surface: Usually rough and granular, medium soft, often 'frosted', glistening.

Internal features: The main body of the colony consists of evenly soft but dense cellular material made coherent by a viscous extracellular matrix.

Colour and opacity: Medium blue, opaque.

Attachment to agar: Free or lightly attached, may leave a slight indentation in the agar surface when moved with a wire loop.

Polysaccharide production: Usually visible as pools of overflowed, watery material on the agar surrounding the colony. Less frequently, a drop of soft polysaccharide is seen emanating from the top of the colony.

Note: This colony type resembles the morphological features of Streptococcus mutans strain JC-2 described by Carlsson.⁽¹⁷³⁾

Colony type 2.

Colony form: The outline of the colony is irregular, often with an effuse or fimbriate periphery.

Size: 0.5 mm in diameter.

Elevation: Slightly raised, often exhibiting a convex central dome.

* Approximation for all types; applies to well separated colonies, cultured under the conditions given in the text.

Surface: Rough, moderately granular with a dull lustre; soft consistency.

Internal features: The internal substance of the colony appears to be cellular material of finely granular texture, creamy soft peripherally, with a somewhat firmer central mass.

Colour and opacity: Dark blue, opaque centre, lighter blue and somewhat translucent periphery.

Attachment to agar: Lightly attached, occasionally exhibits slight central subsurface growth.

Polysaccharide production: Not visible.

Colony type 3.

Colony form: The colony outline is usually irregular, often with markedly undulated margin. Resembles a mulberry.

Size: 0.5 - 1.0 mm in diameter.

Elevation: High convex to conical, frequently heaped-up.

Surface: Contained, rough, finely granular, glistening and 'frosty' surface which may range from friable to softly coherent consistency.

Internal features: Evenly distributed, finely granular, dense, cellular material makes up the substance of the colony. The consistency varies from friable to softly coherent, depending on the amount of extra-cellular matrix present.

Colour and opacity: Dark blue, opaque.

Attachment to agar: Firmly attached, with usually star-shaped subsurface growth penetrating deep into the agar.

Polysaccharide production: Occasionally visible as a watery drop on the top of the colony, also as an overflow, but it is not always evident.

Note: This colony type resembles Colony type 1, the most consistent distinguishing features being its darker colour and penetrating subsurface growth.

Colony type 4.

Colony form: Circular colony outline with entire margin, dome-shaped silhouette.

Size: 0.5 - 1.0 mm in diameter.

Elevation: Medium to high convex, often with bevelled edge.

Surface: Smooth, glistening and very hard.

Internal features: Highly patterned, frequently star-shaped, cellular growth, most concentrated in the centre of the colony, is distributed within a hard extracellular matrix, forming a strongly coherent, firm colony. A high extracellular : cellular material ratio is characteristic of this colony type.

Colour and opacity: Dark blue, opaque cells, transparent extracellular material.

Attachment to agar: Very firmly attached; it is practically impossible to remove the colony without disrupting the agar.

Polysaccharide production: Large quantities of hard, transparent polysaccharide are produced.

Note: The basic features of this colony type, which resembles Streptococcus sanguis, are:

1. very hard smooth polysaccharide coat
2. central cellular patterning.

Colony type 5.

Colony form: Usually circular outline with crenated edge, variable silhouette, mostly truncated cone.

Size: 1 - 2 mm in diameter.

Elevation: High convex, with central plateau and/or crater.

Surface: Generally coarse and granular surface, roughest in the crater area when present, covered by glistening and usually rubbery-hard extracellular material.

Internal features: The cellular growth, usually in the shape of a truncated cone or crater, is embedded in a hard, extracellular matrix, forming a strongly coherent firm colony.

Colour and opacity: Dark blue opaque cells, transparent-translucent extracellular material.

Attachment to agar: Very firmly attached; difficult to remove from agar surface; often exhibits subsurface growth which leads to partial embedding of the colony in the agar. Large colonies tend to cause multiple splits in the agar, which radiate outwards from the edge of the colony.

Polysaccharide production: The cells appear to be wholly enclosed in the copious amount of hard transparent-translucent polysaccharide formed. However, viscous, soft or even watery polysaccharide is frequently present in the central crater or is exuded in large enough quantities to form overflow pools surrounding the colony.

Note: Some features of this colony type are characteristic of Streptococcus sanguis, while others, like the production of soft polysaccharide and colony form are reminiscent of certain strains of Streptococcus mutans.

Colony type 6.

Colony form: Circular colony outline with entire margin and conical or umbonate silhouette.

Size: 2 - 6 mm in diameter.

Elevation: Varies from umbonate, medium convex to high conical. May reach 2 mm in height centrally.

Surface: Smooth, glistening and very hard.

Internal features: Highly patterned cellular growth, mostly arranged in concentric rings or embedded in a hard, extracellular matrix, forming a strongly coherent, firm colony. The extracellular : cellular material ratio is typically high.

Colour and opacity: Dark blue, opaque cells, transparent extracellular material.

Attachment to agar: Securely attached; it is extremely difficult to remove the colony without disrupting the agar.

Polysaccharide production: Large amounts of hard, transparent polysaccharide are produced.

Note: This colony type is very similar to Colony type 4, the main distinguishing features being size and cellular pattern.

Colony type 7.

Colony form: Circular to irregular "gummy" colony, with crenated or lobate edge.

- Size: 3 - 5 mm in diameter.
- Elevation: High convex to heaped-up, sometimes with plateau, frequently with convex, bevelled edge.
- Surface: Variable from rough to smooth, always glistening, soft rubbery consistency.
- Internal features: The firm cellular well or crater is surrounded by viscous or gummy extracellular material containing randomly suspended clumps of cellular components.
The colony is moderately coherent.
- Colour and opacity: Light to medium blue, opaque.
- Attachment to agar: As a rule, the colony is firmly attached, with evidence of subsurface growth; infrequently, free colonies without visible subsurface growth were observed.
- Polysaccharide production: Massive amounts of viscous to gummy opaque polysaccharide are produced.
- Note: This colony type resembles Streptococcus salivarius.

Colony type 8.

- Colony form: Circular colony outline with entire edge.
- Size: 0.5 - 2 mm in diameter.
- Elevation: Raised to low convex, sometimes umbonate tendency.
- Surface: Soft, finely granular and glistening, usually smooth, occasionally scalloped, resembling beaten copper.
- Internal features: The colony consists mainly of cellular material which is, as a rule, uniformly soft, of a smooth buttery or very finely granular consistency, never coherent, rarely dry and friable.

Colour and opacity: Medium to dark blue, opaque.

Attachment to agar: Mostly free, sometimes very lightly attached.

Polysaccharide production: Not visible.

Note: This colony type resembles Streptococcus mitis.

Colony type 9.

Colony form: Dewdrop-like colony with a circular outline and an entire edge.

Size: 1 - 4+ mm in diameter, with tendency to flow.

Elevation: Low to medium convex.

Surface: Soft, smooth and glistening.

Internal features: Sparse cellular growth, evenly suspended in watery extracellular material, giving rise to a non-coherent colony.

Colour and opacity: Clear or light blue, transparent to translucent.

Attachment to agar: Free, no visible subsurface growth.

Polysaccharide production: Massive amounts of watery, transparent polysaccharide are produced.

Note: This colony type was found only in approximately three and nine per cent of the UL2 and pooled plaque samples respectively. The organism was a Gram positive, catalase negative coccus occurring in chains. The possibility that it is a Leuconostoc spp., resistant to the inhibitory effects of tellurite, crystal violet and trypan blue of the Mitis-salivarius agar will be tested. In the meantime, however, it has been included in the result processing.

Colony type 10.

- Colony form: Irregular colony outline, with undulating or erose margin.
- Size: 1 - 2 mm in diameter.
- Elevation: Mostly flat or slightly raised colonies, some with a papillate centre.
- Surface: Evenly soft, finely granular periphery changing to rough and papillate surface centrally, exhibiting a dull lustre.
- Internal features: Mainly cellular material of medium soft, granular consistency, often somewhat firmer in the centre than at the periphery. Non-coherent, tends to lose all form when touched.
- Colour and opacity: Light to medium greyish-blue, opaque.
- Attachment to agar: Free, with no subsurface growth.
- Polysaccharide production: Not visible.
- Note: This colony type resembles the 'non-cariogenic' human streptococcal strain CHT described by Zinner and Jablon⁽¹⁹⁸⁾. It is probably Streptococcus mitis, and usually can be subcultured without variation in form, a characteristic shared by few of the other colony types.

Colony type 11.

- Colony form: The colony outline is circular, with an entire, or less frequently, undulating to lobate edge, with a high dome or truncated cone silhouette.
- Size: 1 - 2 mm in diameter.

Elevation: High convex to conical, umbonate with an off-centre peak which may be replaced by a small crater or plateau.

Surface: Glistening smooth, wet and soft.

Internal features: The colony consists mainly of cellular material which is moderately coherent, creamy soft throughout and is arranged at times in the form of a crater, without visible accumulation of extracellular products.

Colour and opacity: Very dark blue to black, opaque.

Attachment to agar: Lightly attached, without visible subsurface growth.

Polysaccharide production: No discrete emanation of extracellular polysaccharide has been observed.

Note: Various subforms classified within this category had the most obvious common features of soft texture, almost black colour, umbonate, craters to conical form and lack of visible emanation of polysaccharide.

Colony type 12.

Colony form: Mostly circular, sometimes irregular colony outline with raised, slightly undulating or lobate margins.

Size: 1 - 2 mm in diameter.

Elevation: Low central area surrounded by raised periphery.

Surface: Firm to hard and glistening, smooth or slightly granular centrally, granular to coarse with lobate tendency at the periphery.

Internal features: While some patterned central cellular material is usually evident, most cellular growth occurs in

a lobate peripheral ring embedded in a firm to hard extracellular matrix, forming a strongly coherent colony.

Colour and opacity: Medium to dark blue, opaque cellular material and translucent to opaque matrix.

Attachment to agar: Firmly attached, cannot be removed without some of the agar, which may be moated adjacent to the periphery of the colony.

Polysaccharide production: Firm, rubbery to very hard, clear polysaccharide is produced.

Note: One of the variants covered by this classification closely resembles Streptococcus type 5 described by Schamschula and Charlton. (20)

Colony morphology was examined using a wide-field dissecting microscope (Carl Zeiss, Oberkochen, Germany) with direct and oblique reflected lighting at 16 x magnification. To ensure that no part of a plate was overlooked, a grid, 0.5 x 0.5 cm square, was attached to the undersurface of the plate and colonies were classified square by square. As a rule, plates containing 40 - 200 colonies were used for classification. On rare occasions, when none of the plates cultured from a specimen fell within these limits, classification was attempted on the basis of the most suitable plates. When no suitable plates were found, the dilution and culture procedures were repeated.

Not less than two and usually four plates were classified for each specimen, always by the same graduate assistant. After appropriate adjustments for bringing the plates to a common

dilution level, the mean number of colonies in each category was calculated and expressed as:

- a. the proportion of the total number of colony forming units of that colony type, as the percentage of all colony types isolated from a given sample.
- b. the total number of colony forming units per mg wet weight of plaque.
- c. the total number of colony forming units x mg wet weight of plaque collected.

To enable comparisons in retrospect, plates containing typical samples of the principal colony types were preserved by two drops of formalin per plate (approximately 0.1 ml of 40 per cent formaldehyde in water, Townson & Mercer (N.S.W.) Pty. Ltd., Sydney, N.S.W.) and kept under airtight seal for direct reference. In addition, photographs were taken of colonies typical of their category.

Some 10 - 12 representative colonies were selected from each of the 12 categories for further study. These colonies were subcultured on Mitis-salivarius agar, purified by streak-culture passages on sheep-blood agar plates, after which the colony characteristics were again confirmed on Mitis-salivarius agar. From the last subculture, a colony was streaked on to a sheep-blood agar slope and incubated under anaerobic conditions (95 per cent nitrogen 5 per cent carbon dioxide) for 24 hours at 37°C, after which the growth was harvested and freeze-dried in a suspending medium of horse-serum broth containing 7.5 per cent glucose.

Sometimes, colonies of bacteria other than Streptococcus spp. appeared on the plates. Intensive Gram staining and the catalase test were used to differentiate these from colonies of Streptococcus spp. The most frequently found organisms, extraneous to our present interest, were Gram negative bacilli, but less often, colonies of Neisseria spp., Staphylococcus spp., Corynebacteria spp., Bacillus spp. and Escherichia spp. were also seen. On the very rare occasions when coliform bacilli or enterococci appeared on the culture plates, the colonies were dark brown, smooth and slightly raised, corresponding to the appearance described by Chapman. (202) Isolated colonies of Staphylococcus spp. were whitish or pale blue, smooth and slightly raised with a circular outline and an entire edge. Where warranted, adjustments were made to the total counts, but otherwise, for the purposes of the present study, organisms other than Streptococcus spp. were disregarded.

The tests developed and employed to estimate the error of the counting and classification of streptococci were different for the UL2 and pooled plaque samples, reflecting the exact procedures used to obtain the specimens in the field.

In the case of the UL2 samples, all the plaque collected from a subject was homogenized in the suspending medium, and an aliquot of the homogenate was used for preparing the serial dilutions and cultures. In the case of the pooled plaque samples, only a small portion of the mixed pool of plaque collected from a subject was placed in the suspending medium for homogenization and subsequent microbiological processing.

A large superfluous specimen of plaque collected in New Guinea and treated in the same manner as the field specimens, was used to estimate the error of the UL2 method. Treated as individual samples, eight 0.1 ml aliquots of the homogenized plaque suspension were serially diluted, plated, cultured, counted and classified. The mean number of colony forming units of streptococci per mg wet weight of plaque was found to be 1.75×10^8 , S.D. = $\pm 0.32 \times 10^8$, $n = 8$. Expressed as percentages of the total number of colony forming units, the mean values and standard deviations for the six colony types present were: 2: $\bar{x} = 7.2$, S.D. = ± 3.3 ; 3: $\bar{x} = 4.8$, S.D. = ± 4.5 ; 6: $\bar{x} = 6.8$, S.D. = ± 3.5 ; 8: $\bar{x} = 2.6$, S.D. = ± 1.6 ; 10: $\bar{x} = 76.6$, S.D. = ± 5.5 ; 11: $\bar{x} = 2.0$, S.D. = ± 1.7 ; $n = 8$ in all cases.

The error associated with the counts and classifications based on pooled plaque was estimated using plaque collected from nine Sydney school-children. The specimens were pooled and thoroughly mixed in an atmosphere saturated with water vapour. Eight aliquots of the material were successively removed and weighed to the nearest 0.001 mg using the procedure employed in the field (sub-section 3.2.3.5.). The aliquots were suspended and homogenized separately, and were stored and processed as eight individual field specimens.

The mean number of colony forming units of streptococci per mg of wet weight of plaque was found to be 5.54×10^8 , S.D. = $\pm 1.72 \times 10^8$; $n = 8$. Expressed as percentages of the total number of colony forming units, the mean values and standard deviations for the seven colony types present were: 2: $\bar{x} = 1.4$, S.D. = ± 1.5 ; 4: $\bar{x} = 10.0$, S.D. = ± 5.7 ; 5: $\bar{x} = 9.0$, S.D. = ± 4.8 ;

7: $\bar{x} = 18.7$, S.D. = ± 4.2 ; 8: $\bar{x} = 47.7$, S.D. = ± 10.5 ; 10: $\bar{x} = 9.3$, S.D. = ± 6.5 ; 12: $\bar{x} = 3.8$, S.D. = ± 2.7 ; $n = 8$ in all cases.

Because of their resemblance to S. mutans on primary isolation, or because of their importance in subsequent statistical analyses, preliminary tests were carried out for the presumptive identification of Colony types 1, 2, 3, 5 and 10. Isolates of these colony types were examined for:

- a) Colony morphology on Mitis-salivarius agar following repeated passages in nutrient broth culture containing traces of glucose.
- b) Fermentation of mannitol, sorbitol and inulin.
- c) Adherence to glass surfaces when grown in broth culture containing 5 per cent sucrose.

3.3.2.5. Water analyses.

The water samples were obtained by a survey team who also carried out food and soil sampling in villages included in this investigation.

As a rule, the samples consisted of river water, but a lesser number of specimens were obtained from water holes, swamps, lagoons and creeks, as a provision for supplementary information. The samples were collected by filling clean polyethylene containers of one gallon capacity at a location where the villagers obtained their drinking and household water, regardless of water level or flow rate at the time of collection. A single sample was taken at each site. River water was sampled at 14 of the 16 villages included in the investigation. The two villages where no river water was collected were Iniai (No. 6) and Sangriman (No. 13).

Iniai could not be approached because of hazardous water conditions, while Sangriman, a village situated on a swampy lagoon approximately 2 km from the Blackwater River, had tribal land extending to the Korosameri River, and the principal source of drinking and household water could not be ascertained.

Following collection, the containers were sealed and transported to the laboratory by surface freight.

In preparation for the analyses, the sediment was thoroughly suspended by inverting the bulk containers repeatedly. A 20 ml aliquot was then transferred from each sample into new 25 ml capacity polypropylene containers (25-P, Disposable Products, Adelaide, S.A.) which were previously decontaminated using an anionic-cationic detergent, as described in sub-section 3.3.2.1.1. A 2 ml aliquot was then transferred from each sub-sample into a 5 ml capacity polypropylene container (pre-cleaned in the manner stated above) for fluoride determinations.

The sub-samples, now containing 18 ml of water, were acidified by adding 0.5 ml of 15.8 M nitric acid (Aristar) and left standing for 3.5 hr to reverse any adsorption to the container walls that may have occurred during the distribution procedure. Five blank samples, consisting of 18 ml glass-distilled, deionized water, were prepared to correspond with this stage of the preparation.

The samples were then transferred into 15 x 130 mm borosilicate glass test tubes (H.B. Selby) and evaporated at 90 - 100°C in a stainless steel-lined oven ("Qualtex", Watson Victor). The dried samples were then heated in the presence of 0.5 ml of 15.8 M nitric acid (Aristar) at 80° - 100°C in an aluminium heating

block for one hour, followed by evaporation to dryness. This procedure was repeated once, after which a residue of white solids was obtained. This residue was dissolved, while warming for 0.5 hr at 60° - 80° C, with 0.5 ml of 6.3 M nitric acid (Aristar). The samples were then diluted with 4.0 ml of glass-distilled, deionized water to give a basic sample volume of 4.5 ml, transferred to polypropylene containers (5-P), and kept frozen until required for the analyses.

3.3.2.5.1. Calcium determinations.

The aliquots used for calcium determinations consisted of 0.5 ml of the basic sample diluted to 5.0 ml with one per cent lanthanum background solution.

The calcium content of the samples was determined by atomic absorption spectroscopy, using identical instrumentation and experimental conditions as described for enamel in sub-section 3.3.2.1.2.

Blank values.

The blank values were identical with the 0 ppm standard, indicating that all measurable contributions were made by the lanthanum background solution.

Replicate analyses.

All analyses were made in duplicates. The estimate of precision was based on the differences between 14 duplicate pairs, as described in sub-section 3.3.2.1.2. The standard deviation of a single determination was found to be ± 0.04 ppm.

Recovery of known amounts of calcium added to the samples.

The test material was prepared by adding 1.0 ml of 3.3 ppm standard to 1.0 ml of water sample. The estimations were carried out for four such samples with a range of original calcium content of 1.7 to 6.7 ppm. The mean recovery was 102.0 per cent, S.D. = ± 2.6 per cent.

Calculation of results.

Following subtraction of the blank value, the calcium content of the water was calculated from the ppm value determined and from the dilution factor.

3.3.2.5.2. Strontium determinations.

Strontium analyses were carried out by flameless atomic absorption spectroscopy, using identical instrumentation and experimental conditions as described for enamel in sub-section 3.3.2.1.4.

Blank values.

The mean blank value was 1.5 ppb.

Replicate analyses.

The analyses were made in duplicates. The estimate of precision was based on the differences between 14 duplicate pairs, using the method described under 3.3.2.1.2. The standard deviation of a single estimation was found to be ± 1.8 ppb.

Recovery of known amounts of strontium added to the samples.

The test material was prepared by adding 5.0 μ l of 8.5 ppm strontium standard to 2.0 ml of water samples with the strontium content ranging from 0.0184 to 0.0422 ppm. Five analyses gave a mean recovery of 99.0 per cent, S.D. = $\pm$ 5.3 per cent.

Calculation of results.

The strontium content of the water was calculated from the value determined and from the dilution factor, following subtraction of the blank value. The results were expressed as ppb ($\text{g}/1 \times 10^9 \text{ ml}$).

3.3.2.5.3. Fluoride determinations.

In preparation for analysis, the 2.0 ml samples of water were diluted with an equal volume of "Total Ionic Strength Adjustment Buffer" ("TISAB", Orion Research Inc., Cambridge, Mass., U.S.A.) at pH 5.2 containing 0.4 per cent cyclohexylene di-nitrilo tetraacetic acid ("Titriplex IV", E. Merck G.m.b.H., Darmstadt, Germany).

The ionized fluoride concentration of the samples was determined using a combination fluoride electrode (Model 96-09, Orion). Otherwise, the instrumentation was identical to that described in sub-section 3.3.2.1.9.

Standards.

A stock solution of 0.1 M sodium fluoride was prepared by dissolving 4.199 g of oven-dried sodium fluoride (Analar grade, B.D.H. Ltd., Poole, Dorset, U.K.) in distilled, deionized water. Standard solutions were prepared by dilution with distilled, deionized water, to contain 1×10^{-4} M, 1×10^{-5} M, 5×10^{-6} M,

1×10^{-6} M, 5×10^{-7} M and 1×10^{-7} M fluoride ion.

The fluoride ion activity of the samples was determined from a calibration graph, which was prepared by diluting the standard solutions with an equal volume of "TISAB" buffer, so that a direct reading of the fluoride concentration in the water sample could be made. For calibrations, the electrodes were immersed in the standard solutions in order of increasing concentrations.

A typical calibration graph is shown in Figure 23, page 202.

Samples.

The diluted samples were agitated during the estimations by bubbling a fine stream of fluoride-free air through the solution. The electrode potential at infinite time was estimated by taking readings at one minute intervals over a ten minute period, and plotting the electrode potential in millivolts against time, on a time-response chart (Product 90-00-91, Orion). The determinations were carried out in a random order. Standards were interposed between each 3 - 4 samples. Ninety per cent of the samples contained more than 1×10^{-7} M fluoride.

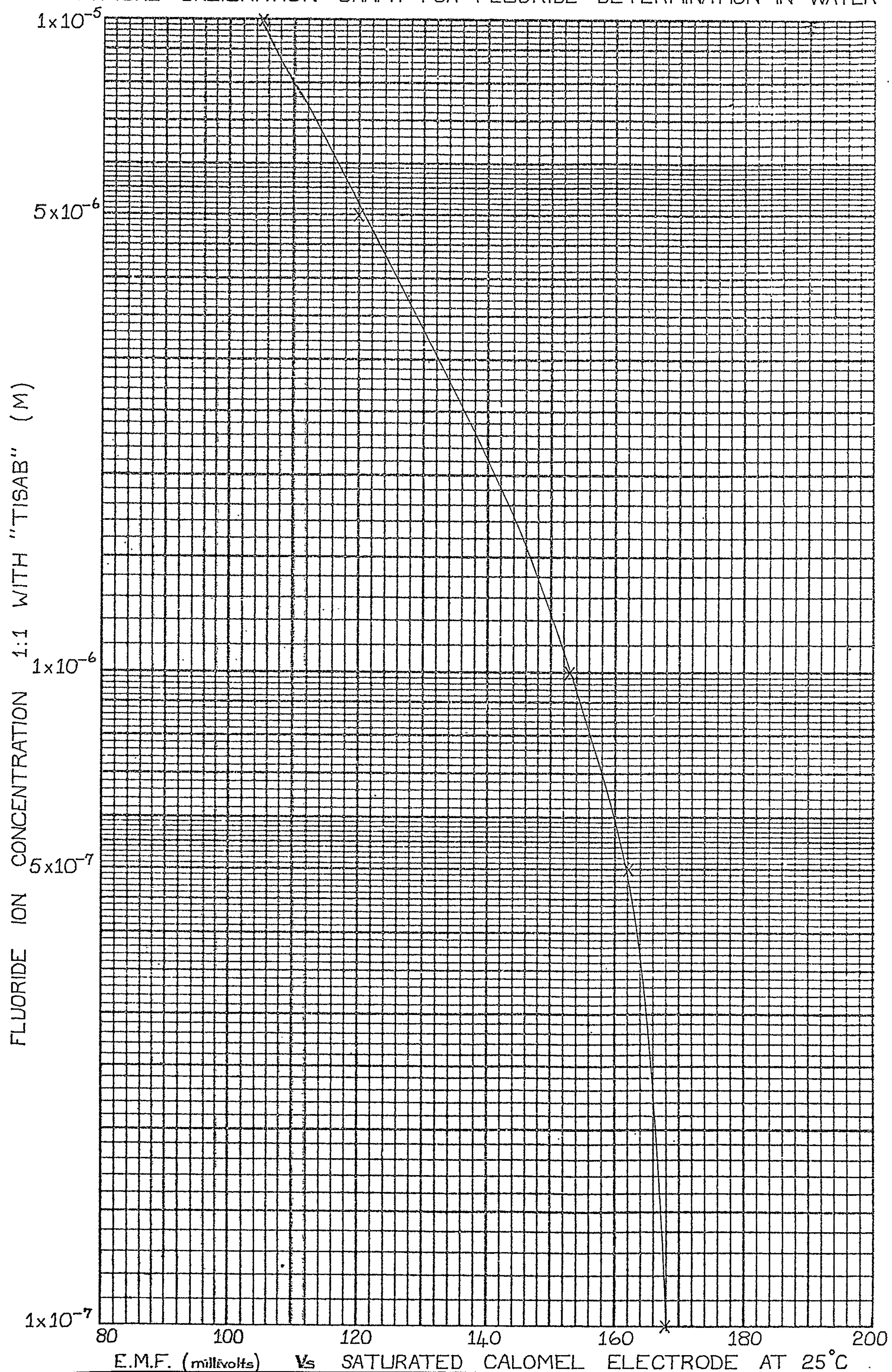
Recovery of known amounts of fluoride added to the samples.

The mean recovery of 19 ng of fluoride added to each of ten water samples as 10 μ l of a 1×10^{-4} M fluoride standard, was 99.7 per cent, S.D. = ± 1.2 per cent.

Precision of the method.

A water sample was divided into ten 2.0 ml aliquots. The mean molarity of the aliquots was 0.6×10^{-7} , corresponding to

TYPICAL CALIBRATION GRAPH FOR FLUORIDE DETERMINATION IN WATER



24.1 ppb fluoride in the water, S.D. = ± 1.2 ppb, $n = 10$.

Blank values.

"TISAB" buffer diluted with an equal volume of distilled, deionized water, contained less than 1×10^{-7} M fluoride, at the limit of detection with the system used.

Calculation of results.

The fluoride content of water, expressed as parts per billion ($\text{g}/1 \times 10^9 \text{ ml}$) was calculated by multiplying the observed molarity $\times 10^{-6}$ by 19.

3.4 Statistical methods.

3.4.1. Introduction.

Statistical methods used in the course of this investigation can be grouped into two major categories:

- 1) Tests concerning the precision or the accuracy of the field and analytical methods employed.
- 2) Examination and statistical treatment of the data in accordance with the specific aims stated in Section 1.2.

The statistics used for the first category consisted of standard methods. Appropriate comments were made about the tests, where they were employed, in Sections 3.1., 3.2 and 3.3.

This Section deals with the second category of procedures, considered in the light of the specific aims of the investigation.

3.4.2. Data summary.

In order to satisfy the first specific aim and as an initial step towards examination of the data, a summary of all observations was made.

This took the form of tables setting out the numbers of subjects and the means and standard deviations for each variable, separately, by village and by sex. A careful study of this table may result in the detection of patterns or stratification of the variables studied. This may suggest specific problems, which may require close attention before the details of statistical analyses to be carried out are finalized.

In addition, having completed more detailed analyses using advanced techniques, further examination of the tables may reveal previously unnoticed trends or configurations for some variables, which support the findings. In respect of other results, it may have to be concluded, that no indication of the findings is apparent in this form of data presentation.

3.4.3. Correlation matrix. Selection of variables for statistical analyses.

The second specific aim of the study called for confirmation or rejection of the hypothesis that "sets of the intra-oral variables measured have a significant capacity to 'explain' variation in individual caries experience". Such a hypothesis may be examined using stepwise multiple linear regression analysis. However, when the pool of variables includes a large number, some form of selection based on relevance, in the sense of statistical association, is necessary.

Thus, in the second phase of the analyses, a correlation matrix involving all individual variables was prepared. The matrix was examined to determine, in the correlation sense, the extent to which individual variables are related to measures of caries experience.

In making use of the correlation matrix, it was appreciated that the product-moment correlation coefficient, (r), has definite limitations. For example, it measures only the extent to which a straight line relationship fits the true relationship between the two variables concerned. It is clear that not all biological data behave in this way and it may be that a curvilinear relationship is more appropriate. However, when the correlation is definite but weak, graphical examination of the variables is not likely to disclose the shape of the relationship. As there is no known mathematical method to find the true relationship of the variables, in practice it is necessary to experiment with simple transformations of the variables, to determine whether the correlations can be improved or not. Such transformations were carried out whenever it was considered appropriate.

Another limitation of using the r values of the matrix as a basis of selection of variables for further investigation, arises from the fact that two variables which appear on the surface to show promise because of their intercorrelation, may not both be relevant, because one may be a reflection of the other. The degree to which one variable reflects the other will vary and is measured by the intercorrelation between the two. This can occur with many more than two variables and, where a large number of variables is being scrutinized, the problem of comprehending the

implications of all interrelationships is beyond practicality.

Furthermore, it should be borne in mind that conventional usage of a correlation coefficient would demand, in itself, a statistical test of its significance. However, Johnson and Leone (203) pointed out that when large numbers of tests of significance are being applied at the same time, the probabilities associated with the conventional significance levels are considerably increased and some spurious results may be produced. In the present study, instead of statistical significance, the relative magnitude of the different correlation coefficients was the basis on which a variable was selected for further study. In this context the significance levels are irrelevant. However, the overall significance of the contribution of the variables chosen was examined in the multiple linear regression analyses, subsection 3.4.4.

As long as its limitations are not overlooked, the correlation coefficient is a useful indicator of the relevance of a variable, particularly in the early stages of an investigation. The study of the correlation matrix may lead to the selection of subsets of variables, that show promise for 'explaining' caries experience when examined collectively.

Considering the number of pairs of observations available (generally in excess of 200), the value of r which would be needed to demonstrate statistical significance at the $P = 0.05$ level is approximately 0.15. As mentioned earlier, the method assumes linearity of relationship between variable pairs, but the reality of this assumption is questionable. For this reason, it was

decided to use a less stringent approach and arbitrarily accept those variables, whose correlation coefficient with measures of caries experience was of the order of 0.1 or more, as being of further interest.

A very large number of closely related variables were derived from the examination of the streptococcal flora of pooled and UL2 plaque samples. In order to keep computer processing requests at a manageable level, a decision had to be made to use data based on either pooled or UL2 plaque in further statistical analyses. Results of preliminary analyses, which led to the use of the UL2 data, are given in Section 4.4.

3.4.4. Stepwise multiple linear regression analysis.

In accordance with the second specific aim, it was decided to employ stepwise multiple linear regression analyses, using the independent variables selected by the previously described procedure, for 'explaining' variation of a dependent variable representing caries experience. This type of analysis provided the means for comparing the contributions of independent variables singly or collectively.

The main objective of the analysis was to decide whether or not a significant proportion of the variation in caries experience could be 'explained' (in a statistical sense) by variation in the independent variables.

In multiple regression analyses, two basic approaches are used for fitting the independent variables into the process when large numbers of variables are available.

One approach is to include all available (or selected) independent variables and successively delete those which contribute least to the 'explanation' of the variation in the dependent variable at each step of the analysis. This can be wasteful from the point of computer time used, particularly if the number of variables is large and many of them show little relevance.

The other approach is to include, as a first step, that independent variable which has the highest product-moment correlation coefficient with the dependent variable, then to add other independent variables which in association with the one already included cause the greatest increase in the proportion of the variation explained in the dependent variable. This procedure continues in a stepwise manner until either a satisfactory proportion of the variation is explained or a predetermined number of variables have been included in the analyses. This was the procedure used in all multiple linear regression analyses referred to in this thesis. The actual stopping rules supplied in computer programs contain an element of arbitrariness, because there is no universal agreement as to the point where the analysis should be stopped. Experience has shown that a liberal attitude is advisable and it was decided to halt the process not sooner than when ten variables had been added or when the increase in the proportion of variables explained was not significant at the 10 per cent level.

The relative importance of a variable, in a statistical sense, is indicated by the contribution it makes to the value of the multiple correlation coefficient, (R) , and by the order in which it is included in the analysis. This follows from the fact that

the criterion for choosing a variable for inclusion in the analysis is that it produces an increase in the R value greater than any other independent variable.

Interrelationships between variables are also taken into account. Thus, if an independent variable is merely a reflection of another, only one of these will be included in the analysis.

As with other regression analyses, this form of analysis assumes linearity of relationship between dependent and independent variables. Therefore transformation of independent variables were also supplied for investigation.

A statistical test of significance, based upon the value of R achieved was used to decide whether or not, and the extent to which, variation in caries experience from one individual to another could be explained by variations in the values of the independent variables.

While examination of the size and sign of the regression coefficients may suggest mechanisms by which different values of the dependent variable are produced, causal interpretation of the results could not be justified. Indeed, it is not clear that the method is capable of such use and it was not the intention to attempt to use it for this purpose in the present work.

3.4.5. Discriminant function analysis.

The third specific aim of this work was: "to examine the extent to which individuals with materially different caries experience can be classified as such, on the basis of the information contained in the independent variables alone".

Discriminant function analysis is a suitable means to satisfy this aim. The basic procedures are described in texts. (204) (205) (206) Essential to the understanding of discriminant analysis is the concept that the number of variables observed, P , can be thought of as a P -dimensional vector and consequently a point in space of P dimensions for a single individual. When the number of variables considered is over three, the space is of more than three dimensions and cannot be represented graphically.

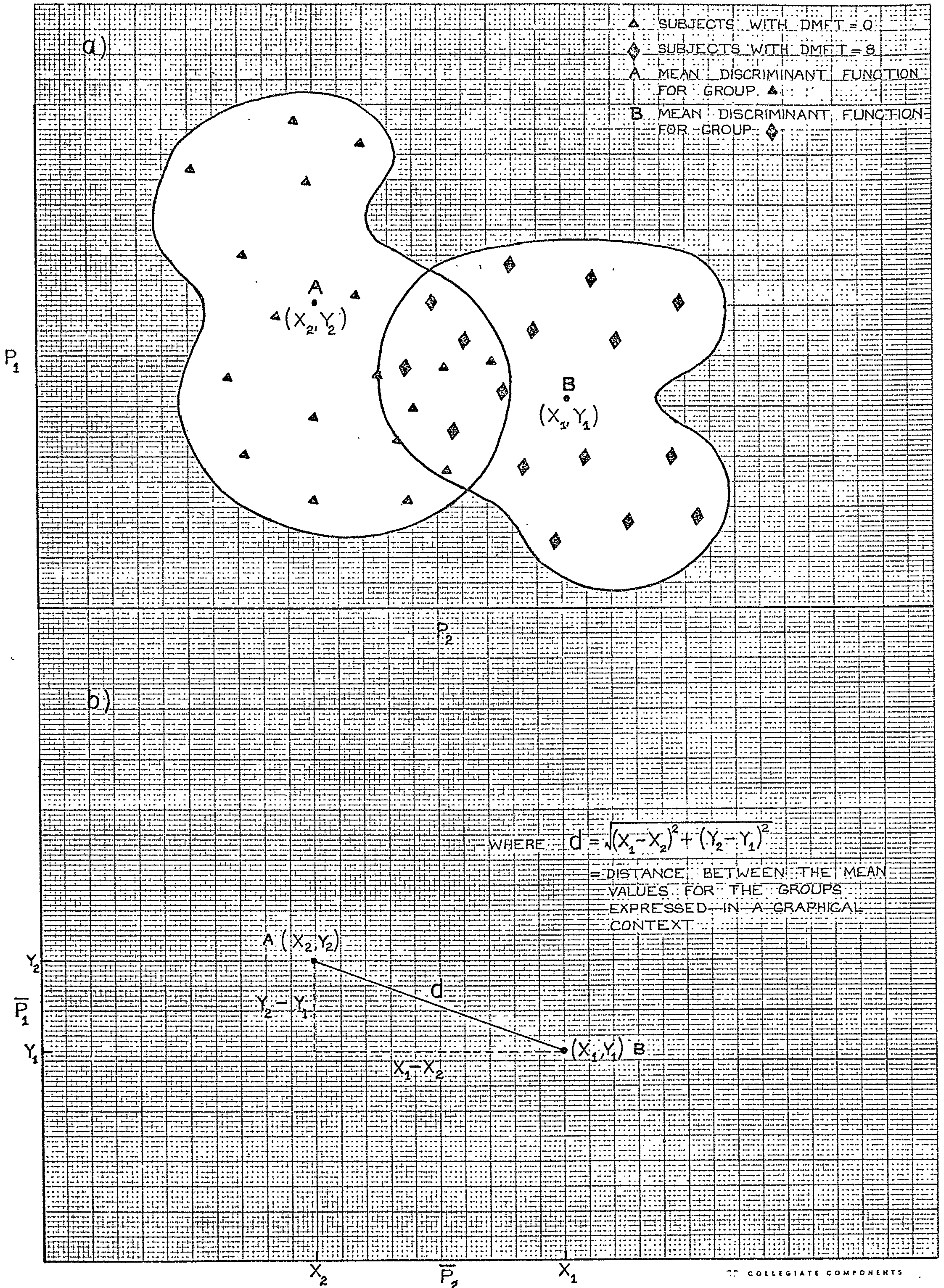
All individuals are capable of such definition and together constitute a conglomerate of points in this space. In addition, there corresponds to each of these points the established caries experience of the individuals concerned.

For example, if only those subjects who are caries-free and those who have, say, 8 DMFT are considered, one could visualize a representation of the points in two dimensions ($P = 2$), as indicated in Figure 24, page 211. Diagram a) is an obvious oversimplification but it illustrates that the points corresponding to the different DMFT scores do not form separate clusters but overlap. If one were to plot the mean point for those with 0 DMFT and the mean point for those with 8 DMFT, the distance between these points, d , can be calculated (Diagram b). The concept as illustrated with two points in two dimensions, can be extended to a space of any number of dimensions.

In discriminant analysis the dependent variable (in this case caries experience) is used only to divide the subjects into groups or strata. The analysis is not dependent on a linear relationship between the dependent and independent variables. It

TWO-DIMENSIONAL REPRESENTATION OF THE BASIC PRINCIPLE
OF DISCRIMINANT FUNCTION ANALYSIS

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operates by providing an expression (the discriminant function), for each individual, from transformations and linear combinations of chosen independent variables which maximise d , and thus achieve the greatest possible separation of clusters which represent individuals with common chosen characteristics. The extent to which the clusters still overlap, which has been minimized in the process, is the extent to which the different levels of caries experience cannot be explained by the independent variables under consideration.

The discriminant function is also evaluated for the mean of each group. The standardized distance between the groups, D , equals the difference between the mean discriminant function values. It is obtained by dividing d by its standard deviation. Having obtained the mean discriminant function for the groups, a critical value is chosen, say midway between the means. This value is regarded as the point of division between the groups and it is postulated that, if the discriminant function of a subject is less than the critical value, the subject belongs to group a); otherwise the subject belongs to group b). Since it is known a priori to which group a subject belongs from the value of the dependent variable (caries experience), the percentage of subjects misclassified on the basis of the values of the independent variables can be found.

Whether or not the analysis has provided a successful means for effectively separating subjects of different caries experience, based on information contained in the independent variables alone, may be shown by graphical illustration of the percentage of cases which were misclassified and by the distribution of individual

discriminant function values.

In accordance with the third specific aim of the present study, interpretation of the results will not be carried beyond this point. However, it is intended to examine, in the future, other aspects and possible uses of discriminant function analysis when applied to the present data and to other aspects of caries aetiology research.

CHAPTER 4.

RESULTS AND DISCUSSION.

4.1 Introduction.

The statistical analyses described in sub-sections 3.4.2., 3.4.3., 3.4.4. and 3.4.5. are directly related to the specific aims of the study. The outcome of these analyses is presented and discussed in a corresponding order in Sections 4.2, 4.3, 4.4 and 4.5. Results of exploratory analyses or sorting procedures are included in the relevant sections.

A few selected features of the results, which are outside the scope of the specific aims, are also discussed because the author did not want to avoid pointing out trends or associations which may be regarded as controversial or unexpected. Comments on these findings also demonstrate the fact that extensive use of computer processing did not replace close personal examination of the data by the author.

It is strongly emphasized, however, that it was never intended to discuss all ramifications of the investigation within this presentation. The amount of information contained in the data precluded even passing reference to many substantial aspects. A case in point is the detailed evaluation of the interrelationships between the independent variables, which will form the basis of several separate investigations over a period of some years.

Earlier in this work, the question was raised concerning the simple linearity of the relationships between the various

measures of caries experience and the independent variables. This was explored using correlation and regression analyses. The transformations of the independent variables used were square roots, logarithms and at times the products of selected variables. It was found generally, that no worth-while benefit would be derived from the use of transformed variables. It was not intended that the nature of the relationships would be explored to any depth in this work, and to ensure that the interpretation of the outcome of the analyses could proceed in a least complicated way, the use of transformed variables was abandoned.

Results of water analyses which could not be treated together with individual data are presented and discussed in the Data Summary, 4.2.15.

4.2. Data Summary.

The investigation involved 301 individuals from 16 villages. Disregarding some inevitably missing data, 114 variables were measured for each individual. A complete listing of some 30,000 observations would be superfluous and impractical and therefore is not supplied. However, it is intended that eventually this information will be made available to workers in related fields of research.

The basic features of the sample are presented in the Data Summary, Appendix, Part 1. The 'Variables' of Table 1 correspond to 'Categories' in the data summary tables which are arranged in the sequence of category numbers. The summary tables show the number of subjects for whom data are available and the mean value and standard deviation for each variable examined, by sex, village

and village group. The***** sign indicates that no data are available.

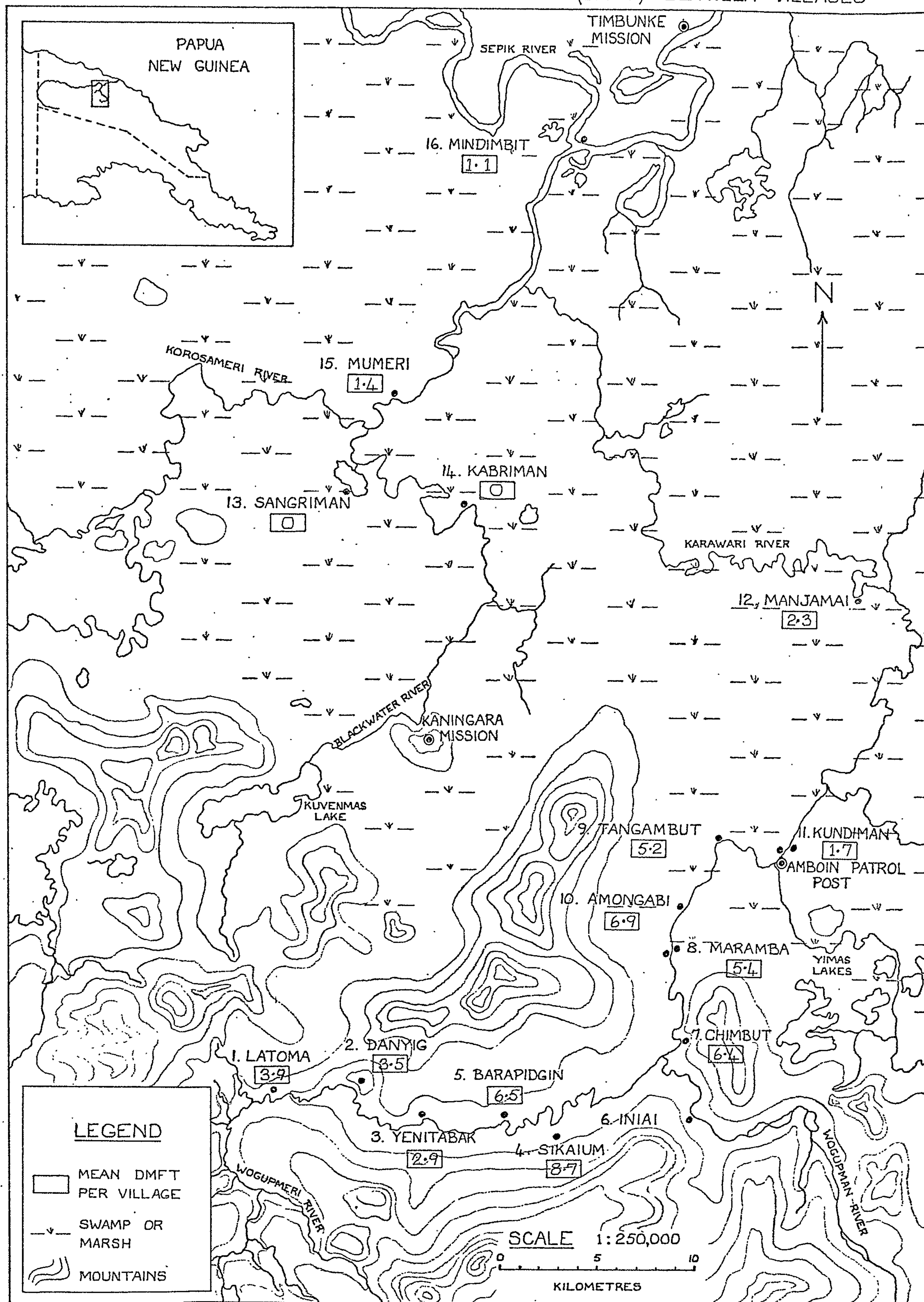
4.2.1. Dental caries (Categories 10, 12, 14 and 229).

The distribution of caries experience within the sample (mean DMFT per person per village) is illustrated in Figure 25, page 217.

The formation of village groups was based on mean caries prevalence levels. Examination of the tables representing various expressions of caries experience (Categories 10, 12, 14 and 229) shows a clear separation of villages into four distinct caries prevalence strata. It became apparent in the early stages of the field examinations, that the presumed 'high' caries villages were not homogenous in this respect, when it was observed that caries prevalence in the three Villages (1, 2, 3) situated in the upper reaches of the Wogupmeri River was appreciably lower than in Villages 4 - 10. A corresponding difference was noted in respect of the severity of carious lesions. The difference in caries prevalence was even more pronounced between Villages 4 - 10 and the villages situated on the lower reaches of the Karawari and Korosameri Rivers and on the Sepik River, designated Nos. 11, 12, 15 and 16, while all subjects examined in Villages 13 and 14 were found to be caries-free. Thus, instead of the originally envisaged three village groups, the villages were formed into four groups on the basis of actual mean caries prevalence levels found.

It can be seen from the tables that individuals in the villages chosen for the study did in fact provide the range and

DISTRIBUTION OF MEAN CARIES EXPERIENCE (DMFT) BETWEEN VILLAGES



contrast in caries prevalence sought for the purposes of the investigation. The existence of caries contrasts within villages is indicated by the standard deviation of the mean values. As an example, in Village 16, which had the lowest mean caries prevalence of all villages where caries was found (1.1 DMFT per person), the range of caries prevalence was 0 - 8 DMFT per person. In contrast, in Village 4, where the mean caries prevalence was the highest at 8.7 DMFT per person, the corresponding range was 0 - 17 DMFT.

The difference between the overall mean DMFT and DMFS scores was small, (0.3), indicating that few carious teeth had more than one distinct lesion. The highest difference was noted in Village 10, (31 per cent of DMFT value), while in villages with low caries prevalence, the differences were of the order of 10-20 per cent.

The distribution pattern of caries prevalence within and between families was examined in detail by the author. Isolated instances were detected where caries prevalence in a family was materially higher or lower than could be expected from the mean caries experience in their village or village group, but the number of such families was not sufficient to warrant further consideration in the context of the specific aims of the present work. However, this aspect will receive further attention in the future, when information concerning the composition of garden soils and food becomes available.

It is of interest to examine here, briefly, the relationship between the availability of Western-type foods and the caries experience found in the villages. Two mission stations and a

Government patrol post, shown in Figure 25, page 217, were within a day's travel (by canoe) from some of the villages in the survey area, where 'trade stores' had, at times, a very limited selection of processed or canned foods available. The village opposite and slightly down-river from Amboin Patrol Post was Kundiman (No. 11, mean DMFT per person 1.7); Mindimbit (No. 16, mean DMFT per person 1.1) and Kabriman (No. 14, mean DMFT per person 0.0) were closest to Timbunke and Kaningara Missions respectively. In contrast, the villages where caries prevalence was high, had the least access to these 'trade stores', not only because of distance but also because of difficult river conditions. Thus, for all practical purposes, there was an inverse relationship between access to 'trade stores' goods and caries prevalence. This observation supports the previous assertion that Western-type foods did not make an appreciable contribution to caries in this population and certainly no contribution to the contrasts between high and low or zero caries rates.

It was reported previously that it was not possible to achieve complete balance in sex and age distribution throughout the sample. In Village Groups 1, 2 and 3 the number of males exceeded the number of females by two, ten and one respectively, while Group 4 included six more female subjects than males. The mean caries prevalence of females was approximately 20 per cent lower than that of males for the whole sample. This order of difference, enhanced by the fact that there were six more females than males in the caries-free group, was not considered critical for the types of analyses employed. Moreover, examination of the data summary tables for all expressions of caries experience, indicated

no consistent tendency for either sex to show greater caries experience than the other at the village or village group level. Therefore, the analyses were allowed to proceed without adjustments.

Several comments are appropriate with regard to age distribution in the sample. It is fully appreciated that, with regard to the development of caries, the period of exposure of teeth to the oral environment, that is, 'time since eruption', is a more relevant variable than calendar age. (207) (208) In the present study, however, there was no practical possibility for taking the former factor into consideration. Moreover, constraints imposed by the limited size of the population, necessitated the selection of subjects from a wider age range (12 - 24 yr) than was originally intended. For these reasons, careful consideration was given to the possible effect of age on the analyses.

Examination of the table summarizing age (Category 2) showed that the mean age in 15 villages ranged from 14.9 yr to 19.3 yr. (Village 6 was represented by two subjects only and was therefore excluded from the analyses.) Comparison of mean age with mean caries prevalence, on a village group basis, indicated an inverse relationship between age and caries:

Village Group	DMFT	DMFS	Severity score	Age (yr)
2	6.4	8.0	60.8	16.6
1	3.4	4.8	38.7	17.0
3	1.5	1.8	12.3	17.3
4	0.0	0.0	0.2	18.2

Thus, it was not surprising to find that the normally expected positive association between expressions of caries

experience and age did not materialize in the correlation matrix. (See Appendix, Part 2.)

Two alternatives were open to deal with the situation. It would have been possible to select a sub-sample of subjects completely balanced with respect to age, for further analyses. This procedure would have resulted in a drastic reduction in the number of subjects and therefore the alternative of including age as a variable in subsequent analyses was chosen. Age was submitted to the multiple linear regression and discriminant function analyses with other variables. The results indicated that age did not contribute appreciably to the progress of R values or to the classification of the subjects in the respective analyses and thus did not have an effect on analyses related to the specific aims of the study.

4.2.2. Tooth colour and enamel surface wear (Categories 7 and 9).

Both variables were considered as accessory measurements, taken because of their relevance to other independent variables rather than because of direct relevance to caries. The values listed in the summary table are fairly uniform and do not disclose remarkable tendencies. To the author's knowledge no corresponding data exist in the literature for comparison.

4.2.3. Plaque quantity (Categories 16, 19 and 203).

It has been generally accepted that the presence of plaque on the coronal and root surfaces of teeth is indispensable for the causation of the corresponding types of carious lesions, (3) (209) (210) and for the occurrence of common forms of periodontal disease. (211) (212) Epidemiological studies (213) (214) (215) (216) have

demonstrated convincing associations between soft deposits on tooth surfaces and manifestations of gingivitis and periodontal disease, but the relationship between the quantity of plaque present on tooth surfaces and caries incidence in man is less clear. Keyes⁽²¹⁷⁾ stated that "the pathogenic potential of plaques can range from harmless to highly odontopathic". Recent clinical observations⁽¹⁵⁾ and epidemiological surveys⁽²¹⁸⁾⁽²¹⁹⁾ have revealed populations where the prevalence of coronal caries was low, in spite of the presence of large quantities of plaque and high sucrose intake. Although a follow-up study⁽²²⁰⁾ to the surveys⁽²¹⁸⁾⁽²¹⁹⁾ could not explain the unusually low prevalence of caries on the basis of the properties of plaque samples examined, the very existence of the situations described above, suggests that the 'nature' of plaque as well as its quantity is of aetiological importance.

In this context, the term "nature" is intended in its widest sense, including all aspects of composition and activity. The period for which plaque is attached to a specific site, particularly if it remains undisturbed, is equally important, as it may or may not allow time for the development of an optimum functional balance within a potentially destructive ecological system or for odontolysis to occur. In the author's view, the existence of minute, well consolidated, highly cariogenic plaques and voluminous soft deposits of minimal odontolytic potential, as well as plaques representing the reverse of these conditions and any degree of transition between the extremes, is a distinct possibility. Thus, it is well recognised that examination of plaque quantity sheds light on only one aspect of a complex situation.

In developing procedures concerned with plaque, the material to be regarded as such had to be considered. Reviews published as a result of a symposium⁽²²¹⁾ on plaque contained numerous and often contradictory interpretations of the term, indicating lack of agreement about its exact meaning. McHugh⁽²²²⁾ defined plaque as "the soft, tenacious bacterial deposit which forms on the surface of a tooth". A definition of considerably different meaning was given by Schroeder and de Boever⁽²²³⁾ who designated plaque as "multiple microbial colonization and microbial products - exclusive of any kind of bacterial nutrients - which gather, adhere and grow on tooth surfaces". Criteria widely employed in the past, included differentiation from "materia alba", itself a poorly defined substance, and restriction of the term to material retained after spraying the teeth with water, without specifying, however, such fundamental details as the force of the water jet and duration of the spraying.

In the present work the view was taken that, soft deposits which were retained on teeth after vigorous mastication for saliva collection and were not recognisable as food (or betel) debris, were representative of the immediate environment of the teeth at the time of examination, and as such, were of interest. This attitude was in agreement with Egelberg's⁽²²⁴⁾ view that "....It cannot be correct to select a portion of plaque according to its physical properties only...."and that "....All plaque must be considered as a structural and functional entity...."

Before examining details of the summary tables, attention is drawn to the fact that no UL2 plaque quantity data are available for most subjects in Villages 1 - 6 and for the 13 subjects in

Village 15. The original reason for measuring this variable was to make possible the examination of interrelationships between the mass, content of cultivable streptococci and pH-related activity of the same specimen of plaque. Because of breakdown of the pH measuring equipment (sub-section 3.2.3.6.), no plaque activity determinations could be made for the first 66 subjects examined or for subjects in Village 15. Therefore, the collection of UL2 plaque specimens was abandoned for these subjects. When the results were first examined, it became apparent that associations with caries, based on UL2 data, were invariably stronger than those based on pooled plaque data. However, a subsequent effort to collect the missing data failed because of technical and logistic difficulties, and available resources did not permit yet another attempt.

Examination of the summary tables for Categories 16, 19 and 203, disclosed no striking differences or consistent trends exhibited by the mean values for the villages. On a village group basis, Categories 19 and 203 showed possible trends of positive associations with caries. All three categories indicated that the mean plaque quantity was higher in the villages with high and medium mean caries prevalence than in villages with low or zero mean caries prevalence. *

	<u>Category</u>		
	16	19	203
Village Groups 1 + 2	5.58	.32	2.16
Village Groups 3 + 4	5.02	.17	1.30

* For the sake of convenience, villages in Groups 2, 1, 3 and 4 are referred to as "high", "medium", "low" and "zero" caries villages, respectively.

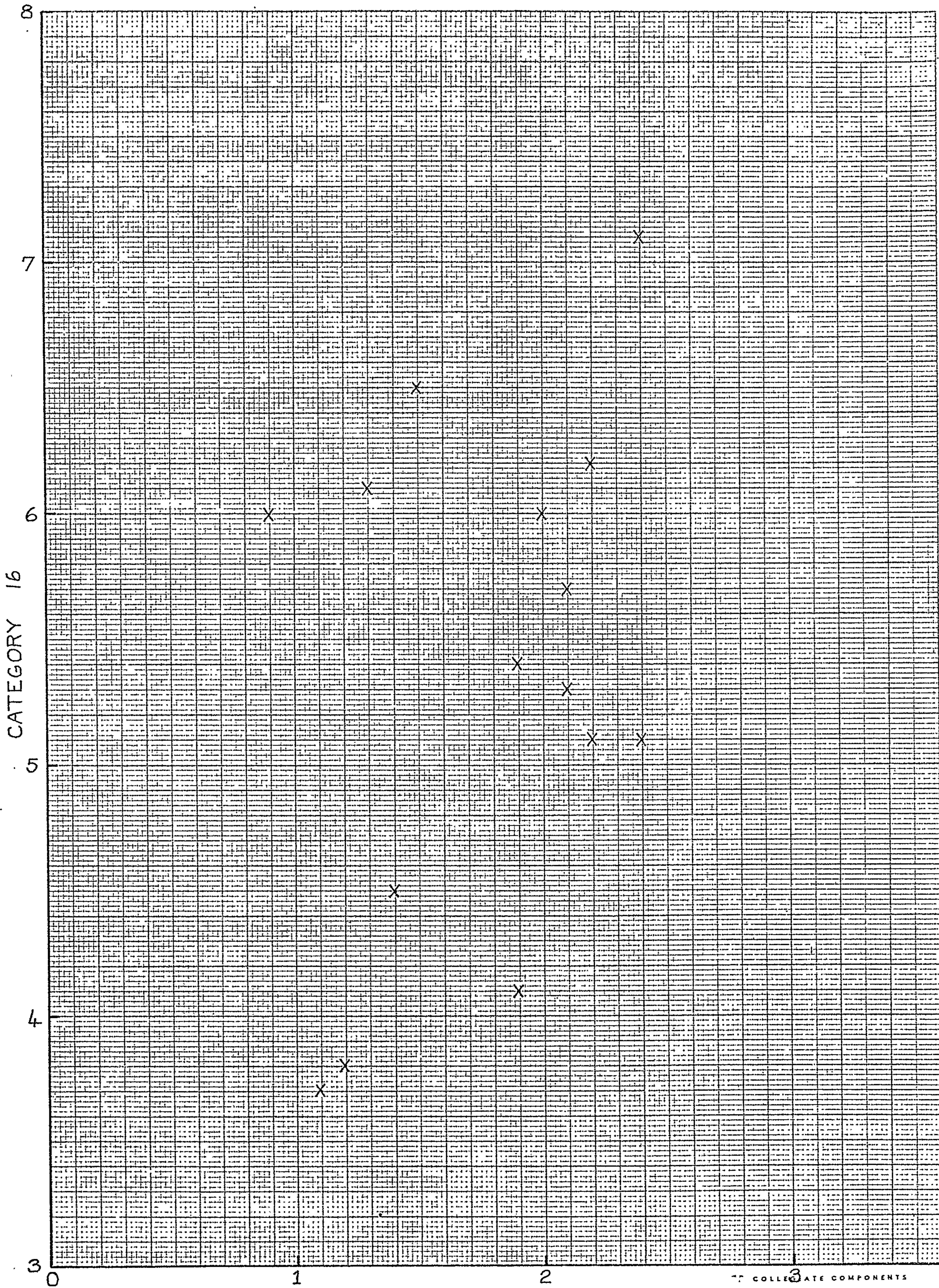
For all subjects, males had more plaque than females by all categories, but this was not always so in terms of villages or village groups; cf. Category 16, Villages 3, 9, 14 and 15 and Village Group 1.

Indices commonly used in epidemiological surveys for the measurement of soft deposits on teeth^{(225) (226) (227) (228) (229) (230) (231)} are primarily based on the area or position on the tooth surface occupied by the material. In the present work, actual wet or dry weights of plaque were used and, as far as it could be ascertained, no directly comparable data exist in the literature. The finding by Loesche and Green⁽²³²⁾ of a high correlation between the clinical scores of Kobayashi and Ash⁽²²⁷⁾ and the dry weight of plaque suggests, however, that indirect comparison of the data presented with results of epidemiological surveys, is a theoretical possibility.

Greene and Vermillion⁽²²⁶⁾ demonstrated close similarity between oral hygiene scores obtained by considering 6, 12 or all tooth surfaces for an individual. Comparison of village means for Categories 16 and 203 and examination of the scatter diagram shown in Figure 26, page 226, suggested a much lower degree of similarity between the dry weights of pooled plaque samples (1) and (2). This is also indicated by the relatively low r value for these variables in the correlation matrix (Appendix, Part 2).

As a period of 11 months elapsed between the collection of the two plaque samples, the lack of close correlation between Categories 16 and 203 could indicate that plaque distribution in an individual over a long period of time and in the present sample,

SCATTER DIAGRAM OF PLAQUE WEIGHTS



was considerably less consistent than that in the short-term study of 15 subjects by Cumming and Löe.⁽²³³⁾ Alternatively, it is possible, but less likely in view of Greene and Vermillion's report,⁽²²⁶⁾ that the sites used for collecting the second plaque specimen were a poor reflection of plaque quantity on all tooth surfaces.

4.2.4. Dissolution rate of surface enamel (Category 22).

It is generally believed that, the solubility of surface enamel in acid influences susceptibility or resistance to caries,⁽²³⁴⁾ although controversial results have been reported.⁽²³⁵⁾⁽²³⁶⁾⁽²³⁷⁾ Brudevold and McCann⁽²³⁸⁾ concluded that "a relationship between enamel solubility and caries resistance has been established only in the case of teeth from high fluoride areas".

The solubility of surface enamel of deciduous teeth collected from a fluoridated area was found to be lower than that of controls from a low fluoride area by Jenkins, Armstrong and Speirs.⁽²³⁹⁾ The same workers were unable to demonstrate similar associations for permanent enamel. Cutress and Malthus⁽²⁴⁰⁾ found the solubility of enamel of permanent teeth obtained from a fluoridated area to be significantly less than that of enamel from a fluoride-deficient area, although corresponding differences in respect of deciduous enamel were not statistically significant. The latter study⁽²⁴⁰⁾ also demonstrated an inverse association between the actual fluoride content of surface enamel and its solubility. Indirect evidence from several other studies⁽⁶⁰⁾⁽²³⁹⁾⁽²⁴¹⁾⁽²⁴²⁾ supports this finding.

As opposed to the beneficial effect of fluoride in enamel,

carbonate has been suggested to predispose to caries possibly by increasing the solubility of enamel. (243) (244) (245) Selective loss of carbonate from surface enamel during the initial stages of caries attack, demonstrated by Hallsworth, Weatherell and Robinson, (246) is in agreement with this view. The contention is further supported by the results of Lambrou, (247) who found that previously decalcified enamel slabs, which were allowed to re-calcify in the presence of carbonate, were more soluble in acid buffer than controls re-calcified in the absence of carbonate. In a recent publication, Driessens (248) described means whereby the incorporation of foreign ions can change the solubility of apatite and proposed that the incorporation of fluoride ions stabilizes the apatite crystals by reducing the incorporation of sodium and carbonate ions. Cutress (86) examined the relationship between enamel composition and solubility, using teeth collected from a variety of geographical areas and racial groups. In most cases, solubility was inversely related to the fluoride content and directly related to the carbonate content and carbonate : fluoride ratio of the enamel. Both carbonate content and solubility were lower in permanent teeth than in deciduous teeth.

A mineral salt will continue to dissolve in an aqueous solvent until the product of its ions dissolved in the solution reaches a constant. Thus, the term 'enamel solubility test' implies conditions under which equilibrium is reached between the enamel and the solvent employed. When extracted teeth, biopsy specimens or enamel powder are tested in the laboratory (as in most of the investigations mentioned above), the time taken for the test is of little consequence and equilibrium conditions can be

attained with relative ease. However, in an in-vivo situation, and particularly under the field conditions of the present investigation, it would have been impractical to test solubility in its true sense. Therefore a simple test of short duration was employed and the results represent the dissolution rate of surface enamel under standard conditions.

Examination of the data summary showed that the mean values were uniform for most villages and the coefficient of variation was of the order of 5 - 12 per cent. Notable exceptions were Villages 1, 2, 3 and 7. In Villages 1 and 2 the standard deviation was unusually high for males and in Villages 3 and 7 the same was true for females. Examination of individual results showed that the apparently high variability was caused by one aberrant result in each of the villages, in that subjects 3, 18, 27 and 73 had calcium values of 8.5, 91.2, 77.9 and 36.8 μg , while the overall mean value was 21.4 μg . Back-tracking the experimental procedure and calculations did not yield an explanation for the unusual values and no material was available for repeat determinations. The calcium values for Variable 220 were examined for the four subjects and were found to be within the normal range. In the light of the latter information, it was considered highly probable that the aberrant results were caused by some form of experimental error and arrangements were made to omit the figures from further analyses. It is of interest to note that when subjects 3, 18, 27 and 73 were omitted from the calculation, the mean values and standard deviations for the respective subgroups came within the expected limits, with corresponding changes in the values for the villages affected and for the whole sample:

<u>Village</u>	<u>Males</u>		<u>Females</u>		<u>Overall</u>	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
1	18.8	2.38			19.9	2.35
2	20.6	1.73			20.7	1.48
3			20.3	1.89	20.3	2.30
.....						
.....						
7			21.6	2.44	21.5	1.61
.....						
.....						
Total	20.9	2.01	21.1	2.05	21.03	2.03

The foregoing paragraphs show that the data summary can serve as a means for identifying aberrant results. Further examination of the summary did not reveal notable trends in village or village group means. Mean values for males and females were closely comparable. The overall mean value indicates that the average depth of the biopsy was 6.13 μ m.

4.2.5. Plaque pH and activity measurements (Categories 23, 24, 25 and 26).

The production of acid from fermentable carbohydrates by bacteria localized on tooth surfaces, has been recognized by Miller⁽²⁴⁹⁾ as a fundamental process in the causation of caries. In his classical experiments, Stephan^{(250) (251)} exposed plaque in-situ on anterior tooth surfaces and in carious cavities to a rinse of 10 per cent sucrose solution and demonstrated a sharp drop in pH from resting values of about 6.5 to approximately 5.0 within 2 - 3 minutes, followed by a slow return over some 40 minutes to the resting pH. Apart from one dissenting opinion,⁽²⁵²⁾

it has been accepted that, in general, the application of fermentable substrates is followed by the production of acid at a faster rate than it can be buffered or diffused out of the plaque, causing a drop in pH. Exceptions have been demonstrated in subjects with low resting pH values, where increased salivary flow, following the ingestion of sweets, may cause a rise of plaque pH. (253)

In subsequent experiments, Stephan⁽²⁵¹⁾ observed that in caries-active persons the resting pH and the terminal pH were lower than in caries-free or caries-inactive persons.

In further investigations, Stephan⁽²⁵⁴⁾ and others⁽²⁵⁵⁾ have shown that the presence of substrates which yield basic metabolic end products (such as ammonia from urea), increases the pH of plaque. It has been shown by Kleinberg⁽²⁵⁶⁾ that the extent and duration of rise or fall in plaque pH depends on the concentration and period of availability of the substrate and on the type of substrate applied. Other observations, based on extensive unpublished data⁽²⁵⁷⁾ indicate, however, that the quality of the plaque has an additional strong influence in this regard. Results of plaque pH measurements following consumption of common carbohydrate foods, instead of sucrose application, and tests involving other tooth surfaces,⁽²⁵⁸⁾ were in agreement with Stephan's findings.

Since the first experiments of Stephan,⁽²⁵⁰⁾ in-situ plaque pH measurements were used extensively for studies of plaque metabolism in man⁽²⁵⁹⁾⁽²⁶⁰⁾⁽²⁶¹⁾⁽²⁶²⁾⁽²⁶³⁾ and in animals⁽⁴⁸⁾⁽²⁶⁴⁾⁽²⁶⁵⁾ and for examining the effectiveness of potential agents for

the control or modification of acid production by plaque bacteria. (264) (266) (267) (268) (269) Development of in-vivo pH radio telemetry⁽²⁷⁰⁾ has made continuous plaque pH measurements possible on interdental surfaces, but it was limited to persons having the edentulous spaces needed for instrumentation. A modified technique⁽²⁷¹⁾ without this limitation was still too complex for a field study involving a large number of subjects. Therefore the simpler method described previously was employed.

For reasons stated previously (sub-section 3.2.3.6.), plaque pH measurements were carried out for a reduced number of subjects. However, it can be seen from the summary tables that data are available for 220 subjects in respect of Category 23, and for 212 subjects in each of the remaining three categories; of the 212 subjects, 73, 86 and 50 were residents in the high, low and zero caries villages, respectively. Thus, comparisons are possible at all caries prevalence levels.

For Categories 23, 24 and 25 the village means were found to be uniform with overall coefficients of variation of 8.9, 8.1 and 10.6 per cent, respectively. Males had lower mean pH values than females in respect of all three categories on a whole sample basis. The same was true for village groups, but a few exceptions were noted within villages. The mean resting pH of 6.15 for the whole sample was lower than that expected from the literature (6.5 - 7.0). However, in the past, most plaque pH measurements have been carried out on very small groups of subjects. As for many other parameters measured in this investigation, no comparable published data exist in respect of in-situ plaque pH determinations made in a substantial human sample which was representative of the

population from which the sample was drawn.*

It is of interest to note that 20 seconds after the supply of substrate the mean pH for the whole sample had fallen from 6.15 to 5.78 and that at 80 seconds the mean pH value was 5.19. In one of the villages where measurements were made for all subjects (Village 12), a mean pH value of 4.8 was reached.

Thus, it would appear that the acid producing potential of plaque was not lacking at any of the caries prevalence levels and, for the whole sample, it was as great or greater than that found in other investigations, which usually involved only a few individuals.

No consistent trends were noted for villages or village groups in respect of Category 23. For Categories 24 and 25 there appeared to be a weak but consistent trend of inverse relationship between pH values and caries at the village group level:

Village Group	Category 24		Category 25	
2	5.65	(22)	5.03	(93)
3	5.83	(15)	5.24	(58)
4	5.89	(13)	5.33	(47)

(The figures in brackets indicate the molarity of hydrogen ion concentration $\times 10^{-7}$ corresponding to the pH values.)

As expected, the figures for Category 26 were far from uniform and the coefficients of variation were, as a rule, very

* Charlton, Schamschula, Blainey and MacDonald⁽²⁵⁷⁾ collected similar data in a longitudinal investigation of N.S.W. school-children. (Unpublished study).

high. It will be recalled that the results for this category were calculated after converting pH units to hydrogen ion concentration. This procedure shows the changes in hydrogen ion concentration in plaque in the correct perspective.

Because of the logarithmic relationship between pH and hydrogen ion concentration, differences which seem small when they are expressed in pH units are shown in their true magnitude and are accentuated progressively with increasing distance from neutrality.

For example, two subjects with resting pH values of 6.5 and 6.0, and a fall of 1.0 pH unit in one minute, would show rates of change of hydrogen ion concentration of 27 and 90 units, respectively. Similarly, variation in the slope of the line of best fit (Figure 5, page 59), resulting in a change of say, 0.3 pH units from 4.3 to 4.0 at the end point, would result in a difference of 498 units in plaque activity.

Examination of the mean values for Category 26 did not reveal noteworthy trends.

4.2.6. Streptococci in plaque (Categories 29 to 104).

A very considerable proportion of the total research effort directed towards the aetiology and prevention of dental caries has been concerned with the streptococcal component of the plaque flora for the reasons outlined below.

Streptococci constitute a substantial proportion of the cultivable microbiota found on the surfaces and in occlusal fissures of teeth. (272) (273) (274) (275) (276) (277) (278) Single cells and aggregates resembling streptococci have been found on

tooth surfaces as soon as 20-60 minutes after prophylaxis⁽²⁷⁹⁾
⁽²⁸⁰⁾ and although the relative proportions of organisms change
 with the ageing of plaque,⁽²⁸¹⁾ streptococci tend to remain
 predominant at most sites.

Most species of streptococci can lower pH to about 5.0 -
 4.0 and many are sufficiently aciduric to continue metabolism
 under these conditions. They have been isolated from carious
 cavities as early as the last century, and their presence at
 various stages of caries development has been demonstrated by
 numerous workers. (For reviews concerning these aspects see
 Burnett and Scherp.⁽²⁸²⁾⁽²⁸³⁾) Since the isolation of the pre-
 viously unknown S. mutans from carious lesions by Clarke,⁽¹⁹⁴⁾
 interest has been maintained in the presence of various species
 of streptococci in carious enamel and dentine as shown by several
 recent reports.⁽¹⁶⁴⁾⁽¹⁶⁶⁾⁽¹⁶⁹⁾⁽²⁷⁸⁾⁽²⁸⁴⁾

During the last two decades, the possible importance of the
 formation of extracellular homopolymers of glucose (dextrans),
 fructose (levans) and intracellular storage polysaccharides
 (anylopectin - glycogen) by plaque microorganisms, has received
 considerable attention. Many species of streptococci have the
 ability to form one or more of these polysaccharides, a property
 by no means unique to streptococci. Dextrans are not readily
 metabolized and are thought to be of importance in promoting the
 formation and maintenance of plaque. Levans can be degraded by
 plaque microorganisms and may serve as a reservoir of fermentable
 carbohydrate as do the intercellular storage polysaccharides.
 Relevant aspects of the aetiological importance and metabolism
 of bacterial polysaccharides have been reviewed by Fitzgerald,⁽²⁰⁹⁾

Critchley and associates, (285) Guggenheim (286) and Newbrun. (287)

The role of extracellular polysaccharides in facilitating attachment of streptococci to tooth surfaces has been generally recognised. However, streptococci (and other bacteria) which do not normally produce these substances, also attach to oral tissues and possible alternative mechanisms have been extensively investigated. Following demonstrations of clumping of bacteria by undefined constituents of saliva, (288) it was found that after interaction with saliva, different streptococci exhibit selective affinities for various oral tissues. Thus, in the presence of saliva, S. mitis and S. sanguis are more readily adsorbed to enamel surfaces than S. mutans unless the enamel is exposed to dextran. (289) (290) (291) (292) Aspects of the aggregating capacity of saliva and bacterial adherence have been the subjects of recent reviews by Gibbons (293) and van Houte. (294)

Several Streptococcus spp. have been shown to induce caries in a variety of experimental animals under appropriate dietary conditions. Following failures with other microorganisms tested, Orland and his associates (295) induced caries in germfree rats after infecting the animals with an enterococcus now thought to have been a strain of S. faecalis. (296) Streptococcus mutans has been used extensively for inducing caries in experimental animals. The role of this organism in animal caries and its implications have been discussed in several excellent reviews. (3) (297) (298) (299) Although not consistently present in human dental plaque, (300) (301) S. salivarius was also shown to be capable of producing caries in hamsters. (302) A recent report, (303) preview

of a pending publication,⁽³⁰⁴⁾ and personal communications⁽³⁰⁵⁾ confirmed that under appropriate conditions S. sanguis is capable of inducing carious lesions in rats.

Examination of the streptococcal flora of plaque has been the subject of extensive epidemiological studies. It appears that attention has been focussed on S. mutans and several investigations demonstrated positive associations between the prevalence or relative proportion of this organism in the plaque microbiota and the prevalence or incidence of caries in man.⁽¹²⁾
 (20)(162)(164)(165)(171)(306)(307)

Several of the studies listed above⁽¹⁶²⁾⁽¹⁶⁴⁾⁽¹⁷¹⁾ indicated that there was an increase in the number and/or relative proportion of S. mutans in plaque with the development of new smooth surface carious lesions. Littleton, Kakehashi and Fitzgerald⁽¹⁶⁹⁾ have found that the mean proportion of "specific caries-inducing streptococci" in plaque collected from carious lesions, was over 40 per cent compared to 4 per cent in plaque from non-carious sites. The percentages were expressed as the proportion of all cultivable streptococci, using the method of Jordan, Krasse and Möller.⁽¹⁶³⁾ A recent study by Ikeda, Sandham and Bradley⁽³⁰⁸⁾ involved longitudinal examination of selected tooth surfaces for the presence of S. mutans. The authors found that "the initiation of a carious lesion on a tooth surface was closely associated with a history of a previous S. mutans infection of the surface; in the absence of such a history no caries occurred". Preliminary results of a study of 18 Naval recruits, who were free of caries at the time they entered the Service, indicated that only those who harboured S. mutans developed carious lesions after one year.⁽³⁰⁹⁾ Recently,

the localization of S. mutans on proximal tooth surfaces was examined in two groups of children with contrasting caries prevalences of 1.4 and 7.6 mean DMFS per person.⁽³¹⁰⁾ Apart from finding consistent patterns of localization within an individual's mouth, it was reported that "the number of tooth surfaces infected with S. mutans parallels the caries experience of these populations."

The circumstantial evidence linking this organism with human caries is substantial. However, one cannot help recalling the times when lactobacilli were regarded by many as the most likely potentially cariogenic organism. One should ask, whether we are once again focussing attention on a specific organism, and all but neglecting other members of the plaque flora or the possibility of non-specific odontolysis as the result of interactions of the plaque microbiota in general.

It has been pointed out already that streptococci other than S. mutans can cause caries in animals. The same has been shown for lactobacilli⁽³¹¹⁾ and for actinomyces with⁽³¹²⁾⁽³¹³⁾ or without concomitant periodontal disease⁽³¹⁴⁾⁽³¹⁵⁾ and is probably true for other members of the plaque flora not yet tested or not tested under appropriate conditions. It should not be overlooked that test conditions applied in most animal experiments in the past (i.e. very high sucrose diet) favour S. mutans and may be inhibitory to other organisms.⁽²⁹⁶⁾ Moreover, there is no reason to believe that, an organism which cannot establish itself, or shows little or no activity in monoinfection or in relative gnotobiosis induced in, say, a rat, behaves in the same way when it is supported by an entire ecological system, which develops

according to the advantages and limitations offered on a given human tooth surface.

One should also consider that most workers have found that S. mutans makes up but a small part of the streptococcal component of the plaque flora, usually under 10 per cent, which makes it diminutive compared with the total number of microorganisms present in plaque.

Some epidemiological data also point to the fact that S. mutans may not be an indispensable contributor to human caries. Mikkelsen and Poulsen⁽³¹⁶⁾ examined the composition of interproximal plaque flora in relation to the development of caries in a longitudinal study involving 54 children who were caries free at the beginning of the study. No association was found between the ratio of the number of S. mutans to the number of microaerophilic streptococci and the development of carious lesions. In concluding their presentation to the meeting (ORCA 1973) the authors stated: ".... Streptococcus mutans in the present study represents only about 1 per cent of the cultivable flora...." and "....This low incidence of Streptococcus mutans in plaque related to the other results of the present investigation indicates that future investigations of the plaque flora during caries development in humans must include a study of the total plaque flora instead of being limited to a study of separate more or less caries-conducive microorganisms...."

Preliminary results of a study involving persons suffering from xerostomia following irradiation therapy of the head and neck, indicate an increase of caries experience without a detectable

increase in the S. mutans fraction of plaque. (317)

In studies of plaque streptococci conducted over several years, the author and associates have been able to demonstrate associations between the presence and relative proportions of S. mutans in plaque and caries prevalence. (20) However, also in the author's personal experience, there were so many instances where S. mutans was not detectable prior to the initiation of, or in association with, the development of carious lesions, that he cannot help sharing the scepticism expressed by Jenkins (210) about unduly limiting plaque studies to a potential specific pathogen.

Turning to the present work, all variables representing streptococcal counts and classifications have been included in the data summary, although data based on pooled plaque were not included in the statistical examination of the results, for reasons stated in sub-section 3.4.3. and Section 4.4.

As would be expected for parameters subject to very high biological variation and to considerable experimental error, the results show high variation between villages, village groups and sexes. This is reflected by the fact that the coefficient of variation is rarely less than 100 per cent and is frequently higher.

The mean values of streptococcal counts in pooled and in UL2 plaques at 10.4×10^8 and 13.2×10^8 colony forming units per mg plaque were higher than expected. In a previous study, (20) the author and associates have found corresponding values of 1.4×10^7 and 4.8×10^7 , although differences in the methods used for

quantitation and preservation of viability, could account for a considerable proportion of the differences between the values. Gibbons and associates⁽²⁷⁵⁾ examined the total number of bacteria in five samples of plaque. The mean values found using microscopic counts and anaerobic culture methods were 2.5×10^{11} and 4.6×10^6 microorganisms per gram of plaque material.

Because of the very high standard deviations, it was unlikely that examination of the summaries could have disclosed the presence of aberrant individual results in subdivisions of the sample. As an alternative test, individual results were examined to ascertain whether any of them were outside the limits of practical possibilities. This aspect was tested by calculating the theoretical weight of viable streptococci found per unit weight of plaque material. For this purpose, streptococci were regarded as spherical cells of unit density with a diameter of the order of 0.5 - 0.7 μm . The latter figures were based on microscopic examination using phase contrast and Gram staining of Gram positive coccal forms found in plaque smears collected from a similar population in New Guinea. Examination of electron micrographs of plaque⁽³¹⁸⁾ indicated that in densely packed situations, the cells were of a cuboidal shape occupying practically all (up to 95 per cent) of the available space. On this basis, the maximum number of streptococci per mg wet weight of plaque could be $5.6 - 15.3 \times 10^9$. Higher results than the mean of these values (10.4×10^9) were obtained for 7 pooled and 5 UL2 plaque samples. These results were attributed to unknown experimental error and were rejected before constructing the data summaries. The likelihood that approximately this number of

outlying results would occur was indicated by the standard deviation when the accuracy of the method was tested (sub-section 3.3.2.4.).

Much consideration was given to the possible reasons for the unexpectedly high counts. It was known that the counts obtained were, on the average, 25 - 30 per cent higher than the real values, because the calculations were based on the weight of plaque 31 seconds after collection, by which time a reduction of weight occurred due to loss of moisture (see sub-section 3.2.3.5.). This effect could have been enhanced by delay in weighing of a number of samples, although no specific instances were noted in the field.

With regard to the composition of the samples, it is not unreasonable to assume that plaque which is rarely exposed to sucrose, contains little extracellular polysaccharide and has a higher microbial density than plaque subject to frequent sucrose exposure. This would be in accordance with the fact that sucrose consumption in the present sample was negligible compared to Western societies.

There are no data in existence concerning the proportions of all plaque microorganisms for the complete sample or for comparable populations. However, Drs. J.M. Hardie and G.H. Bowden of the London Hospital Medical College examined aliquots of 15 of our homogenized plaque samples, using culture methods applicable to the whole flora. In this limited subsample, very small numbers of organisms usually isolated from plaque material, such as Actinomyces and Neisseria, were found. Admittedly, prolonged storage and two stages of processing and transport may have been detrimental to some organisms, although in the author's personal experience, storage under the conditions used is suitable for

Actinomyces species. It is of interest to note that the streptococcal counts carried out by Drs. Hardie and Bowden corresponded closely with our results.

The foregoing considerations, together with the large experimental error, could have been regarded as an explanation for the elevated counts. However, the author was not satisfied with the explanation, and the possibility that growth had occurred during non-frozen storage of the specimens was considered, in spite of the fact that repeated tests in the laboratory failed to demonstrate growth in the VMGII - sucrose medium at ambient temperatures of up to 37°C over periods of up to 1 week. A review of procedures suggested, that the only time when the samples were stored unfrozen for periods sufficient for growth to occur, was during the day the samples were collected. The procedure used to test this aspect was based on the experimental results. It was assumed that, if growth did occur between plaque collection and freezing of the specimens, subjects who had plaque collected early during the day's work, would have higher mean counts than subjects who had plaque collected later during the day. Thus, the subjects were stratified into eight levels according to the times of day when the plaque samples were collected, and the mean values of streptococcal counts were calculated for each stratum. The outcome is shown in Table 3, page 244.

The fact that, against all expectations, growth did occur in the samples, was clearly indicated by the figures shown in Table 3. Although a statistical test was hardly necessary to prove that

TABLE 3.

The mean number of colony forming units of streptococci isolated from pooled and UL2 plaque samples, stratified according to the time of day when the samples were collected.

	Time stratum *							
	8	7	6	5	4	3	2	1
Mean no. of strep/mg pooled plaque x 10^8	12.5	16.0	13.3	9.2	7.9	2.6	2.7	1.9
S.D.	14.3	18.7	14.9	10.5	11.3	2.5	4.9	1.4
Number of subjects	26	56	58	51	32	11	20	15
Mean no. of strep/mg UL2 plaque x 10^8	19.1	23.0	11.7	13.2	7.0	2.5	3.4	1.4
S.D.	21.3	23.2	13.8	19.1	14.7	1.9	4.4	1.0
Number of subjects	15	38	46	39	22	7	8	6

* 8 = Before 10 a.m., 7 = 10 - 10.59 a.m., 6 = 11 - 11.59 a.m.,
 5 = 12 - 12.59 p.m., 4 = 1 - 1.59 p.m., 3 = 2 - 2.59 p.m.,
 2 = 3 - 3.59 p.m., 1 = 4 p.m. and later.

this was indeed so, the relationship between individual streptococcal counts and the time elapsed between the collection and freezing of the samples was examined using regression analysis. Despite the fact that the relationship between the mean counts and time was not linear (Table 3), the r values of 0.310 and 0.306 obtained for the pooled and UL2 samples were significant at the $P < 0.001$ level. The number of observations for these analyses were 267 and 177 respectively.

As laboratory tests failed to produce a similar effect in the same medium, the reasons for growth having occurred in the field are not clear. It is suspected, however, that some compositional variation in the particular batch of medium used in the field may have been responsible. However, laboratory records showed no departure from the normal routine of medium preparation.

As a next step, it was necessary to examine whether or not the growth had led to a change in the proportion of various colony types. To test this aspect, the individual values for the proportion of each colony type (expressed as the percentage of all colony types isolated from a given sample) were regressed against the times of collection of the respective specimens. The analyses were carried out separately for pooled and UL2 plaque samples. For each colony type, all plaque samples from which that colony type was isolated were included in the analyses, provided that the time of sample collection was known. The results, which show the correlation coefficients, significance levels and number of observations in respect of each colony type, are shown in Table 4, page 246.

TABLE 4.

Product-moment correlation coefficients between the time of plaque collection and the percentage of 12 streptococcal colony types isolated from pooled and UL2 plaque samples.

Colony type (per cent)	Pooled plaque			UL2 plaque		
	n	r	P	n	r	P
1	70	0.11	N/S	36	0.20	N/S
2	200	0.06	N/S	73	0.16	N/S
3	80	0.14	N/S	62	-0.16	N/S
4	201	-0.03	N/S	119	0.17	N/S
5	183	-0.20	0.05	78	-0.13	N/S
6	53	0.05	N/S	38	0.01	N/S
7	39	0.07	N/S	14	-0.01	N/S
8	269	0.01	N/S	178	0.11	N/S
9	39	0.27	N/S	11	-0.32	N/S
10	259	-0.03	N/S	158	0.05	N/S
11	150	-0.01	N/S	79	-0.16	N/S
12	199	-0.07	N/S	144	-0.05	N/S

It may be observed from Table 4, that the correlation coefficient for Colony type 5 in pooled plaque was statistically significant at the $P < 0.05$ level. However, it should be noted that the value of r obtained was small and negative (-0.20), indicating an inverse association with time elapsed. Statistical significance was probably achieved as a consequence of the fact that the number of observations was large. In addition, tests of significance were being conducted at the same time for 24 related sets of data, thus magnifying the danger of obtaining a single spurious result. This possibility has been discussed in subsection 3.4.3., with reference to the findings of Johnson and Leone. (203)

It is also clear from Table 4 that the sign of r , indicating increase or decrease of the relative proportions of colony types with time elapsed, was positive and negative for approximately the same number of colony types, and it was frequently opposite for the same colony type in pooled and UL2 plaque. Thus, it was concluded that there was no consistent or significant pattern in the relative proportions of the 12 colony types in relation to the time of plaque collection.

Complications arising from the growth of streptococci during the holding stage, raised the question of whether or not it was justified to use the data for subsequent statistical analyses. It appears that the relative proportions of the 12 colony types remained unaffected by the growth and need not be considered further in this context.

In respect of total streptococcal counts, one must conclude

that the absolute values obtained were not the true values. Because of the close association between the time of day and the streptococcal counts, it may have been possible to calculate and apply a correction factor which would have led to a better approximation of the actual values. This would have required arbitrary decisions, both as to the shape of the relationship involved and as to the point on the time scale to which all values would have had to be adjusted. However, it is clear from Table 3 that the size of the standard deviation is closely related to the mean value in each case, and therefore, if further analyses were to be based either on mean values or on lines of regression derived from individuals, such adjustments seemed unnecessary.

The effect of different extents of growth in individual cultures on possible associations between the total number of streptococci in plaque and caries experience was also considered. In this respect, it was realised that the additional experimental error arising from this source would reduce the strength of correlations, but would not produce spurious associations unless the error were in some way associated with caries experience. This latter aspect was examined by calculating the mean caries experience of subjects stratified according to the time of day their plaque samples were collected. The results are shown in Table 5, page 249.

It appears from Table 5 that plaque collection took place for subjects throughout the day, in such a way, that there was no tendency of association between the caries experience of the subjects and the time of plaque collection. This was confirmed

TABLE 5.

The mean caries experience of subjects stratified according to the time of plaque collection.

	Time stratum *							
	8	7	6	5	4	3	2	1
Mean caries experience (DMFT)	1.8	3.5	3.7	2.9	4.4	0.8	3.4	4.3
S.D.	2.9	4.4	4.6	3.2	4.1	1.2	3.5	4.2
Number of subjects	32	62	62	54	27	11	22	17

* The time categories used were the same as shown in Table 3, page 244.

by means of a regression analysis using individual data for 288 subjects. The product-moment correlation coefficient relating caries experience (DMFT) to time, 0.074, was not significant. Because of the high correlation between various expressions of caries experience (DMFT vs. DMFS $r = 0.98$, DMFT vs. Severity score $r = 0.94$) there was no need to conduct similar analyses for DMFS and the severity score of carious lesions.

From the foregoing considerations it was concluded that no bias would arise from the experimental error associated with the streptococcal counts, and the data were submitted for analyses.

Examination of the summary tables showed that the most prevalent Colony types were 8, 10, 12 and 4, in decreasing magnitude, accounting between them for approximately 80 per cent of cultivable streptococci in both the pooled and UL2 plaque samples. The least prevalent types were 1, 7 and 9, together making up 2 and 1 per cent of the colony types in the pooled and UL2 samples respectively. The remaining 18 - 19 per cent was divided fairly evenly between Colony types 2, 3, 5, 6 and 11. The prevalence of colony types in the villages was all but complete, because disregarding Village 6 where only two subjects were examined, all colony types except 9 were isolated from one or the other of UL2 or pooled plaque samples in all the villages. Colony type 9 was not found in Village 9.

It should be mentioned here that aliquots of all the homogenized plaque suspensions were sent to Dr. D. Bratthall at the Faculty of Odontology, at the University of Göteborg, for immunofluorescent screening for the presence of S. mutans. Cells

which were classed as S. mutans according to Dr. Bratthall's criteria, (178) were found in 50.4 per cent of the specimens and were present in all villages. In less than a quarter of the samples did S. mutans account for over 1 per cent of the total microscopic count of bacteria, and only in 8 per cent of the samples did it reach 5 per cent or more of the total microbial population.

The summaries were examined for possible trends with caries on a village group basis. No obvious trends were noted in the figures based on pooled plaque samples. In examining the UL2 plaque results, Village Group 1 was disregarded because data were available for only a few subjects in those villages. Trends were noted for one or another expression of several colony types. The most notable was Colony type 5, which showed a trend of positive association with caries prevalence in all three forms of expression (Categories 73, 85 and 97).

4.2.7. Hypoplasia index, mean score (Category 201).

The summaries support the conclusion reached in the field that the incidence of hypoplasia was low, and with a few exceptions, hypoplastic lesions were mild throughout the sample. The prevalence was approximately equal for the sexes. No consistent trend with caries was revealed by the summary, but it was noted that the mean prevalence of hypoplasia was highest and lowest in the high and zero caries villages, respectively.

4.2.8. Mesio-distal crown diameters of upper anterior teeth (Categories 205 - 207).

The values shown by the summary tables were generally

uniform and within the limits of expectations. The size of the coefficients of variation, greatest for the lateral incisor tooth at 10.7 per cent for the whole sample, indicated no remarkable variability in the mesio-distal diameter of the teeth measured. Overall, the measurements for males were greater than for females, but the differences were small and the opposite was true in some villages for each of the three teeth measured.

A comparison of the present results for Melanesians, with corresponding measurements for three different racial groups reported by Lavelle⁽³¹⁹⁾ is shown below:

<u>Racial Group</u>	<u>Tooth</u>	<u>Male</u>		<u>Female</u>	
		M-D diam(mm)	S.D.	M-D diam(mm)	S.D.
Melanesians (R.G.S.)	Central I.	8.63	0.70	8.34	0.69
	Lateral I.	6.79	0.69	6.76	0.76
	Canine	7.88	0.70	7.47	0.53
Caucasoid (Lavelle)	Central I.	8.79	0.45	8.54	0.41
	Lateral I.	6.32	0.56	6.21	0.18
	Canine	7.53	0.38	7.35	0.59
Negroid (Lavelle)	Central I.	9.33	0.59	9.21	0.44
	Lateral I.	7.61	0.63	7.48	0.38
	Canine	8.32	0.62	8.21	0.52
Mongoloid (Lavelle)	Central I.	8.67	0.42	8.57	0.59
	Lateral I.	6.99	0.77	6.85	0.62
	Canine	8.07	0.66	7.94	0.54

Generally, the measurements for the Melanesian group are more closely comparable to Caucasoids and Mongoloids than to Negroids. It is to be noted, however, that Lavelle carried out

the measurements on casts and, with 40 subjects in each, his groups were considerably smaller than the Melanesian group in this investigation.

There is considerable evidence from correlations between parents and offspring and among siblings, that tooth size is determined primarily genetically. (320) (321) However, recent studies (322) (323) showed a reduction in the correlation of tooth size between immigrant parents and their offspring, suggesting the operation of an environmental component.

Because tooth size may be an indicator of genetic groupings and because of the possible operation of a genetic factor directly, or indirectly through customs, habits or other environment-associated variables in the causation of caries, particular attention was paid to possible trends shown by villages or village groups. However, no consistent trends were revealed by the summary.

4.2.9. Variables related to the betel-chewing habit (Categories 208, 209 and 213).

Betel-chewing is a widespread habit practised from early childhood in communities living in the coastal areas, foothills and islands of Papua New Guinea. Observation of betel-chewing led the author to consider the possible effect of the practice on the initiation and development of carious lesions. A literature search revealed little factual information on this topic, (114) (115) but exchange of views with investigators who also observed the habit (Dr. D.A. Anderson, Dr. D.E. Barmes, Mrs. B. Blainey, Dr. G. Charlton, Dr. I.J. Møller), supported the possible relevance of

betel-chewing to caries. Of particular interest was an independent observation made by Møller⁽³²⁴⁾ who found that in an Indonesian community where women chewed betel and men did not, the caries prevalence of males was significantly higher than that of females.

In considering the following summary of the author's views concerning possible mechanisms through which betel-chewing may exert a protective effect against caries, it should be kept in mind that the frequency of betel-chewing usually increases with age until at least young adulthood.

- 1) Betel-chewing reduces the frequency of food intake with corresponding reduction in the frequency of substrate supply to the plaque flora.

- 2) Betel-chewing induces a copious flow of saliva, apparently much more so than mastication of common foods, with a resultant increase in its protective effects.

- 3) Because of the coarse fibrous nature of the Areca nut (and of the pepper or bark used with it) and the coarseness of the home-calcined lime, the practice tends to remove or disrupt plaque.

- 4) It is well known that plants often contain bactericidal or bacteriostatic compounds - such as essential oils, phenol, cresol and creosote. The release of such substances from the nut, pepper or bark could result in modification of the activity or composition of the plaque flora. It is also known that plants often concentrate minerals. The findings of Nanda and Kapoor⁽³²⁵⁾ show that some varieties of Areca nut contain as much as 12 ppm fluoride. Thus, betel-chewing may result in a high fluoride

intake and in a topical application at each chewing.

5) Hydration of the lime (CaO) into calcium hydroxide results in a strongly alkaline environment; acids present at the tooth surface are likely to be neutralized and the ionic equilibrium at the enamel-plaque fluid interface shifts towards incorporation of calcium ions into the apatite crystals.

6) Dissolved lime would contribute to a common ion effect (Ca^{++}), slowing down the dissolution rate of enamel.

7) Bibby⁽³²⁶⁾ suggested that the presence of brown-black stains on the teeth was associated with a decreased frequency of caries. Sutcliffe⁽³²⁷⁾ reviewed several investigations with similar findings. The dark, strongly adherent film that forms on the enamel surfaces of betel-chewers may have a protective effect in several ways: a) by separating bacteria from the enamel surface, b) through its organic content, by forming a barrier to acids produced by the plaque flora, c) by serving as a reservoir of calcium ions. On the other hand, the possibility that the film can contribute to a framework maintaining bacteria on the tooth surface, as was recently demonstrated for black stain by Theilade and associates,⁽³²⁸⁾ cannot be discounted.

8) Betel-chewing contributes to attrition which in turn can physically eliminate slowly progressing carious lesions. Alternatively, the habit may result in the enlargement of the surface opening of lesions by fracturing undermined enamel and may thus contribute to the slowing down or arresting of the progress of odontolysis.

It is not intended to suggest that betel-chewing, as such, could be a caries preventive measure. However, the points listed suggest many implications and areas to be explored regarding the aetiology and prevention of caries.

The summary concerning the number of Areca nuts chewed per day (Category 208) showed considerable individual variation as one would have expected. Males and females appeared to be equally addicted to the habit. It is noteworthy that the mean number of nuts used per day was almost three times greater in Village Groups 3 and 4 than in Village Groups 1 and 2.

This was not reflected by the mean colour of saliva (Category 213), which may only mean that subjects in the lower villages may have rinsed their mouths more thoroughly before saliva collection than subjects in the top-river villages.

4.2.10. Viscosity of saliva (Category 214).

The relationship between the viscosity of saliva and caries experience received the attention of workers soon after the turn of the century. Three independent early investigations reviewed by Ericsson and Stjernström⁽¹¹⁷⁾ resulted in the conclusion that high viscosity of saliva was, as a rule, associated with elevated incidence of caries. Similar conclusions were reached in investigations reviewed by Afonsky.⁽¹⁵⁸⁾

Lack of interest in this field by contemporary workers (during the last decade) was indicated by the fact that viscosity was not discussed in the extensive review of the relationship between relevant properties of saliva and caries by Mandel.⁽³²⁹⁾

Together with a large number of other variables measured, Shannon (330) reported on the viscosity of stimulated parotid saliva of 384 subjects in relation to flow rate, but made no reference to the caries experience of the subjects.

The summary tables indicated marked individual variation in the viscosity of saliva, reflected by coefficients of variation of the order of 50 per cent. The variation between villages was also considerable, without exhibiting consistent trends. The mean values for males and females were comparable. Field observations were confirmed by the summary in that, contrary to the commonly held view, the highest mean viscosity was exhibited by the caries-free Village Group 4.

4.2.11. Bicarbonate content and buffering capacity of saliva
Categories 215, 216, 217, 218 and 219.

The carbonic acid - bicarbonate content of saliva as it enters the oral cavity, depends partly on the carbonic anhydrase content of the salivary glands⁽³³¹⁾ and partly on the partial pressure of carbon dioxide in the glands during secretion.⁽³³²⁾ Thus, the concentration of bicarbonate increases with gland activity and with the flow rate of saliva. Wah Leung⁽³³³⁾ and Lilienthal⁽¹¹⁸⁾⁽³³⁴⁾ demonstrated that bicarbonate was the most important buffer within physiological pH ranges in activated whole saliva. The results indicated that the bicarbonate system was responsible for approximately 65 - 80 per cent of the buffering capacity.* Phosphate was found to be the only other buffer of

* The high contribution made by the bicarbonate system to the buffering capacity of saliva is also evident from the present data. The product-moment correlation coefficients between the bicarbonate content and the two measures of the buffering capacity of saliva were 0.72 and 0.77. See Appendix, Part 2a, p.12.

importance.

The relationship between the buffering capacity of saliva and caries experience has been the subject of many investigations. Ericsson⁽⁴⁵⁾ evaluated the results of 21 studies and later reviewed the literature on this subject.⁽³³⁵⁾ He concluded that the inverse relationship between the buffering capacity of saliva and caries susceptibility was well established. The author and associates came to a similar conclusion in a three year study of 400 New South Wales school children⁽²⁵⁷⁾ wherein the buffering capacity of saliva showed consistent inverse correlation with caries prevalence. Andersson⁽³³⁶⁾ examined the pH and buffering capacity of saliva of 7 - 13 year old children in stimulated and unstimulated specimens. He found that the saliva of girls had a lower buffering capacity than the saliva of boys, and suggested that this fact may contribute to a higher caries prevalence of girls. However, no figures of caries experience were given. Marlay⁽³³⁷⁾ determined the buffering capacity of activated whole saliva collected at monthly intervals over two years from 30 adolescent girls. She found that rises in caries increments were preceded by a fall in salivary buffering capacity in 75 per cent of the sample. The mean time interval between the lowest value in buffering capacity and the peak of caries increment was nine months.

The carbonic anhydrase activity of saliva of caries-free and caries-active children, the latter with 10 or more carious teeth, were compared by Szabó.⁽³³⁸⁾ The carbonic anhydrase activity was significantly higher in caries-free than in caries-active children living in an institution. Corresponding differences were not

significant for children living at home. The author attributed the differences in the findings to the heterogeneity of the home environment and stressed the influence of nutrition and diet on the composition and properties of saliva. Nutritional and dietary factors are known to influence the buffering capacity of saliva. (45) (339) Flow rate has a similar influence (340) and tends to show considerable variation for individuals, particularly under the psychological stress of clinical examination or saliva collection. These factors may well account for findings of seemingly contradictory or ambiguous relationships between the buffering capacity of saliva and caries.

The summary for the bicarbonate content of saliva (Category 215) showed high variation in individual values reflected by an overall coefficient of variation of 57 per cent. The range of village means (0.004 - 0.010 meq/ml) was also considerable, but all values were well within expected physiological limits. (329)

(330) (341) (342) No consistent pattern emerged on examination of the village means, although the medium and high caries village groups had lower mean values than the low and zero caries groups. The mean bicarbonate content of saliva was higher for males than for females. This was reflected in a similar relationship for both parameters of buffering capacity (Categories 218, 219). Summaries in respect of the latter two categories had values and variation of the expected order and did not disclose any remarkable trends associated with the caries distribution of the sample. The number of subjects for whom data are available for the two parameters differs in Villages 1 and 11, because of technical difficulties encountered in the field.

4.2.12. Variables associated with the composition of surface enamel (Categories 220, 230, 231, 232, 233, 234, 235 and 236).

Results of a previous study involving a primitive population in Papua New Guinea⁽¹⁰⁾⁽¹³⁾ demonstrated associations between the concentrations of major and trace elements in soil, food and water, and caries prevalence. Similar associations were shown between a variety of trace elements and caries experience by several investigations conducted in different parts of the world.⁽³⁴³⁾⁽³⁴⁴⁾⁽³⁴⁵⁾⁽³⁴⁶⁾⁽³⁴⁷⁾⁽³⁴⁸⁾⁽³⁴⁹⁾⁽³⁵⁰⁾⁽³⁵¹⁾⁽³⁵²⁾⁽³⁵³⁾⁽³⁵⁴⁾⁽³⁵⁵⁾

Furthermore, epidemiological studies have detected considerable differences between the caries experiences of comparable populations exposed to similar levels of fluoride in the drinking water.⁽³⁴⁸⁾⁽³⁵¹⁾⁽³⁵⁶⁾⁽³⁵⁷⁾⁽³⁵⁸⁾ There could be many reasons for these differences, but the general conclusion drawn from the studies was that trace elements other than fluorine may contribute to resistance or susceptibility to caries in man. Several comprehensive reviews deal with relationships between trace elements and caries.⁽³⁵⁹⁾⁽³⁶⁰⁾⁽³⁶¹⁾⁽³⁶²⁾⁽³⁶³⁾⁽³⁶⁴⁾⁽³⁶⁵⁾⁽³⁶⁶⁾

In the design of the present investigation, it was reasoned that, if the elements which appeared to be of importance in the former study⁽¹⁰⁾⁽¹³⁾ did in fact influence individual susceptibility or resistance to caries, they must, in some form, exert an effect which would be manifest in the oral cavity. Hardwick⁽³⁵⁹⁾ stated that one of several possible ways in which trace elements may act was "....By their incorporation chemically in the hard dental tissues either during tooth formation or post-eruptively, thus rendering the tooth less susceptible to the carious process...."

The author feels this statement needs qualification, in that certain constituents of enamel may contribute to an increase in caries susceptibility. For example, carbonate as a constituent appears to be a liability, (246)(367) and the same may be true for certain trace elements. However, our knowledge concerning the exact composition and limits of variation in the concentrations of minor constituents of enamel are limited and, in the words of Losee, Cutress and Brown (368) "the influence of enamel composition on the carious process cannot be fully interpreted until the composition of the enamel is further documented". This is particularly true for surface and immediate subsurface enamel, to a depth of, say, 5 - 20 μ m, from the point of initiation and possible reversal (369) of decalcification. However, this is the area where the greatest gaps exist in our knowledge. The reason for this is that the amount of material required for conventional analytical techniques necessitated the use of bulk samples, usually involving the whole depth of enamel from one or more tooth surfaces and frequently the pooling of samples. Analyses of this type, limited to extracted teeth, have made substantial contributions to our knowledge of the composition of whole enamel, but the results are meaningless in terms of surface composition. In-vivo biopsy methods, mostly of the abrasive or etch type, have been used for over a decade. (For bibliography see sub-section 3.2.3.12.). These methods have made possible the examination of surface enamel composition in relation to individual caries experience. However, these studies were, as a rule, restricted to the determination of the fluoride content of surface enamel because the determination of trace constituents was precluded by the relatively large amounts of material required for the available analytical techniques.

The application of flameless atomic absorption spectroscopy to the determination of a range of major and minor elements employed in the present study, has made it possible for the first time to examine the relationship between the concentration of these elements in surface enamel and individual caries experience. The total amount of enamel used for the determination of eight elements was, on the average, 1 mg per subject. The amount needed for the determination of the four metals for which flameless atomic absorption was used, was less than 0.1 mg. Experiments are now in progress to further reduce the amount of material needed and to extend the scope to other elements. Of the latter, lead and copper determinations appear feasible already.

It follows from the previous paragraphs that no data are in existence which would be suitable for absolute comparison with the results presented, in spite of the fact that numerous investigations of enamel composition have been carried out. (For bibliography see sub-section 3.3.1.1., for reviews see⁽⁸⁶⁾(147)(148)(368)).

Under the circumstances, results obtained in recent studies by Cutress⁽⁸⁶⁾ and Losee, Cutress and Brown,³⁶⁸⁾ both known to the author for their particularly careful design and meticulous execution, were selected for comparison. Cutress⁽⁸⁶⁾ examined the composition of enamel removed from intact lingual surfaces of extracted premolar teeth obtained from specified geographically distant sources. The enamel was removed in three successive layers, each approximately 30 μ m thick, using a perchloric acid etch. The teeth and surfaces examined by Cutress⁽⁸⁶⁾ were different from those included in the present study and his results cannot be related to individuals because of pooling of samples. The fact

that the mean biopsy depth in the present study was much less than in Cutress' work, would also impair the validity of comparisons for elements which vary in distribution with depth.

Losee, Cutress and Brown⁽³⁶⁸⁾ examined the composition of sound whole enamel removed from the cuspal two-thirds of the buccal surface of 28 caries-free first bicuspid teeth. The teeth were extracted from individuals of 12 - 24 years of age, resident in 24 communities of 16 States of the U.S.A. Analyses were carried out by spark source mass spectrography for 66 minor or trace constituents, of which 35 were found to be present in measurable quantities. Once again, inter-data comparisons are only possible with appropriate reservations.

The first data summary table in the present series deals with the calcium content of the enamel biopsy specimen, Category 220. The amount of enamel removed by the biopsy procedure and, in turn, the concentrations of other elements determined, were based on this parameter.

Taking that the weight of enamel equals the weight of calcium x 2.7, the average amount of enamel removed by the four biopsies was 3.40 mg. The average depth of a single biopsy calculated from the formula given in sub-section 3.2.3.12. was 15.9 μ m.

The figures show remarkably little variation. Even if one takes into consideration that a rigidly standardized technique had been used, the uniformity of the values is striking. Mellberg and associates⁽³⁷⁰⁾ modified the abrasive biopsy technique described by Brudevold, McCann and Grøn⁽⁸³⁾ by making provisions

for defining the biopsy area and for controlling the polishing speed and the pressure applied. In spite of the modifications, the average amount of enamel removed from a defined area varied from 59.0 to 195.5 μg between ten operators. It appears that the acid biopsy method is far superior to the abrasive technique when control of the biopsy depth and the amount of enamel removed is a principal requirement. Similar conclusions can be reached from a recent evaluation of an acid biopsy technique by van der Merwe, Retief, Barbakow and Friedman. (371)

Because of their uniformity, the calcium values obtained in the biopsy could be regarded as a second parameter for the dissolution rate of enamel. In this context, it is of interest to compare the results with those obtained for Category 22. It will be noted that in each case the highest mean calcium values were obtained for Village Group 1.

The mean magnesium content of surface enamel (Category 230) was found to be 0.13 per cent of the dry weight. This is in good agreement with the values given by Cutress, (86) who found a range of 0.11 - 0.18 per cent in the outer 30 μm enamel layer. The lower and upper ends of the range represented Maori and Texas teeth, respectively. A considerably wider range of 0.04 - 0.3 per cent magnesium was found by Losee and associates (368) in whole enamel. The mean value reported by Losee's group (0.16 per cent), is consistent with the finding that magnesium concentration increases with increasing depth from the surface of enamel. (147)

The variation in the magnesium content of enamel was small in the present sample, indicated by the narrow range of village means (0.12 - 0.15 per cent) and by the low overall coefficient

of variation (17 per cent). The average values for males and females were almost identical. No consistent trends were detected in the village or village group means.

Literature reports dealing with the potassium content of enamel are limited. Bowes and Murray⁽¹⁴²⁾ reported it to be 0.05 per cent (500 ppm) or less but remarked that "...We are doubtful as to the presence of K, the values obtained being almost within the limits of experimental error...." However, the result proved to be a very good approximation. Lowater and Murray⁽⁶⁸⁾ were unable to detect potassium in sound enamel, but qualitatively demonstrated its presence in mottled enamel. The range given by Brudevold and Söremark,⁽¹⁴⁷⁾ 500 - 3000 ppm, appears to be too high in the light of recent findings. The mean values reported by Haataja, Pohto, Kleemola-Kujala and Hansson⁽³⁷²⁾ and by Losee and associates⁽³⁶⁸⁾ were almost identical at 401 and 400 ppm.

The mean potassium content of surface enamel (Category 231) found in the present study was 345 ppm, which is in general agreement with the previous two findings. The mean values for village groups showed a trend of positive association with caries experience. However, the differences between village group means were small and well within the range of variation exhibited by the sample, reflected by an overall coefficient of variation of 28 per cent. The same is true for the difference between the mean values for males and females.

It is pointed out that two subjects for whom no data were available were included in the "female" and "total" columns of

the "number of subjects" division of the summary tables for Categories 231, 232, 233, 234 and 235 (subjects 66 and 212 in Villages 3 and 12 respectively). The same is true for Category 236 in respect of subjects 123M and 156F in Villages 2 and 9 respectively. These errors did not influence mean values and standard deviations, but it should be noted that the data were based on 299 subjects and not on 301 subjects as shown in the summaries.

The phosphorus content of surface enamel (Category 232) was determined for two reasons:

- 1) To permit the determination of the total amount of enamel in the biopsy specimen, based on calcium determination, using an independent method. Comparison of the results would lead to the detection of aberrant individual values.
- 2) To permit the determination of calcium : phosphorus ratio in surface enamel.

The second point is beyond the scope of the stated specific aims of this investigation, but it will be discussed briefly because it represents an area of special interest. As far as it could be ascertained, no published data were available concerning individual variation in the Ca : P ratio of surface enamel determined in a sample of substantial size. Murray⁽³⁷³⁾ examined the ratio in enamel and dentine in a variety of species including man. She found that "in numerous samples of human teeth" "the values fell between 1.92 (lowest dentine) and 2.15 (highest enamel)". Later in the same paper she quoted the value of 2.2 for enamel of newly erupted human premolars. Bowes and Murray⁽¹⁴²⁾

have reported a mean Ca : P ratio of 2.15. The range reported by Brudevold and Söremark,⁽¹⁴⁷⁾ 1.92 - 2.17 by weight, was based on average findings of several investigators. Using thin sections of enamel, Weatherell, Robinson and Hallsworth⁽³⁷⁴⁾ and Robinson, Weatherell and Hallsworth⁽³⁷⁵⁾ found the mean Ca : P ratio to be 2.1 (w/w), S.D. = ± 0.1 . The ratio remained fairly constant despite considerable variations in the calcium and phosphorus content of enamel. In contrast, Little, Cooper and Rowley⁽³⁷⁶⁾ reported ranges of 1.4 - 2.3 and 1.7 - 2.2 (w/w) in altered (pre-carious) and sound enamel, respectively. Söremark and Samsahl⁽¹⁴⁴⁾ analysed 15 samples of whole enamel for calcium and phosphorus content. Calculations based on the figures gave the mean Ca : P ratio as 2.04, but no estimate could be made of the extent of variation between samples.

Cutress⁽⁸⁶⁾ examined the Ca : P ratio in enamel removed from premolar teeth grouped according to geographical origin. The lowest and highest ratios found in the 30 μ m deep surface layer were 1.94 and 2.31, in Maori teeth and in teeth obtained from Niue Island, respectively.

An unusually high mean value (2.79) and a very wide range (1.70 - 4.97) were found by King and associates,⁽⁸⁰⁾ who estimated the calcium and phosphorus content of 43 in-vivo biopsy specimens.

The mean Ca : P ratio of 2.14, (S.D. = ± 0.07) found in this investigation was based on 299 pairs of individual calcium and phosphorus values. All the enamel surfaces samples were sound and there was relatively little variation in the depth of sampling; mean 15.9 μ m, coefficient of variation 25 per cent. Considering that some of the variation in the Ca : P ratio was due to known

experimental error, (sub-sections 3.3.2.1.2. and 3.3.2.1.8.), it appears that individual variation, which is bound to exist in the ratio, was remarkably small in the present sample. These results also support the validity of calcium and phosphorus determinations.

No aberrant results were detected on comparison of the calcium and phosphorus values. The amount of enamel removed by the four biopsies, calculated from the amount of calcium found in the pooled biopsy specimens, was 3.40 mg. A similar calculation based on the phosphorus content of the specimens and on the assumption that enamel contained 17.5 per cent phosphorus, ⁽⁸⁶⁾ gave the corresponding mean value as 3.35 mg.

The determination of the barium content of surface enamel (Category 233) was subject to high experimental error for reasons detailed in sub-section 3.3.2.1.6. Thus, it was not surprising to find that of all elements determined, the barium content of enamel exhibited the greatest variability. This was reflected by the unusually high standard deviations shown by the summary table. The high mean value for the four male subjects in Village 1 was conspicuous even against the considerable overall variation in the sample. Examination of the individual results showed that subjects 4 and 33 had values of 127 and 525 ppm respectively. The analytical records were then checked and it was found that the analyses for these samples were carried out on different days and that the barium content of the samples analysed at the same time was within the 'normal' range.

Repeat analyses for these samples produced almost identical values. Under these circumstances it was considered improbable

that the high values were caused by experimental error, but the possibility that contamination had been responsible could not be discounted. The results were not withdrawn from further statistical analyses because, in the author's experience, the likelihood of sample contamination with barium was comparatively low and no experimental proof could be produced that contamination did in fact occur. It is of interest that Cutress⁽⁸⁶⁾ also found similar seemingly aberrant results, reproduced below.

<u>Source of sample</u>	<u>Enamel layer *</u>	<u>Barium content (ppm)</u>
Hastings	1	19
	2	17
	3	500
Texas	1	361
	2	74
	3	57
Niue Island	1	110
	2	18
	3	12

* Each layer approximately 30 μ m thick, layer 1 outermost.

Cutress carried out the analyses by spectrographic assay and, as in our case, the purity of the graphite used is always suspect. However, Losee's group⁽³⁶⁸⁾ also employed spectrographic analyses and their results for barium showed a lower mean value and relatively little variation (4.2 ppm; S.D. = $\pm$ 0.60). The present results are in general agreement with Cutress' findings.⁽⁸⁶⁾ The summary tables did not disclose any trends of associations. The possibility that barium-rich areas occur in enamel appears worthy of investigation.

The mean strontium content of surface enamel, 100 ppm (Category 234) and the range of individual variation (overall coefficient of variation = 30 per cent), established for the present sample, was comparable to other findings. Brudevold and Söremark⁽¹⁴⁷⁾ gave the range of 25 to 600 ppm based on data representing a variety of geographical areas. The range of strontium concentration found by Cutress⁽⁸⁶⁾ in the surface 30 μ m enamel layer was 97 - 495 ppm, while Losee and associates⁽³⁶⁸⁾ found a mean value of 81 ppm and a range of 26 - 280 ppm in whole enamel.

It was noted with interest that the two subjects in Village 6 who lived in an area some 6 - 10 miles from the Wogupmeri River had twice the amount of strontium in the enamel than the average value for the sample. Village 6, a small hamlet situated on a tributary of the Wogupmeri River, was visited during the survey and its inhabitants and environment did not appear different in any way from the villages and their populations situated on the main stream. The case of these two subjects was not considered coincidental, but an indication that environmental variations which may not be readily apparent can cause remarkable variation in the parameters studied.

The summary tables did not disclose any consistent trends in the mean strontium values by villages or village groups.

It has been reported⁽⁶⁶⁾ that the zinc content of enamel is highest at the surface and decreases progressively towards the dentino-enamel junction. Comparison of the results obtained in the present study with the data of Cutress⁽⁸⁶⁾ and Losee and

associates⁽³⁶⁸⁾ supports the previous finding.⁽⁶⁶⁾ The mean zinc content of surface enamel (Category 235) to an average depth of 15.9 μm , was found to be 798 ppm in the present study. The surface layer of enamel sampled by Cutress⁽⁸⁶⁾ was 30 μm thick and the range of zinc concentration found was 240 - 580 ppm. Losee and associates⁽³⁶⁸⁾ who sampled the whole thickness of enamel, reported a mean value of 199 ppm.

Examination of the summary table did not disclose any noteworthy trends.

Before discussing the fluoride content of surface enamel (Category 236), the reader is reminded that the determinations were carried out using surface enamel obtained in the dissolution rate test, representing a surface layer of 6.1 μm average thickness.

One of the possible mechanisms through which fluoride confers protection against caries, is increasing the resistance of teeth to acid dissolution through its presence in the enamel, although other mechanisms have been suggested and opinions regarding their relative importance are still divided.⁽²⁹⁹⁾⁽³⁷⁷⁾⁽³⁷⁸⁾⁽³⁷⁹⁾

As a result, numerous investigations of the fluoride content of surface enamel have been carried out.⁽²¹⁾⁽⁵⁵⁾⁽⁵⁷⁾⁽⁵⁸⁾⁽²⁴⁰⁾⁽³⁸⁰⁾⁽³⁸¹⁾⁽³⁸²⁾⁽³⁸³⁾⁽³⁸⁴⁾⁽³⁸⁵⁾⁽³⁸⁶⁾⁽³⁸⁷⁾⁽³⁸⁸⁾ The general aims of these studies were to gain a better understanding of caries susceptibility or resistance, and to monitor fluoride uptake in connection with water fluoridation and topical fluoride applications, to help in assessing the mode of action and potential of various forms of these widely practised preventive measures. (See ⁽²⁹⁹⁾⁽³⁸⁹⁾⁽³⁹⁰⁾⁽³⁹¹⁾⁽³⁹²⁾⁽³⁹³⁾⁽³⁹⁴⁾ for general reviews, and ⁽³⁹⁵⁾⁽³⁹⁶⁾⁽³⁹⁷⁾⁽³⁹⁸⁾⁽³⁹⁹⁾⁽⁴⁰⁰⁾ for cost-benefit aspects).

Apart from interest in the fluoride content of enamel for the reasons mentioned, variation in the concentration of one element may be associated with changes in the concentrations of other elements, and with alteration in the properties of enamel. (380) Combined or additive protective effects by fluoride and other protective agents have been reported. (401) (402) Thus, a considerable body of information is available concerning the fluoride content of surface enamel, but inter-study comparisons of findings are difficult, because small variations in sampling techniques may lead to marked differences in the results.

In this respect, sampling depth appears to be the most critical variable, because the concentration of fluoride is known to decrease from the enamel surface inwards. (59) (65) There is a particularly sharp drop in fluoride concentration in the immediate vicinity of the enamel surface of newly erupted or unerupted (unworn) teeth. (61) Wear or attrition of the enamel surface results in the removal of layers of fluoride-rich outer enamel. Therefore, lack of information regarding the wear status of the teeth amounts in effect to an unknown depth of sampling.

While wear results in loss of fluoride, the enamel surface also takes up fluoride from its immediate environment. The rate of uptake must depend primarily on the availability of fluoride from the environment, but it is also influenced by other factors such as the amount, composition and activity of plaque covering the enamel surface. (21) (403) (404) The balance of loss and uptake of fluoride depends on the combined effect of a large number of factors, some of them almost certainly unknown. Our present

knowledge is insufficient even to approximate the net result for any given set of environmental conditions. It is probable, however, that the balance is more favourable at protected enamel sites which are subject to less wear, and may be covered with more plaque than at exposed surfaces. It is clear then, that the site of sampling will also influence the amount of fluoride found. Variations in the fluoride distribution in enamel with age, and from site to site, have been demonstrated by Weatherell, Robinson and Hallsworth.⁽⁵⁴⁾

It is also known that structural variations in enamel are accompanied by variations in fluoride content.⁽⁶²⁾⁽⁶³⁾⁽³⁷⁶⁾ Thus, the presence of hypoplastic areas, white and brown spots and defects can introduce significant variations in the results. Further variation can result from the presence of localized sources of fluoride such as silicate restorations.⁽³⁸⁰⁾ Recently, Aasenden and Brudevold⁽⁴⁰⁵⁾ reported small (10 per cent) but significant differences between the surface enamel fluoride content of 13.5 year old boys and girls, irrespective of low (0.1 ppm) or high (5 - 7 ppm) exposure to fluoride in the drinking water. The higher fluoride values obtained at all fluoride levels for boys, were attributed to the longer pre-eruptive maturation time of enamel in boys than in girls.

In view of the experimental variations discussed in the previous paragraphs, it would be futile to compare the present results to those obtained in other studies. It is pointed out, however, that the design of this study catered for all sources of known variations.

It would be equally futile to attempt a close estimation of

the likely range of the fluoride content of human surface enamel. In the experience of the author and his associates, all enamel samples analysed contained some fluoride, although concentrations as low as 20 ppm were found. As far as the author could ascertain, the highest values found in the absence of previous clinical fluoride applications were reported by Baume and Vulli  moz,⁽⁴⁰⁶⁾ who demonstrated concentrations approaching 7200 ppm fluoride in the enamel of Polynesian natives. The investigators attributed the high fluoride levels to a continuous topical enrichment of the enamel with dietary fluorides. There is disagreement concerning the amount of fluoride that would be desirable to have in enamel from a preventive point of view. Englander⁽⁴⁰⁷⁾ suggested values ranging from 700 - 2000 ppm.

Over twenty references listed in the bibliography of this text deal with enamel biopsy techniques. For reviews see also⁽⁵⁸⁾ (238) (371). Considering the efforts expended in developing, improving and evaluating biopsy techniques principally aimed at the determination of fluoride in enamel, it is surprising how little epidemiological information is available concerning the relationship between the fluoride content of surface enamel and individual caries experience. One cannot help thinking that some findings, which appeared controversial or contrary to expectations, may not have been reported.

Englander and Mellberg⁽⁴⁰⁸⁾ could not demonstrate a correlation between caries experience and the fluoride content of enamel in the deciduous dentition of 138 kindergarten children. Keene, Mellberg and Nicholson⁽⁴⁰⁹⁾ reported an inverse association between the fluoride content of surface enamel of upper central incisor teeth

and caries experience, in a sample of 108 Naval recruits.

The mean fluoride content of 3459 ppm for 47 caries-free recruits was significantly higher than the corresponding mean of 1945 ppm for 29 men with 12-26 DMFT. ($P < 0.01$). The lesser mean biopsy depth for the caries-free group (2.79 μm), compared with those who had 12 and more DMFT (3.61 μm), could have accounted for a considerable proportion of the difference. De Paola and associates⁽⁴¹⁰⁾ have found a significant inverse association between the fluoride content of surface enamel and caries experience in a sample of 1447 children aged between 12 - 15 years. The product-moment correlation coefficient of -0.384 between $\sqrt{\text{DMFS}}$ and the corrected log fluoride content of the enamel corresponded to 15 per cent of the variation in caries experience explained by variation in the fluoride content of enamel. A similar trend was indicated by the results of Little, Losee and Barrett⁽⁴¹¹⁾ who examined the fluoride content of the surface enamel of bicuspid teeth extracted from persons with known caries experience.

The summary table for Category 236 showed that the mean fluoride content of surface enamel was 415 ppm for the whole sample. As expected, the variation between individuals and between village means was high, reflected by a coefficient of variation of 85 per cent. The difference between the mean values for males and females, 410 ppm and 421 ppm respectively, was minor compared to the overall variation. Some remarkable features of the summary were: 1) The mean value for Village 2 was considerably lower than the mean values for surrounding villages. 2) There appeared to be a trend of inverse association between village group means and the order of village groups, which in turn was a rough indication of the

distance of the villages from the source of the Wogupmeri River, Village Group 1 being closest to the source. 3) It follows from the previous point that the mean fluoride content of enamel was higher for the medium and high caries villages than for the low and zero caries villages:

Village Groups 1 + 2	478 ppm
Village Groups 3 + 4	353 ppm

4) The mean fluoride content of surface enamel was considerably different in Villages 13 and 14, where all subjects examined were free of caries. Examination of enamel surface wear data (Category 7) suggested that wear has made a contribution to the overall difference; the mean wear ratings for Villages 13 and 14 were 4.1 and 4.4 respectively. However, mean surface wear values for females in the two villages were practically identical (3.94 and 3.91) yet the fluoride values were considerably different (418 and 262 ppm). This indicated that factors other than wear also had a strong influence on the fluoride content of surface enamel.

Subjects in Villages 13 and 14 may have been free of caries because of their resistance, or because not all necessary predisposing factors were operating (lack of challenge). Provided that the former was true, the difference between the mean fluoride contents of enamel in the two villages suggests that, in the present population this parameter was not a major determinant of freedom from caries, unless approximately 200 ppm fluoride in enamel represented a level of absolute protection against the disease in this environment. That this was not so, is clear from the fact that the mean fluoride content of enamel was 405 ppm in the 'high caries' Group 2.

The relationship between fluoride and caries experience in the present sample will be discussed further in sub-sections 4.2.14. and 4.2.15. as more relevant information is presented.

4.2.13. The calcium content of saliva (Categories 239 and 240).

The desirability of collecting all saliva specimens at the same time of the day, because of possible diurnal variation of certain constituents, was recognised in the project design. It was reported in sub-section 3.2.4.1. that the field conditions did not permit uniformity in this respect. Two relevant points are raised here.

The existence of true diurnal variation in the calcium content of saliva is a controversial issue. Nydahl, Carr and Singer⁽¹⁰⁷⁾ could not detect diurnal variation in the total or ultrafilterable calcium content of stimulated whole saliva samples collected from ten individuals. However, the period of observation, 8.30 a.m. - 3.00 p.m., was relatively short. Dawes and Ong⁽¹¹¹⁾ also failed to detect diurnal variation in the calcium content of unstimulated parotid saliva, collected from seven subjects, about five times each day, for 11 - 17 successive days, although they demonstrated a diurnal pattern for other metals and for protein and chloride. In contrast, Ferguson and associates⁽¹¹⁰⁾ found a circadian rhythm in the calcium content of stimulated whole saliva. Ahrens and Lücke⁽¹⁰⁸⁾ found diurnal variations in unstimulated parotid and submandibular-sublingual saliva. The peak calcium values were obtained at 7 a.m. and 7 p.m. with a plateau in between. Nydahl and associates⁽¹⁰⁷⁾ may have failed to detect diurnal variation

because their collection times had not covered a sufficiently long time span. If diurnal variation in the present samples was similar to that found by Ahrens and Lücke,⁽¹⁰⁸⁾ the results would not have been affected, as no specimens were collected before 7 a.m. or after 6.30 p.m. in this investigation.

The second and more important point is that saliva specimens were collected throughout the working day from all subjects, and there was no common pattern or trend between the caries prevalence of subjects and the time of saliva collection. Under these circumstances the procedure would not have produced spurious associations even if diurnal variation existed, but the correlation (if any) between the calcium content of saliva and caries experience could appear poorer than it is in actual fact.

The calcium content of stimulated whole saliva is influenced by a great number of factors which include the diet (calcium intake), the excretory status of the individual, possible diurnal variations, secretion rate, period of stimulation and proportional contribution of major and minor salivary glands. Relevant aspects of saliva secretion and composition have been discussed in several recent reviews and key articles.^{(107) (108) (109) (111) (154) (158) (329) (341) (412) (413) (414)}

Investigations concerned with the relationship between total salivary calcium levels and caries experience produced controversial results.⁽¹⁵⁸⁾ In his recent review of the subject, Mandel⁽³²⁹⁾ remarked that this was not surprising "...in view of the many factors that affect calcium concentrations, especially in whole saliva" Thus, it is not unusual to observe considerable

variation of the salivary calcium values from person to person. The 'normal range' for the total calcium content of mixed saliva is correspondingly wide for most populations, at approximately 3.0 - 8.0 mg per cent (30 - 80 ppm), in both stimulated and unstimulated specimens. (153) (154) (415) (416) (417) (418)

The overall mean total calcium value found in the present investigation was 81 ppm and two village means were over 100 ppm. The high values suggested the presence of calcium from an extraneous source, while the standard deviations indicated high variation between individuals and villages. With respect to Category 239, it appeared that the standard deviation was unusually high for males in Village 5 and for females in Village 15. A similar situation was observed in respect of Category 240, where an additional high standard deviation was noted for females in Village 13. Examination of individual values in these groups showed that 11 subjects distributed between these groups had unusually high calcium values. The mean calcium content of saliva for these subjects was 206 ppm and 2.09 per cent of solids. The mean saliva colour of the same subjects was 7.1 and the average time elapsed since the last betel-chewing was 1.3 hr. These observations supported the contention that the high salivary calcium values for this population reflected to a large extent calcium retained in the oral cavity after betel-chewing. Further supporting evidence was obtained by comparing the mean calcium content (ppm) of the saliva of 49 subjects, who were known to have chewed betel within half an hour of saliva collection, with the mean calcium content of the saliva of 49 randomly selected subjects who did not chew betel at all, or did not do so on the day of saliva collection. The respective mean calcium values were 102

and 71 ppm, $t = 2.55$, $P < 0.01$.

These results are in accordance with the report of Mukherjee (419) who observed considerable elevation in the calcium content of saliva following betel-chewing. The mean calcium values for males and females were comparable. Because of the high variation within the sample it would have been difficult to discern trends but it was noted that Village Group 4 had the highest mean calcium values according to both categories. This was attributed to betel-chewing rather than to physiological causes.

4.2.14. The fluoride content of saliva (Category 243).

Martin and Hill (420) examined the fluoride content of activated whole saliva of children included in the Evanston study. The authors found mean values of 0.25 and 0.20 ppm fluoride for children aged 6 - 8 years and 12 - 14 years respectively. Other early investigators (421) (422) found similar values, which led Afonsky (158) to conclude that "....Irrespective of the levels usually present in the drinking water, the fluorine concentration in the saliva varies from 0.08 - 0.25 ppm". However, on comparing the fluoride content of saliva of persons resident in different localities, McClure (423) found average values considerably less than 0.1 ppm, and indications that higher than usual fluoride concentrations in the reticulated water (3.8 - 4.0 ppm) were reflected in the fluoride content of saliva. Results of recent studies of whole saliva (424) and parotid fluid (129) (425) suggested that, in the absence of exogenous administration, or unusually high fluoride levels in the water supply, the expected range was between 0.01 and 0.05 ppm

although the number of subjects studied was small. The fluoride content of unstimulated parotid saliva (and probably also of whole saliva) is higher than the fluoride content of stimulated saliva, (425) although it is still within the range given above.

In contrast with calcium, the fluoride concentration of stimulated saliva does not change with variations in the rate of flow. (425)

The effect of oral administration of single doses of fluoride, on the fluoride content of stimulated parotid saliva, was studied by Grøn, McCann and Brudevold. (129) The authors found maximum values of 0.06, 0.2 and 0.3 ppm within 30 - 60 minutes after the ingestion of 1, 5 and 10 mg fluoride. Thereafter, the concentrations decreased slowly, approaching the resting value 2 - 6 hours after ingestion, depending on the amount of fluoride ingested. Similar results were obtained by Shannon and Edmonds (426) who also examined the excretion rate in urine and found it to be a near-linear function of the amount of fluoride administered. Büttner (427) found highly significant correlation and an approximate ratio of 4 : 1 between fluoride levels in plasma and saliva, following administration of small and large doses of fluoride (0.5 - 100 mg) to 6 young adults. Maximum levels were reached almost simultaneously about 30 minutes after ingestion, followed by an exponential decrease over 6 - 8 hours.

The presence of fluoride in saliva makes the transfer of fluoride from saliva to enamel theoretically possible and it has postulated that saliva was a source of enamel fluoride. (380) In view of the very low fluoride level in saliva the practical

significance of salivary fluoride in this context seems doubtful. The constant availability of salivary fluoride to plaque, which is known to accumulate fluoride, (403) (404) probably through bacterial action, (428) may be of considerably more significance. The contention that transfer of fluoride from plaque to enamel may occur, is supported by the association found between the amount of plaque and the fluoride content of surface enamel by Charlton and associates (21)

The overall mean value and range shown in the data summary (as ppb fluoride = $\text{g}/10^9 \text{ ml}$) were within the expected limits. It was apparent from the standard deviations that there was considerable variation in the fluoride content of saliva from person to person and between villages. The small difference between the overall mean values for males and females seemed to be of no consequence in view of the variability of the results. However, the mean values for the top-river villages (with the exception of Village 4), and for Village Group 1, were appreciably higher than for the rest of the sample. The pattern of mean fluoride concentration in saliva by villages and village groups was remarkably similar to the corresponding pattern of the fluoride content of surface enamel (Category 236).

For example, the difference in the mean fluoride content of enamel between the two caries-free Villages 13 and 14, discussed in 4.2.12., was accompanied by a difference, in the same direction, in the mean fluoride content of saliva. Both differences were statistically significant.

Village	Mean fluoride content	
	Surface enamel (ppm)	Saliva (ppb)
13	414	19.7
14	211	15.2
	t = 2.90	t = 2.01
	P < 0.01	P < 0.05

The overall similarity of pattern between fluoride in enamel and in saliva is not considered coincidental. The fact, that it could be demonstrated as clearly as it was, despite the large number of factors that influence these variables, was not only gratifying but it also inspired confidence in the experimental procedure. The implications of these findings are discussed in the next sub-section, following presentation of the results of the water analyses.

4.2.15. The fluoride, strontium and calcium content of household water.

It has been demonstrated unequivocally that water-borne fluoride at appropriate levels confers a degree of protection against dental caries. (1) (8) (429) Associations between the concentration of other major and trace minerals in water and human caries experience have been examined in several studies. (10) (346) (348) (350) (353) (355) (401) (430) (431) (432) (433) (434) (435) As yet no element or combination of elements emerged, to which a protective effect, comparable to that of fluoride, could be attributed.

Water analyses were not necessary for accomplishing the specific aims of this study. However, because of their value as background information, it was decided to report the fluoride, strontium and calcium content of drinking water samples.