THE RELATIONSHIP OF EXTRINSIC DARK TOOTH STAINS TO DENTAL CARIES

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THESIS

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INTRODUCTION

The presence of coloured extrinsic stains upon the teeth and the relationship of these stains to dental disease has attracted the attention of dental research workers since the last century (Miller, 1894).

Results have been published to demonstrate that one particular colour of extrinsic tooth stain is related to a higher rate of dental caries than other colours (Sutcliffe, 1967).

Green stain upon the teeth has been shown to be associated with a higher incidence of dental caries than dark stain for both the deciduous dentition (Mellanby et al., 1957; Pedersen, 1946), and the permanent dentition (James, 1964; Sutcliffe, 1967).

In particular the significantly lower caries experience of children who have extrinsic dark tooth stain as compared to children who have no, or other coloured, extrinsic tooth stain has been noted (Bibby, 1931; Pedersen, 1946; Mellanby and Coumoulos, 1946; Shourie, 1947; James, 1963 and 1964; Sutcliffe, 1967).

Pickerill (1923) noted the relationship between dental caries and extrinsic dark tooth stain. He described the appearance and distribution of the stain calling this the "mesenteric line".

Although Bunting (1929) and Leung (1950) did not find children with dark extrinsic tooth stain to have a lower dental caries incidence than children with no stain, the majority of dental epidemiological results would support the statement of Pickerill (1923). (Bibby, 1931; Pedersen, 1946; Mellanby and Coumoulos, 1946; Shourie, 1947; James, 1963 and 1964; Sutcliffe, 1965 and 1967).

In spite of the relationship between extrinsic dark tooth stain and dental caries, very little research has been undertaken into the aetiology and chemical composition of the black material.

Bibby (1931) in an excellent investigation into both aetiology and chemistry of the black material stated prior to his investigations:

> "It is somewhat surprising that more attention has not been given to the relationship between these conditions, which as yet seems to be but poorly understood".

Pickerill (1923) in the same context stated:

> "I regret that I have not had time to work out the association, bacteriologically or chemically. This certainly requires doing ...".

Nonetheless he postulated the presence of the mesenteric group of organisms as the causal agents of black stain upon the teeth.

Leimgruber (1950) stated that:

> "..... the pigment is a substance derived from the 'maturation factor' by polymerization and oxydation".

Unfortunately no further information as to the nature of the 'maturation factor' is available.

Miller (1894) had shown that the dark stain upon the teeth of iron-workers contained iron. However, no further published results into the aetiology and chemical composition of extrinsic dark tooth stain could be found although the need for further research has been stressed.

Commerell (1955) stated the need for further investigations thus:

> "Die noch notwendige Klarung ihrer Entstehung und Zusammensetzung wird sicher einen Schritt weiter fuhren auf dem Wege zur Erkenntnis der biochemischen Zusammenhange in der Mundhohle und damit der Kariesatiologie".

Mellanby et al. (1957) stated in this context:

> "It may be that the varying metabolism of individual children plays a direct part, or acts indirectly via the saliva, but the whole problem obviously needs further research".

James (1963) stated:

> "..... the children with dark stain had a lower overall caries prevalence. This agrees with the impressions of dental clinicians, and is a feature that merits further investigation".

It was decided that in view of the important relationship between dental caries and the presence of extrinsic dark tooth stain that further research into the subject was warranted. The investigations carried out in this work involve epidemiological, bacteriological and chemical techniques, the result of which it is hoped will not only shed light on the aetiological factors operating to produce dark extrinsic tooth stain but also will be of benefit in the field of preventive dentistry.

REVIEW OF LITERATURE

The presence of coloured extrinsic tooth stains and the relationship between these stains and dental disease has been, and still is, attracting the attention of dental research workers. Investigations into why one particular colour of extrinsic tooth stain is related to a higher, or lower, incidence of dental disease, in a chosen population, than other coloured extrinsic tooth stains, have been carried out.

A search for the aetiological factor, or factors, concerned in the production of these coloured extrinsic tooth stains is still being carried out. Miller (1894) investigated coloured deposits upon the teeth with special attention to green and metallic deposits and made the interesting observation that dental caries was no more likely beneath tooth surfaces covered with green stain than tooth surfaces without stain. Leung (1950) supported the above finding whereas Ottolengui (1892) found the green stain to act in a destructive manner.

Investigations of particular interest to the present work were also carried out by Miller (1894) and involved the roll played by iron in the production of coloured extrinsic tooth stain. Specific mention was made of the black colour produced by feeding animals large quantities of iron. The material causing the black colour was given as ferrous sulphide. Tracy (1969) has stated that the extracellular pigment produced by the bacterium, Bacteroides melaninogenicum

is ferrous sulphide. Pickerill (1923) commented on the association between immunity to dental caries and the presence of dark extrinsic tooth stain which he called the "Mesenteric Line". He believed that the mesenteric and other alkali-producing bacteria predominated in the mouths of these patients and that that was the reason why dental caries did not occur. Results from a number of epidemiological surveys have supported a lower dental caries incidence where black stain was present. (Bibby, 1931; Mellanby and Coumoulos, 1946; Pedersen, 1946; Shourie, 1947; Leimgruber, 1950; Commerell, 1955; Mellanby et al., 1957; James, 1963; James, 1964; Sutcliffe, 1965; Sutcliffe, 1967). However, other workers have not found a correlation between the presence of black extrinsic tooth stain and dental caries (Bunting, 1929; Leung, 1950).

Bibby (1931) investigated in detail the nature and aetiology of the black stain material. In an excellent and detailed account of his investigations he found the physical nature of the material to have little structure, being merely a gelatinous substance with no detectable crystalline formations. He was unable to discern any structural details in the few prepared stained sections which had the deposit present. In spite of difficulties in carrying out chemical tests on the small amount of material collected he found the material to contain a form of mucin, small amounts of

calcium carbonate and phosphates. The bacteriological investigations he carried out produced small numbers of cocci and gram negative bacilli plus small gram positive bacilli and thread-forming organisms. Unfortunately Bibby chose to disregard the former group of organisms and concentrate on the latter. He concluded that these latter organisms gave no indication of being the cause of the black stain.

It is a pity that he did not examine the gram negative cocco-bacillary organisms as Oliver and Wherry (1921) had described just such an organism which produced black material on culture medium. This organism, a member of the genus Bacteroides, was called Bacteroides melaninogenicus (Oliver and Wherry, 1921).

Bibby (1931) also investigated the effect of oral hygiene, pathological conditions in the mouth and the effect of prophylaxis, concluding that it was impossible to state that any of these factors was a definite adjuvant to black stain formation. The observation that patients with black stain had less well cared for mouths than patients without stain was made. However, Sutcliffe (1967) did not find children with extrinsic dark tooth stain to have better or worse oral cleanliness than children with no coloured extrinsic tooth stain.

A brown extrinsic deposit upon the teeth was termed 'Brown Pellicle' by Manly (1943). This deposit was described as a recurrent structureless deposit on teeth. The distribution of this material differed from that of dark extrinsic tooth stain in one important clinical aspect, namely the structureless deposit was found preferentially on the labial surfaces of the anterior teeth. Occasionally the central third of the labial surface of anterior teeth was most heavily coated with the cervical third free of material. This distribution picture has not been reported for extrinsic dark tooth stain as described by Pickerill (1923).

Vallotton (1945) reviewed the literature regarding acquired pigmented pellicles of the enamel surface and divided them into two fundamental types.

The first division was anatomical and known as "Nasmyths Membrane" or the "Enamel Cuticle" whilst the other division was an acquired type consisting of various "plaques" or "films". The former was described as a structureless membrane formed during development and eruptive stages and very similar to the structureless pellicle described by Manly (1943). The latter was described as acquired films largely bacterial in origin.

Mellanby and Coumoulos (1946) reported on the incidence of dark extrinsic tooth stains upon the teeth of five year old children. They also reported a lower dental caries rate for these children than for

the rest of the children in their sample. This finding was confirmed by Pedersen (1946) who examined children between the ages of 1 and 7 years thus the relationship between dental caries and the presence of dark extrinsic tooth stain has been reported for both the deciduous and permanent dentition with the weight of evidence showing a reduced dental caries incidence when the dark stain was present.

Shourie (1947) examined over 1,000 boys aged between 13 - 16 years from a district in India. He showed that boys with dark extrinsic tooth stain had a lower dental caries incidence than boys whose teeth had no coloured extrinsic stain. However, Leung (1950) found no reduction in dental caries incidence in children with brown extrinsic tooth stain when compared to children with no coloured extrinsic stain.

Leimgruber (1950) stated that children with "black-stain" had relatively caries-resistant teeth and stated that the "black-stain" was a pigment belonging to the class of melanin pigments. He stated that the deposit was firstly a colourless basic substance secreted by the salivary glands with the pigment derived from the "maturation factor" by polymerization and oxydation. The maturation factor determines the resistance of the hard tissues of the teeth but loses its value when converted to the inactive form which becomes black stain.

He made a very interesting statement that it was because fluoride maintains the "maturation factor" in the active stage, not allowing black stain to be formed, that increased tooth resistance to dental decay and not because hydroxyapatite was transformed into fluorapatite. While it would be unlikely that the last statement would be acceptable today it is of note as James (1963) has shown that the lowest dental caries incidence recorded by him after examining a group of children living in a high fluoride area was found amongst those children with dark extrinsic tooth stain.

Torell (1954) published results of investigation into the effect of ferrous and ferric salts on the resistance of enamel to acid dissolution. He reported that the former increased the resistance of enamel to acid attack, a fact which will be of importance when the chemical composition of the black material from the mouth is discussed.

Commerell (1955) reported astonishment at the dramatic relationship between caries-resistant teeth and the presence of dark tooth stain.

James (1963) found children with dark extrinsic tooth stain to have a lower dental caries incidence than children with no tooth stains after examination of children living in a high fluoride area.

Sutcliffe (1965) in a study of mandibular incisor caries reported that children with brown extrinsic

tooth stain had the lowest dental caries incidence of the total sample except for the first permanent molars and the mandibular first premolars. A further report by Sutcliffe (1967) showed that children with black stain had no better or no worse oral cleanliness than children without stain. However, children with extrinsic black or brown stain did have a smaller total caries experience than children with no stain.

Bibby (1931) had tried to isolate organisms which might prove to be the cause of the dark extrinsic tooth stain. However, he did not investigate the gram negative cocci and gram negative bacilli which he had found in smear examinations. This would certainly have been worth doing as Oliver and Wherry (1921) had described similar organisms which produced black pigment on suitable culture medium. Oliver and Wherry (1921) described these organisms as polymorphous rods approximately 0.8 u x 1 u - 3 u in size, gram negative and non-acid fast. They stated that the bacteria were unpigmented but that the black pigment occurred as an extracellular amorphous mass which they thought was a melanin.

Schwabacher et al. (1947) carried out investigations into the black pigment produced by Bacteroides melaninogenicus and concluded that the pigment was a haematin not melanin. However, these researchers also stated that the pigment was "united in the cells with a bacterial protein to form a parahaematin", and

therefore may have been investigating a different pigment from that of Oliver and Wherry (1921).

In spite of the rigorous purification procedure carried out by Schwabacher et al. (1947) in an attempt to remove all the haemoglobin from the sample grown on blood agar, some may still have been present to influence their results.

This latter fact was commented upon by Tracy (1969) who further investigated the nature of the pigment and stated the pigment to be extracellular thus agreeing with Oliver and Wherry (1921). Spectroscopic analysis carried out by Tracy (1969) on the pigment showed the absorption peak at 250 nm which is not consistant with that of a haem pigment. Chemical analysis carried out by Tracy (1969) on the pigment proved it to have the characteristics of colloidal ferrous sulphide.

The identification of Bacteroides melaninogenicus is initially on the production of black pigment when the organism is grown anaerobically on blood agar culture medium.

MacLennan (1951) divided the genus Bacteroides into three groups, the first, the "Funduliformis", being representative of pleomorphic strains, the second, the "Fragilis", being representative of the non-pleomorphic strains and the third, "Miscellaneous" of which Bacteroides melaninogenicus is a member.

Loesche and Gibbons (1965) suggested a practical method for identifying oral gram negative anaerobic rods stating that Bacteroides melaninogenicus was readily separated by its black pigment production from other colonies after incubation on culture medium. Confirmatory tests, once the organism was shown to be gram negative, were in accordance with the findings of Sawyer et al. (1962) who showed the organism to be proteolytic, to produce hydrogen sulphide, to be mainly indole positive, and not to reduce nitrates or to form catalase, and to ferment some carbohydrates.

Oliver and Wherry (1921) found the organism to ferment carbohydrates but not to attack gelatin.

Gibbons and MacDonald (1961) have shown that a strain of Bacteroides melaninogenicus hydrolysed native rat tail and gingival collagen.

Bladen and Waters (1963) carried out investigations into the ultrastructure of some strains of the genus Bacteroides. They found a triple-layered cell wall completely encircling the organisms and a space of 150 to 250 A^{o} separating the cell wall from the cytoplasmic membrane. The space was crossed frequently by bridges which connected the cell wall and the cytoplasmic membrane. Intracytoplasmic membranous elements were demonstrated but no special discerning features were suggested as diagnostic of these organisms neither was Bacteroides melaninogenicus specifically described as all the organisms investigated

were unidentified species of the genus Bacteroides.

Mergenhagen et al. (1961) have shown Bacteroides melaninogenicus to possess an endotoxin which produces toxic effects in rabbits.

The chemical characteristics of this endotoxin were further studied by Hofstad (1968) who also studied the biological activities of the endotoxin (Hofstad, 1970). He published results suggesting that the endotoxin was a lipopolysaccharide, containing sugar, protein and lipid and which was lethal for mice and prepared rabbits for the local Shwartzman reaction.

Kaufman et al. (1972) showed that the ability of material from Bacteroides melaninogenicus to potentiate infection with other organisms was negated if the material was first heated to destroy the collagenolytic activity. Endotoxin was therefore eliminated as the source of the pathogenic material as heat would not inactivate it under the described conditions.

The role played by Bacteroides melaninogenicus in mixed anaerobic infections has been studied with particular reference to the production of endotoxins and collagenolytic activity as previously discussed.

Courant and Bader (1966) using indirect fluorescent antibody techniques demonstrated the presence of Bacteroides melaninogenicus or its products in inflamed gingival tissues. Takeuchi et al. (1970) also using fluorescent antibody techniques demonstrated specific fluorescence of Bacteroides melaninogenicus in inflamed

gingival tissues.

Oliver and Wherry (1921) isolated the organism from various parts of the body and from sites of infection.

Burdon (1928) also isolated the organisms from similar sites of infection and noted that these organisms grew in very intimate mixture with other bacteria.

Hemmens and Harrison (1942) showed an increase in the numbers of Bacteroides melaninogenicus in material from patients with suppurative periodontitis when compared to normal mouths.

MacDonald and Gibbons (1962) demonstrated that four types of bacteria were required to produce infection in guinea pigs and one organism required was Bacteroides melaninogenicus.

Later work by MacDonald et al. (1963) suggested that Bacteroides melaninogenicus might be the key pathogen in most mixed anaerobic infections of mucous membranes and that the other organisms are producing growth factors necessary to Bacteroides melaninogenicus.

Socransky and Gibbons (1965) showed that mixtures of either oral or intestinal bacteria which were free of Bacteroides melaninogenicus did not produce transmissible infections when inoculated into guinea pigs. However, addition of Bacteroides melaninogenicus to the mixture restored infectivity.

Socransky et al. (1963) did not find a statistically significant difference in numbers of Bacteroides

melaninogenicus in material collected from the gingival margins of normal and periodontally involved patients a finding supported by other published data (Kelstrup, 1966; Morhart et al., 1970; Mackler and Crawford, 1973). The type and number of bacteria in the mouths of individuals of different age groups has been investigated and reported upon from the beginning of this century (Brailovsky-lounkevitch, 1915; Kostecka, 1924).

Dale et al. (1961) found the total viable count of Bacteroides melaninogenicus in gingival material to be 9.5×10^8/gm.

Socransky et al. (1963) found a slightly lower count of these organisms in gingival debris the number being 1.3×10^7 to 5.5×10^9.

De Araujo and MacDonald (1964) examined children and published figures for Bacteroides melaninogenicus in gingival material giving the number as 4×10^5 to 14×10^8/gm. wet weight of debris. However, Berger et al. (1959) failed to isolate Bacteroides melaninogenicus in young children even after the eruption of the deciduous incisors a fact supported by other research workers (Bailit et al., 1964; Kelstrup, 1966). Morhart et al. (1970) did find Bacteroides to be present in gingival debris from children in the 4 - 6 year old age group, and this finding was confirmed by other research workers (Mackler and Crawford, 1973).

Variations in sampling techniques and difficulties in culturing these fastidious organisms may account for

some of the above findings.

Oliver and Wherry (1921) had grown the organisms on blood agar slants under anaerobic condition.

Burdon (1928) disputed that Oliver and Wherry had grown pure cultures due to the latter's technique and the fact that he had noticed that the organism grew in intimate mixture with other bacteria. He also stated the difficulty of obtaining these organisms in pure culture.

Lepper and Martin (1929) had shown the value of adding heart muscle to culture media to facilitate the growth of anaerobes a technique which was later modified by Cowan and Steel (1965).

Evans (1951) published results showing that haematin was an essential growth factor for Bacteroides melaninogenicus. Lev (1958) reported that when Bacteroides melaninogenicus was grown with proteus a much better growth was obtained. He discovered that if Vitamin K was added to the culture medium a better growth of Bacteroides melaninogenicus was obtained. Both the above findings were confirmed by Gibbons and MacDonald (1960).

Postgate (1959) published results suggesting the addition of sodium sulphite and iron citrate to nutrient agar to encourage the growth of Bacteroides melaninogenicus. He pointed out that sulphur in inorganic form was a major participant in the metabolism of these organisms and, of particular interest,

that any blackening caused was due to ferrous sulphide production.

Lev et al. (1971) have reported that succinate enhances the growth of Bacteroides melaninogenicus.

Takazoe et al. (1971) published results which demonstrated that Corynebacterium has an enhancing effect on the growth of Bacteroides melaninogenicus when both are grown together but it may be due to the former's production of menadione.

Loesche et al. (1971) showed that the recovery of Bacteroides melaninogenicus from dental plaque was enhanced by the addition of Kanamycin to the culture medium. Although the numbers of Bacteroides melaninogenicus were reduced the chances of obtaining separate colonies of these organisms to make a pure culture isolation was enhanced.

The anaerobic conditions necessary to promote growth of Bacteroides melaninogenicus were obtained initially using the pyrogallic acid method (Oliver and Wherry, 1921; Burdon, 1928).

Later methods involving the use of anaerobic jars, pumps, metering equipment and cylinders of gas were compared with the Gaspak system whence the latter was found to be only slightly less efficient than the former (Collee et al., 1972).

Loesche (1968) discussed the possible sources from which Bacteroides melaninogenicus could receive nutrients. The organism's need for Vitamin K could

be satisfied by other organisms present in the mouth synthesising this vitamin and by a supply of Vitamin K in the gingival fluids and inflammatory transudates which permeate through into the gingival sulcus. The suggestion that bleeding into the gingival crevice area would provide all the haemin requirements for these organisms is less convincing as these organisms are also isolated from clinically healthy mouths. Certainly the above-mentioned source of haemin is very important but perhaps other sources will be discovered.

The catabolism of amino acids and perhaps organic acids as energy sources by Bacteroides melaninogenicus usually yields equal amounts of acid and base thus maintaining the pH or causing only slight variations. Consequently if a basic pH were maintained it is more likely that calculus formation rather than cavity formation would occur (Kleinberg and Jenkins, 1964).

Sawyer et al. (1962) have shown that when Bacteroides melaninogenicus ferments carbohydrates the pH rarely falls below 5.0 and this fact plus what has already been suggested above gives important information regarding dental disease.

The fact that Bacteroides melaninogenicus cannot be established as a monocontaminant in germ-free animals has frustrated the obtaining of information which could be of value in human dental disease (Gibbons et al., 1964).

Aims of the Present Study

The present study was designed to investigate the relationship between coloured extrinsic tooth stains, with particular emphasis on dark extrinsic tooth stain, and dental disease. The information collected involved the use of epidemiological, bacteriological, histological and chemical techniques with the express intention of achieving the following aims:-

1. Establish the prevalence of dental disease in a group of thirteen year old Glasgow school children.

2. Discover the prevalence of coloured extrinsic tooth stain in the chosen population.

3. Record "Social data" such as toothbrushing habits, sweet eating and dental attendances of the children.

4. Compare the mean dental caries incidence of the total population to the mean caries incidence of each group with coloured extrinsic tooth stain.

5. Compare the mean dental caries incidence of each group of children with coloured extrinsic tooth stain with one another and with the group of children with no coloured extrinsic tooth stain.

6. Establish the relationship between dental disease and toothbrushing and sweet eating habits.

7. Examine the relationship between oral hygiene and gingivitis in the total population and compare the results with each group of children with coloured extrinsic tooth stain.

8. Establish the numbers of Bacteroides melaninogenicus in both a control group and "black stain" group of children.

9. Chemically examine material collected from the teeth of both the "non-stain" and "black stain" groups.

10. Examine under the electron microscope black material collected from the children.

11. Carry out oxygen tension investigations into both the "non-stain" and "black stain" group and compare the results.

12. Investigate fermentation reaction using isolated pure cultures of Bacteroides melaninogenicus.

13. Relate the presence of dark extrinsic tooth stain to the presence of Bacteroides melaninogenicus.

14. Investigate the role played in dental disease by Bacteroides melaninogenicus.

CHAPTER I

CHAPTER I

EPIDEMIOLOGY SECTION

Description of the Sample

The City of Glasgow is a largely industrial city with a population of almost one million. The water supply is deficient in fluoride, the level being only 0.01 part per million. The city was arbitrarily divided into five sections - North, South, East, West and the Central area. From two of these sections, four schools giving Secondary Education were selected, two Roman Catholic and two Protestant schools. Nine further schools were chosen, three from the Centre, three from the West and three from the South. Five of these were Roman Catholic and four were Protestant schools. All the schools were under the control of the Education Department of the City of Glasgow. No private, fee-paying or handicapped children schools were included so that school selection was not random. Another two factors were considered when selecting schools, namely, access for the Mobile Dental Examination Unit and Headmaster permission. The latter was most important as Headmasters had been under considerable dental pressure in the preceding time.

Permission to examine children at the school was also cleared with the Authorities. The schools chosen are as in Table 1. Children aged thirteen years were chosen from the school register. Numbers were further reduced by choosing only those thirteen year old children whose birthday fell between the first and tenth day of any month. A total of 928 children were examined with the number from each school being approximately equal. Table 2 shows that of the total, 445 (47.9%) were male and 483 (52.1%) were female.

TABLE 2

Number of children examined

MALE	FEMALE	TOTAL
445	483	928

Two main reasons led to the choosing of thirteen year old children, namely:-

1. It has been suggested that at this age nearly all children have Bacteroides melaninogenicus present in their mouths (Bailit et al., 1964). Initial work suggested that the prevalence of these bacteria in the mouth increased with age (Bailit et al., 1964). This now seems to be under review as although Bailit et al. (1964), Wilson C. de Araujo and MacDonald (1964), and Kelstrup (1966) did not find this organism to be prevalent in the under four year old children, more

recent work has shown the organism to be present in the under four year old age group (Morhart et al., 1970; Mackler and Crawford, 1973). In fact Mackler and Crawford (1973) found the bacteria to be present in all of the two year old children examined whereas Berger et al. (1959) did not isolate the organism from the oral cavity of young children.

2. By choosing thirteen year old school children the dental data could be carried out on the permanent dentition thus eliminating the need for a separate study of the deciduous dentition.

Methods of Examination

Dental examinations were carried out in a Mobile Dental Examination Unit parked within the school grounds. Two consecutive days were allotted to complete the examinations. The children were examined on a horizontal couch situated within the Mobile Unit as described by Slack (1961) with lighting from a side wall unit. All clinical examinations were carried out by the author.

Prior to the clinical examination each child, on entering the Dental Unit, was interviewed by a trained Dental Surgery Assistant who completed a social questionnaire on one side of a pro-forma.

The answers given by the children were recorded and the results are entirely dependent on the honesty of the child. It is highly probable that closer and more intensive questioning of each individual would produce different answers. However, due to the numbers involved this was thought not to be feasible. Perhaps a random sample from within the group could have been used to check the validity of answers given by the total sample. However, as this is also time consuming and open to the same criticism, the idea was not pursued. The dental clinical examination was then undertaken using a standard mouth mirror and a probe with replaceable sickle heads (Miller and Atkinson, 1951). The probe points were changed after every four examinations (McHugh et al., 1964; Berman and Slack, 1972; Stephen and Sutherland, 1971). Teeth were dried where necessary using a chip syringe. Instruments were stored in a 2 per cent chlorhexidine and spirit solution between examinations. A wall-mounted Anglepoise lamp was used for lighting from a standard 60 watt bulb. Dental data was recorded on the pro-forma by a trained assistant sitting at a desk beside the examiner. The examiner wore a halter microphone connected to a tape recorder to allow all the data to be rechecked at a later date.

The Measurement of Dental Disease

It was thought to be impracticable to take dental radiographs for the whole sample, thus only clinically demonstrable carious lesions were recorded. This technique would certainly fail to detect some interproximal lesions as was shown by Sognnaes (1940) who demonstrated by using four different procedures that an increase in caries incidence arose as more procedures were employed in the examinations. The difference in carious teeth charted between Procedure A using mirror and explorer only, and Procedure D using all techniques plus radiographs was from 39 per cent in Procedure A to 53 per cent in Procedure D. However, the difference between Procedure B which used a mirror and probe plus drying of the teeth as in this study, and Procedure D for carious surfaces was from 21.7% to 25.6% after an examination of 2,006 surfaces. However, Burket (1941) in a study of caries detection using standard examination techniques plus radiographs plus microscopic investigations on 460 extracted teeth concluded that histological studies demonstrated that carious lesions were missed on both clinical and radiographic examinations. Further evidence of the value of histology in caries detection was shown by Miller and Hobson (1956) who examined extracted teeth for occlusal caries.

Using the examination technique described by Jackson (1950) these workers showed that of 12 extracted teeth which demonstrated no evidence of caries by clinical examination, 7 had evidence of caries on histological section. However, in the present survey when a definite pull was required to remove the dental probe from a pit or fissure that area was recorded as carious (Jackson, 1950). To assess the caries involvement of each tooth the penetration scores of McHugh et al. (1964) were used:-

Score 1 = "Sticky Fissure".

Score 2 = Fissure or free surface cavity with softness at base and staining or opacity of the enamel.

Score 3 = Cavity with obvious dentine involvement (all detectable approximal cavities in teeth with approximal contacts were given this score unless there was pulp involvement).

Score 4 = Cavity with obvious pulp involvement.

Only one penetration score was given to each tooth as an index of the size of the largest cavity whilst teeth which were filled and carious counted carious.

were recorded as carious

Oral Hygiene

Many methods for assessing oral hygiene have been used in epidemiological studies, some use classification

into good, fair or neglected (Mansbridge, 1959; Sutcliffe, 1967) or good, fairly good and not good (Schei et al., 1959) or good, fair (plus), fair (minus) or poor (Finlayson and Wilson, 1961) or category 1, 2 and 3 (James, 1964). The Oral Hygiene Index of Greene and Vermillion (1960) later presented as the Simplified Oral Hygiene Index (1964) uses a numerical system to represent the standard of oral hygiene either as, in the former case, using all the teeth or in the latter, selected surfaces of six chosen teeth. The method of scoring was also modified by McHugh et al. (1964). It is obvious that no ideal system for classifying oral hygiene exists but due to two main factors the Simplified Oral Hygiene Index of Greene and Vermillion (1964) was chosen for use in this study. Firstly because it offers a quicker but only slightly less sensitive system than the Full Oral Hygiene Index (Green and Vermillion, 1960). Secondly because it is certainly less subjective than the other methods.

Calculus

As with oral hygiene many methods exist for recording the presence and amount of calculus. James (1963) recorded the presence or absence of calculus, a positive finding being if calculus was present on one tooth surface.

He did not record the calculus as subgingival or supragingival. McHugh et al. (1964) adopted both categories as above but only scored calculus present when two or more teeth had deposits upon them. The method chosen in this investigation was the Simplified Oral Hygiene Index of Greene and Vermillion (1964). This method records the presence of calculus if at least one tooth is affected and further divides it into subgingival and supragingival in origin plus allotting a score:

0 = No calculus present.

1 = Supragingival calculus covering not more than one-third of the exposed tooth surface being examined.

2 = Supragingival calculus covering more than one-third but not more than two-thirds of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth.

3 = Supragingival calculus covering more than two-thirds of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth.

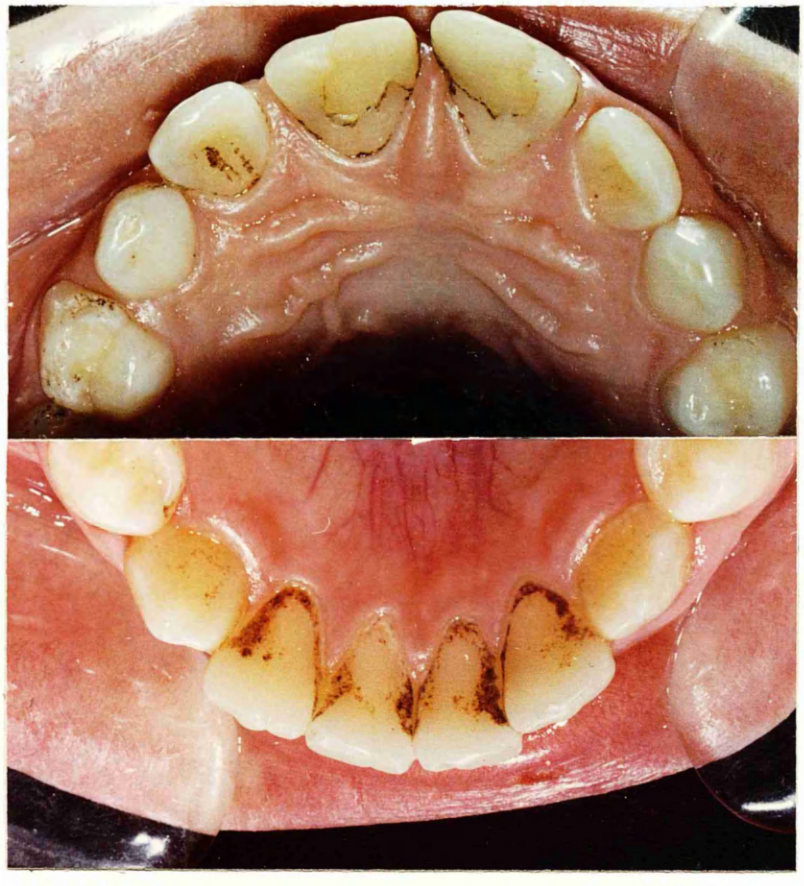
The use of this Index allowed a total score for the Simplified Oral Hygiene Index to be calculated as the Simplified Debris Index was known.

Gingivitis

The clinical difficulties of assessing the presence or absence plus degree of gingivitis in epidemiological studies are underlined by the number and variety of indices in use. A quantitative method of assessing the prevalence of gingivitis by gingival units consisting of a papillary portion (P); a marginal portion (M); and an attached portion (A) is given by the P-M-A Index of Schour and Massler (1948). This system gave a division into two groups, if gingivitis was present or absent, but not degrees of severity. However, in 1950, Massler et al. used the P-M-A Index plus an attempt to give a grading score to the gingival units. They also demonstrated a good correlation between gingival scores totalled after examining the whole mouth with scoring from the anterior region of the mouth only. This latter system has the commendable properties of speed of use in large epidemiological investigations plus easy observation of the anterior part of the mouth.

Russell (1956) used gingivitis in his overall periodontal score. Parfitt et al. (1958) gave a modified P-M-A Index using selected areas plus a scoring system. Jackson (1962) obtained an index of gingivitis by utilizing both clinical and photographic techniques.

PLATE I



Photograph showing the typical appearance and distribution of Extrinsic Black Tooth Stain.

James (1963) also used this technique in one survey but the variables introduced by photography may add more problems than they eliminate. McHugh et al. (1964) used selected gingival areas and allotted a score to each to obtain a gingival score.

The P-M-A Index Massler et al. (1950) confined to the anterior regions only but without a scoring for severity, was chosen in this survey. This method has the advantages of speed and easy observation with little reduction in the overall accuracy. Certainly intensity of inflammation is ignored but this must be balanced against the very real difficulty of diagnosing degrees of severity.

Extrinsic Tooth Stains

The presence or absence of coloured extrinsic stain upon the teeth was recorded. When stain was present it was recorded under its colour, mainly black, brown, green and orange. Stain distribution was also recorded. The differentiation between extrinsic brown stain and the brown pellicle described by Manly (1943) was made on clinical grounds only.

The closest attention was given to the investigation of extrinsic black tooth stain and Plate I shows an example of the stain which was accepted for investigation in this research.

The black stain was most commonly found as a continuous or intermittent thin stained line around the cervical portion of the crowns of teeth, especially posterior teeth. Occasionally the stain was found in the pits and fissures of posterior teeth. In a very few cases the stain was found on the buccal surface of upper and lower incisor teeth. In general the black stain was found as a thin line, rarely more than 1 mm. in width, in close proximity to and closely following the contour of the gingival margin.

Missing Teeth

The children were questioned regarding missing teeth and those not extracted for caries were recorded as missing due to extraction for orthodontic reasons, congenitally absent, or missing due to trauma. In a number of cases no reason for missing teeth could be elicited so that on the clinical examination of the rest of the mouth a decision was made. Consequently some teeth may have been wrongly charted. However, Jackson (1950) shows that only a small percentage of missing first permanent molars was due to causes other than extraction due to dental decay. A tooth was recorded as erupted into the mouth when any part of the emerging crown was visible (Sutcliffe, 1967).

Questionnaire Information

The children were questioned as to how long they had lived in Glasgow, whether or not they had a toothbrush, brushing habits, sweet consumption and types of snack eaten. They were also asked not only if they had a dentist but also how often they attended for visits.

Information from the dental clinical examination and the questionnaire was coded and then transferred to punch cards. A Glasgow University computer programmer transferred the information to a magnetic tape so that all the necessary results plus correlation results could be obtained from the computer. Any further correlations between the different criteria was calculated using a Hewlett Packard 9100A Desk top Computer.

CHAPTER II

CHAPTER II

THE INCIDENCE OF DENTAL DISEASE

Results

Dental Caries

Munblatt (1933) first suggested the division of teeth affected by dental caries into:-

1. The number of teeth lost through decay (missing).
2. The number of teeth with open cavities (decayed).
3. The number of teeth with fillings (filled).

This formed the basis for the Decayed, Missing and Filled Index of Klein et al. (1938) abbreviated to the D-M-F Index and used in this survey to record the incidence of dental caries.

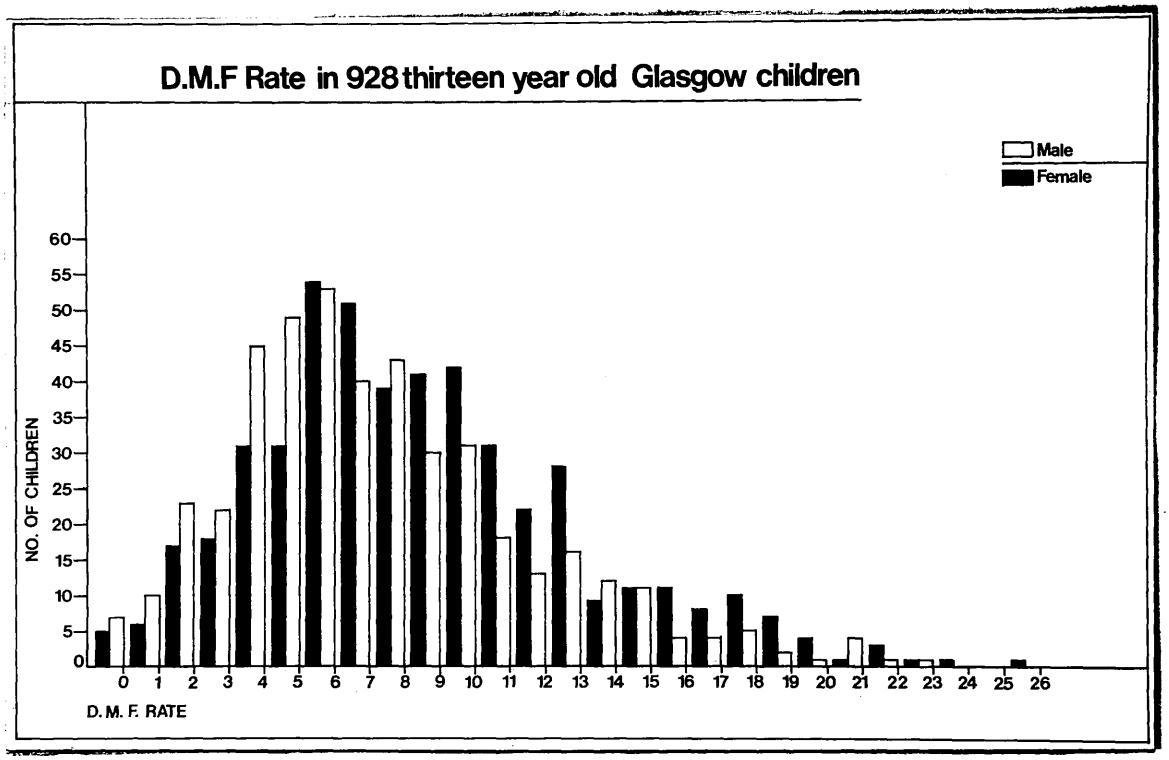
The D-M-F Index (Klein et al., 1938) was calculated from the dental data and results are shown in Table 3.

The mean D-M-F rate for the total sample and for males and females is shown in Table 4 reproduced below.

TABLE 4

Mean D-M-F rate of the total sample

	MALES	FEMALES	TOTAL
Mean D-M-F rate	7.58	8.86	8.25
Standard Deviation	4.27	4.58	4.48
Number of children	445	483	928



Histogram showing the D-M-F rate in 928 thirteen year old Glasgow school children.

Analysis of these figures show that the females had a significantly higher D-M-F rate than the males.

Comparison of mean D-M-F teeth rate

Males 7.58 ± 4.27: Females 8.86 ± 4.58[1]
p/0.001 (t = 4.41) highly significant

The histogram shows the squewed distribution of the number of D-M-F teeth for males and females.

Figures for the D-M-F rate related to the different areas of the mouth are shown in Tables 5, 6, 7, 8, 9 and 10.

The mean D-M-F rate for these areas is reproduced here and serves as a comparison as to how much each area is contributing to the overall D-M-F rate of the boys and girls.

TABLE 5

Mean D-M-F rate $\overline{8 - 4/}$ region

	MALES	FEMALES	TOTAL
Mean	1.72	1.89	1.81
Standard Deviation	0.92	0.91	0.92
Number of children	445	483	928

TABLE 6

Mean D-M-F rate $/\overline{4 - 8}$ region

	MALES	FEMALES	TOTAL
Mean	1.73	1.91	1.82
Standard Deviation	0.93	0.96	0.95
Number of children	445	483	928

1 ± = Standard Deviation throughout text.

TABLE 7

Mean D-M-F rate /4 - 8 region

	MALES	FEMALES	TOTAL
Mean	1.60	1.95	1.78
Standard Deviation	1.12	1.16	1.15
Number of children	445	483	928

TABLE 8

Mean D-M-F rate 8 - 4/ region

	MALES	FEMALES	TOTAL
Mean	1.53	1.85	1.70
Standard Deviation	1.05	1.18	1.13
Number of children	445	483	928

TABLE 9

Mean D-M-F rate 321/123 region

	MALES	FEMALES	TOTAL
Mean	0.80	0.98	0.89
Standard Deviation	1.31	1.44	1.38
Number of children	445	483	928

TABLE 10

Mean D-M-F rate $\overline{321/123}$ region

	MALES	FEMALES	TOTAL
Mean	0.20	0.29	0.24
Standard Deviation	0.72	0.92	0.83
Number of children	445	483	928

Examination of the results of the D-M-F Index for males and females shows that the mean number of teeth decayed in each group is almost equal.

The mean number of decayed teeth of the total sample was 4.33.

Table 11 shows the decay rate in detail with the mean decay rate reproduced below.

TABLE 11

Mean number of teeth decayed

	MALES	FEMALES	TOTAL
Mean	4.31	4.36	4.33
Standard Deviation	3.35	3.43	3.39
Number of children	445	483	928

The mean number of teeth missing for males and females in the sample is shown in Table 12 with the mean number of teeth missing in each group shown below.

TABLE 12

Mean number of teeth missing

	MALES	FEMALES	TOTAL
Mean	0.83	1.04	0.94
Standard Deviation	1.28	1.61	1.47
Number of children	445	483	928

Once again little difference between the number of teeth missing in boys and girls can be seen.

However, when Table 13 is examined and the mean number of filled teeth shown below it can be noted that females have a mean of one more tooth filled than males.

TABLE 13

Mean number of teeth filled

	MALES	FEMALES	TOTAL
Mean	2.46	3.47	2.85
Standard Deviation	3.14	3.81	3.42
Number of children	445	483	928

Table 14 shows the number of decayed teeth for males and females excluding grade 1 cavities and will be compared later in this Chapter with the figures for teeth decayed (Table 11) including grade 1 cavities. The mean figures for the number of teeth decayed excluding grade 1 cavities show a fall of approximately equal proportions in both females and males.

TABLE 14

Mean number of teeth decayed excluding grade 1 cavities

	MALES	FEMALES	TOTAL
Mean	2.74	2.91	2.83
Standard Deviation	2.79	2.97	2.88
Number of children	445	483	928

Tables 15 to 18 show the mean number of grade 1, 2, 3 and 4 cavities in males and females. The largest number of cavities was found in the grade 3 category closely followed by grade 1 category cavities. Both are reproduced in the Tables below.

TABLE 17

Mean number of grade 3 cavities

	MALES	FEMALES	TOTAL
Mean	1.80	1.91	1.86
Standard Deviation	2.07	2.15	2.11
Number of children	445	483	928

TABLE 15

Mean number of grade 1 cavities

	MALES	FEMALES	TOTAL
Mean	1.58	1.43	1.50
Standard Deviation	1.55	1.37	1.46
Number of children	445	483	928

Table 19, 20 and 21 show the average number of teeth assessed as missing for reasons other than dental caries.

TABLE 19

Mean number of teeth lost apart from caries - Orthodontic

	MALES	FEMALES	TOTAL
Mean	0.20	0.30	0.25
Standard Deviation	0.75	0.91	0.84
Number of children	445	483	928

TABLE 20

Mean number of teeth lost apart from caries - Congenitally absent

	MALES	FEMALES	TOTAL
Mean	0.002	0.006	0.004
Standard Deviation	0.047	0.102	0.080
Number of children	445	483	928

TABLE 21

Mean number of teeth lost apart from caries - Trauma

	MALES	FEMALES	TOTAL
Mean	0.04	0.02	0.03
Standard Deviation	0.20	0.16	0.18
Number of children	445	483	928

These figures would suggest that the boys lose more teeth through traumatic injuries than the girls whereas the reverse is true for orthodontic reasons.

Dental Cleanliness

The results of the Simplified Oral Hygiene Index

are given in Tables 22, 23 and 24. Table 25 gives a further division of the figures which will be of use in drawing comparisons with the results of other dental research workers. These figures show that the males had a higher score for the overall Index, i.e. Males 1.27 ± 0.47: Females 1.07 ± 0.45. The results demonstrate that the females had significantly better dental cleanliness than the males.

Comparison of mean Simplified Oral Hygiene Indices

Males 1.27 ± 0.47: Females 1.07 ± 0.45
$p < 0.001$ ($t = 6.83$) highly significant

This total figure was due largely to the difference in the Debris Index for males and females.

Mean Debris Indices

Males 1.14 ± 0.36: Females 0.94 ± 0.34

Comparison of these mean Debris Index figures show that the girls had significantly less debris present $p < 0.001$ ($t = 8.12$).

The males had a slightly higher calculus level than the females but when compared the figures for calculus were not significantly different $p > 0.05$ ($t = 1.29$).

Mean Calculus Indices

Males 0.14 ± 0.24: Females 0.12 ± 0.23

Results showing whether or not calculus was present are given in Table 26 and show that 283 males (63.6%) and 344 females (71.2%) had no calculus present. The total number in the sample without calculus was 627 (67.6%).

Gingivitis

The P-M-A Index (Massler et al., 1950) was used to record the presence or absence of gingivitis and the results are shown in Tables 27, 28 and 29. These can be categorised into the various degrees of gingivitis suggested by Massler et al. (1950).

	P-M-A
Absence of Inflammation	0-0-0
Mild (average or less)	1-0-0 to 2-1-0
Moderate (average to twice average)	3-2-0 to 5-2-0
Severe (more than twice average)	6-2-1 to 8-3-1
Very Severe	9-3-1 and above

The categories of degrees of gingivitis into which the children examined in this survey were placed are shown in Table 30. The divisions of severity of gingivitis used are only slightly different from those suggested by Massler et al. (1950) and are:-

	P-M-A
Absence of Inflammation	0-0-0
Mild (average or less)	1-0-0 to 2-1-0
Moderate (average to twice average)	3-0-0 to 5-4-3
Severe (more than twice average)	6-0-0 to 8-7-6
Very Severe	9-0-0 and above

These divisions allowed for easier computer analysis of the survey data on gingivitis.

Table 30 shows that over 99% of the children had gingivitis present in the anterior region of their mouth, mostly of the severe or very severe type.

From the figures obtained in this investigation it is recorded that the P-M-A Index of the males is on average 8.7-7.4-0.3 whilst for females the figures are 8.0-6.0-0.2. Analysis of the results show that females have better gingival conditions than males but both are still in the severe category.

Comparison of the mean papillary gingivitis score

Males 8.69 ± 1.96: Females 7.99 ± 2.38

$p < 0.001$ ($t = 4.94$) highly significant

Comparison of the mean marginal gingivitis score

Males 7.37 ± 3.17: Females 5.99 ± 3.71

$p < 0.001$ ($t = 6.10$) highly significant

Comparison of the mean attached gingivitis score

Males 0.27 ± 1.05: Females 0.23 ± 1.22

$p > 0.05$ ($t = 0.47$) not significant

Discussion

The mean D-M-F rate for the total sample is 8.25 D-M-F teeth. This figure falls approximately in the centre of the range of D-M-F figures published for children in similar age groups. Mansbridge (1959) examined 4,034 Edinburgh children aged five to seventeen years. Figures derived by the present author from a graph published in the above paper show an approximate mean of 4.5 D-M-F teeth for boys and 5.5 D-M-F teeth for girls in the thirteen year old age group, whereas Mansbridge (1960) found in a sample of 426 twelve to fourteen year old Ayrshire children an average of 10.0 D-M-F teeth.

However, this latter sample (Mansbridge, 1960) was selected from children in the age group who had not less than twenty five permanent teeth erupted at the time of examination. This much higher figure can be attributed to some degree to the rigid choice of patient i.e. each child had more teeth present than a random sample of a similar age group had as shown by Mansbridge (1959) thus a true comparison with the higher D-M-F rate cannot be drawn. James (1961) showed that the mean D-M-F rate for 198 twelve year old children in Norfolk was 6.2 D-M-F teeth. McHugh et al. (1964) found one of the highest recorded mean D-M-F rates for children published to that date. The average D-M-F rate for the 2,905 thirteen year old Dundee children was 10.02 D-M-F teeth. James (1964) found the mean D-M-F rate of 2,070 eleven and twelve year old boys and girls to be 5.5 D-M-F teeth.

Sutcliffe (1965) found the mean D-M-F rate in 986 eleven year old children to be 5.4 D-M-F teeth while in another survey Sutcliffe (1967) found on examining 986 children aged eleven and twelve years the mean D-M-F rate to be 5.67. Stephen and Sutherland (1971) examined a total of 515 Paisley children aged fourteen years and found a very high mean D-M-F rate, the figure being 13.04 D-M-F teeth. Berman and Slack (1972) in a longitudinal study found the mean D-M-F rate for the children examined in the thirteen year old age group to be 9.43 D-M-F-T in the Essex group and 9.51 D-M-F-T in the Kent group. Jackson et al. (1973) published figures for York children aged fifteen years with a D-M-F teeth rate of 8.95 which is very close to that found in this survey.

Although it is not possible to draw exact analogies between different results of epidemiological surveys, the mean D-M-F rate for the chosen population in the Glasgow study shows the same tendency towards a high figure.

It is interesting to examine the results for the number of decayed teeth in each section of the mouth. The four posterior regions contribute almost the same percentage of decayed teeth to the overall decay rate. The upper jaw posterior regions contribute almost the same percentage to the decay rate as do the lower jaw posterior regions.

These figures suggest an even spread of decay for the mouth as a whole. Berman and Slack (1972) in their survey work supported this finding and reported:

> "The bilateral symmetry of caries attack at all ages".

The upper anterior teeth contribute more to the mean decay rate than do the lower anterior teeth. This low rate of decay for lower anterior teeth may add weight to the oft stated clinical comment:

> 'that permanent lower incisor caries in young children denotes a high caries rate'.

Had the figures for the decay rate of the permanent lower incisors been higher in this study then one might have expected a disproportionately higher D-M-F rate for the sample. Jackson (1961) has shown that the mandibular incisors are among those teeth which are least likely to be affected by dental caries and the results from this survey would certainly support those findings. Sutcliffe (1965) in a clinical study of mandibular permanent incisor caries showed little difference in the prevalence of mandibular caries in the sexes but a much higher mean D-M-F rate in those with mandibular incisor caries than those without. The work of Berman and Slack (1972) also reported that in both groups studied the mandibular incisors showed very little caries activity.

Figures for the number of decayed teeth in this sample (4.33) and for decayed teeth less grade 1 cavities (2.83) are very similar to those given by McHugh et al. (1964) who reported 4.54 and 3.36 respectively. These figures show the contribution of grade 1 cavities to the overall decay rate and provide valuable information both on the extent of caries and treatment need as grade 1 or "Sticky Fissure" cavities can be classified as the least urgent for treatment. The figures for grade 3 cavities: males 1.80 and females 1.91 and for grade 4 cavities: males 0.35 and 0.34 for females are also in agreement with those of McHugh et al. (1964) and Stephen and Sutherland (1971) who both reported that grade 4 cavities contributed the smallest percentage to the overall decay rate.

The figures for teeth lost other than for dental caries suggest that most are lost for orthodontic reasons. However, these results depend not only on the answers given by the children to questioning at examination but also on the clinical assessment of the dental state of the child by the examiner. Jackson (1950) showed that only a very small percentage error would be introduced by assigning all missing teeth to the D-M-F rate.

It is interesting to note that although the figures for filled teeth in this study (2.46 for males and 3.47 for females) are slightly lower than those published by McHugh et al. (1964) and Berman and Slack (1972), they are surprisingly close to those published by Stephen and Sutherland (1971) their figures being 2.20 for males and 3.17 for females. It would be interesting to speculate that equal proportions of these populations in Paisley and Glasgow are dentally conscious.

Comparison of the D-M-F teeth rate for males and females (Table 3) shows that the females had a significantly higher D-M-F teeth rate ($p < 0.001$) ($t = 4.41$) and that this is due mainly to the greater number of teeth filled in females. This result is in contradistinction to Stephen and Sutherland (1971) who found that there was no significant difference in the D-M-F scores for males and females in their sample. However, they did find that females had more fillings than did the males. McHugh et al. (1964) found that girls had approximately one more filled tooth than did the boys. The higher caries incidence in girls was studied in an interesting survey by Mansbridge (1959) where he reviewed literature supporting the higher incidence.

His own results showed that the higher caries rate in girls was due in part only to the earlier eruption of teeth in girls and partly 'constitutional in origin'.

Dental Cleanliness

The results of the Simplified Oral Hygiene Index show that the girls had significantly less deposits on their teeth than did the boys ($p < 0.001$). Although direct comparisons with the results of McHugh et al. (1964) and Stephen and Sutherland (1971) are not possible due to their use of a modified Greene and Vermillion (1960) scoring method, nonetheless both these workers showed that females have significantly cleaner mouths than the males. The significant difference in cleanliness between males and females was in the Debris Index ($p < 0.001$) with no significant difference in the calculus score. Although different assessments of oral hygiene are used the results do strengthen the fact that females have better oral hygiene than males (James, 1963 and 1964; Mansbridge, 1959). Figures published by Greene and Vermillion (1964) cover the age group ten to nineteen year olds and are not exactly comparable with the results given in this survey.

However, there is a fairly close similarity between them (Standard Deviations given).

Comparison of mean oral hygiene scores

	Greene and Vermillion	Present Survey
Mean Debris Score	1.5 ± 0.50	1.04 ± 0.36
Mean Calculus Score	0.5 ± 0.48	0.13 ± 0.23
Mean Oral Hygiene Score	2.0 ± 0.87	1.17 ± 0.45

Greene and Vermillion (1964) also reported that no male or female in their survey was free of debris on some teeth. This agrees with Stephen and Sutherland (1971). McHugh et al. (1964) found only one girl to have teeth free of debris whereas James (1964) found 29.6% of girls and 17.3% of boys to have teeth free of debris which was largely in agreement with figures for James (1963) and Mansbridge (1960) who reported 33% in the good category and earlier 50% in the good category (Mansbridge, 1959). Sutcliffe (1965) reported approximately 40% of eleven year old children with oral cleanliness in the good category. The importance of good oral hygiene was demonstrated by Schei et al. (1959) who demonstrated that poor oral hygiene leads to increased alveolar bone loss.

Results in this survey support the findings of other research workers already mentioned that a decrease in oral cleanliness has occurred over the last few years.

Calculus

No significant difference in the mean calculus score was found between males and females and this agrees with the results of McHugh et al. (1964) and James (1965). However, Sinclair and Goose (1966) show a higher percentage of males with calculus than females while Stephen and Sutherland (1971) show a significant difference in mean calculus scores between males and females. The figure for the mean calculus index 0.36 given by Stephen and Sutherland (1971) is midway between the figures already given for Greene and Vermillion (1964) and the present survey.

The percentage of children with calculus present was 32.4% - boys 36.4% and girls 28.8%. These figures are much higher than those published by Sinclair and Goose (1966) for twelve to seventeen year old girls (11.7%) and boys (15.6%). These researchers also concluded that:

> "..... it is unlikely that calculus plays a prominent part in the aetiology of periodontal disease in children".

However, McHugh et al. (1964) found that although the incidence of calculus in boys and girls was not significantly different being present in 25% of

cases there was a positive correlation between the presence of calculus and the mean gingivitis score. Figures published by James (1964) for the presence of calculus are very close to those in this survey i.e. boys with calculus 31.2%, girls with calculus 28.2%. The presence of calculus found in children by Marshall-Day et al. (1955) - 33% was also very similar to that found in this study. Table A summarizes these results.

TABLE A

Incidence of Calculus

Calculus Present	Year	Per cent Males	Per cent Females	Per cent Total
Marshall-Day et al.	1955	39	28	33
McHugh et al.	1964	25	24	25
James	1964	31.2	28.2	29.7
Sinclair and Goose	1966	15.6	11.7	13.6
Present Study		36.4	28.8	32.4

Gingivitis

The results for gingivitis in this survey show that females had much better gingival conditions than males (Table 30). This was true for both papillary and marginal gingiva but not for attached gingiva where no significant difference was found (Tables 27 - 29).

Comparison of the figures in this survey with those of Schour and Massler (1948) show an increase in prevalence for all divisions of the P-M-A Index for both females and males.

Comparison of mean gingivitis scores

	Schour and Massler	Present Survey
Mean Papillary Score	2.4	8.3
Mean Marginal Score	1.1	6.7
Mean Attached Score	0.03	0.3

Although variations in age groups may account for part of these differences a much more likely reason is that Schour and Massler (1948) chose medical and dental students as subjects. A much greater awareness of dental health should be expected from such a group. Schour and Massler (1948) found no significant increase in the amount of gingivitis between males and females. Also 42.4% showed no clinical evidence of gingivitis. The increase in gingivitis is also shown by comparing the Periodontal Score, given for twelve to fourteen year olds, of Russell (1956) with Stephen and Sutherland (1971). The former gives the highest score as 0.20 while the latter gives the mean periodontal score as 0.82. Parfitt et al. (1958) found girls to have better gingival conditions than boys but were not certain that it was due to better oral hygiene.

They stated that the severity and incidence of gingivitis increases with age with peak levels in boys at thirteen years six months and girls at ten years six months thus arguing that between the ages of eleven and sixteen years girls would, by their inference, have better gingival conditions anyway. This they say may be due to the time difference in reaching puberty. This will be discussed again later in this Chapter. James (1963) stated that the gingival conditions of girls were much better than boys. McHugh et al. (1964) found that more boys than girls had gingivitis and that boys had a higher mean gingivitis score. However, Stephen and Sutherland (1971) found that boys had significantly better Russell Index mean scores than did girls. Unfortunately, apart from stating that:

> "a lack of calibration between different author-groups is a possible explanation"

they do not offer any further discussion. This would certainly have been of value as the weight of epidemiological work supports the findings that girls have better gingival conditions than boys.

The finding that over 99% of children examined in this survey had gingivitis present is in agreement with McHugh et al. (1964) who also reported a finding that over 99% of the children they examined had gingivitis present.

Interrelationship of Dental Disease

Results

The result of correlating the Oral Hygiene Index and the D-M-F rate is shown in Table 31. Statistical analysis of these results show that the differences in the D-M-F rate between the Oral Hygiene Index groups are not significant.

Table 32 gives the results of the relationship between the Oral Hygiene Score and the P-M-A Index. Statistical analysis of the results show a significant correlation between poor oral hygiene and the more severe gingivitis scores ($t = 6.23$: $p < 0.001$). A summary of Table 32 is given below in Table B.

TABLE B

Oral hygiene and Gingivitis

Sex	Oral Hygiene Index	Number in Sample	Gingivitis Score
Boys	0	84	7.79 ± 2.56
	1 to 2	360	8.89 ± 1.85
Girls	0	171	7.34 ± 2.76
	1 to 2	312	8.36 ± 2.19
Total	0	255	7.48 ± 2.70
	1 to 2	672	8.64 ± 2.03

Standard Deviations are given with the gingivitis score. Comparison of gingivitis scores in both groups show a highly significant correlation. ($t = 6.23$: $p < 0.001$).

The results of correlating the gingivitis scores with the incidence of calculus are given in Table 33 and are summarized below in Table C. Standard Deviations are given.

TABLE C

Calculus and Gingivitis

Sex		Number in Sample	Mean Gingivitis Score
Boys	Calculus absent	283	8.58 ± 2.12
	Calculus present	162	8.85 ± 1.90
Girls	Calculus absent	344	7.85 ± 2.51
	Calculus present	139	8.34 ± 2.28
Total	Calculus absent	627	8.18 ± 2.37
	Calculus present	301	8.62 ± 2.09

Comparison of mean gingivitis total scores in groups with and without calculus shows a significant difference between the groups ($t = 2.87$: $p < 0.01$).

Discussion

Table 31 shows that there was no significant tendency for children with poorer oral hygiene to have more D-M-F teeth than children with good oral hygiene. This is in agreement with the findings of McHugh et al. (1964). However, Mansbridge (1960) found that children with good oral hygiene had fewer D-M-F teeth than children with poor oral hygiene. Fosdick (1950) and Weisenstein et al. (1954) found that carefully controlled toothbrushing regimes

would reduce the incidence of new dental caries. The contrary was found by Miller and Hobson (1961) who published results which showed a higher number of D-M-F teeth among children who brushed regularly than among those who brushed infrequently. Hein (1954) stated:

> "..... that frequent brushing, even as done by the public, is highly desirable but mainly for reasons other than dental caries".

and the results of this survey would lend support to that statement (Tables 31 and 32).

The high degree of correlation between oral hygiene and gingivitis in both boys and girls suggests that lack of oral hygiene is the factor causing most of the observed differences in gingivitis prevalence and that puberty (Parfitt et al., 1958) is of lesser significance. This finding is in agreement with the published results of McHugh et al. (1964) which show that poor oral hygiene is the most important factor causing gingivitis in boys and girls.

The positive correlation between the presence of calculus and the mean gingivitis score (Table 33) is in agreement with the findings published by McHugh et al. (1964) relating to these two parameters. These researchers summed up the part played by calculus as an aetiological agent in gingivitis by stating:

> "It is evident, however, that calculus is only one factor in the aetiology of gingivitis".

CHAPTER III

PLATE II

Illustration of examination and questionnaire section of pro-forma.

SERIAL NO. _____ (1 - 3) NAME _____ SEX _____ (4)

ADDRESS _____ DATE OF EXAMINATION _____

SCHOOL _____ DATE OF BIRTH _____

CLASS _____ AGE _____ (5)

1. HAVE YOU ALWAYS LIVED IN THIS AREA? (YES, if never away for longer than six months at a time). Yes / No (6) — 1 / 0

2. DO YOU HAVE A TOOTHBRUSH? Yes / No (7) — 1 / 0

3. HOW OFTEN DO YOU BRUSH YOUR TEETH? ___ times/day — 1 2 3

___ times/week — 5/6 (4) 3/4 (5) 1/2 (6)

___ (rarely) — R (0) (8)

4. HAVE YOU BRUSHED YOUR TEETH IN PAST 24 HOURS? Yes / No (9) — 1 / 0

5. HAVE YOU EATEN SINCE YOU LAST BRUSHED YOUR TEETH? Yes / No (10) — 1 / 0

6. AMOUNT SPENT ON SWEETS IN PAST WEEK £ p. (11)

7. DO YOU EAT SNACKS? Yes / No (12) — 1 / 0

8. WHAT TYPE? Cho / Non (13)

9. NUMBER PER DAY 0 1 2 3 4. (14)

10. DO YOU HAVE A DENTIST? Yes / No (15) — 1 / 0

11. DO YOU ATTEND A DENTIST REGULARLY? Yes / No (16) — 1 / 0

EXAMINATION

Examiner _____ Dentures

8 7 6 5 4 3 2 1 | 1 2 3 4 5 6 7 8

ORAL HYGIENE

DEBRIX INDEX SIMPLIFIED

		AMT.		TOTAL
UPPER				
LOWER				
TOTAL				

CALCULUS INDEX SIMPLIFIED — Total Index

ORAL HYGIENE INDEX SIMPLIFIED = _____
(Debrix Index Simplified) + (Calculus Index Simplified)

GINGIVITIS P.M.A. INDEX = _____

3 2 1 1 2 3
3 2 1 1 2 3

	P.	M.	A.
UPPER			
LOWER			
TOTAL			

M. _____

Teeth lost apart for caries

A. _____

Tr _____

EXTRINSIC STAIN

STAIN COLOUR	P.	A.	D.
BLACK			
BROWN			
ORANGE			
GREEN			
NONE			

SUMMARY

Teeth decayed

Teeth Missing — D.M.F. Teeth

Teeth Filled — D.M.F. Surfaces

CHAPTER III

SOCIAL DATA

The results obtained from direct questioning of the children will be, as already mentioned, affected by the truthfulness of the child. Notwithstanding this the conclusions drawn and the comparisons made with the results of other researchers are of value in exemplifying general points. The questionnaire section of the pro-forma is shown in Plate II.

Results of Questionnaire

The children were asked:

a. Do you have a toothbrush?

Table 34 gives the results and shows that 420 boys (94.4%) stated that they had a toothbrush while 481 girls (99.6%) stated that they possessed a toothbrush.

b. How often do you brush your teeth?

Table 35 gives the results, some of which are shown here as percentages.

Stated Frequency of Toothbrushing

Frequency	Per cent Boys	Per cent Girls	Per cent Total
Thrice/day	20	24	22
Twice/day	14	44	29
Once/day	2	8	5
Once to six times/week	28	9	18
Rarely	36	15	26

c. Have you brushed your teeth in the past twenty four hours?

Table 36 shows the results given as percentages here to show that 230 boys (50%) said that they had not brushed their teeth whereas only 155 girls (32%) said that they had not brushed their teeth in the last twenty four hours.

d. Have you eaten since you last brushed your teeth?

Table 37 shows the results and that 372 boys (83%) said that they had eaten since they last brushed while 391 girls (80%) also had eaten since last brushing their teeth.

e. Amount spent on sweets in the past week.

Table 38 gives the results in detail and shows the average amount spent by boys and girls to be:-

Mean amount spent on sweets per week in pence

	MALES	FEMALES	TOTAL
Mean	30.9	31.4	31.2
Standard Deviation	12.8	11.8	12.2
Number of children	445	483	928

The amount spent per week most frequently by both boys and girls was in the 25 - 29 pence range. It is interesting to note that the next most popular amount to spend for both sexes was over 45 pence per week.

f. Do you eat snacks?

Table 39 shows the results and that 276 boys (62%) and 339 girls (70%) did eat snacks.

Table 40 shows results of an attempt to classify the snacks as carbohydrate or non-carbohydrate with 325 (35%) stating that they ate carbohydrate rather than non-carbohydrate snacks.

g. Number of snacks eaten per day.

Table 41 shows the results with the mean number of snacks for boys and girls as given.

Mean number of snacks eaten per day

	MALES	FEMALES	TOTAL
Mean	1.6	1.6	1.6
Standard Deviation	1.5	1.3	1.4
Number of children	445	483	928

h. Do you have a dentist?

Table 42 shows the results and that 356 boys (80%) and 438 girls (90%) said that they had a dentist. However, only 152 boys (34%) and 266 girls (55%) attended the dentist regularly (Table 43).

Discussion

The number of children claiming to possess a toothbrush was 901 (97%) with 481 (99.6%) being girls and 420 (94.4%) being boys. These figures are very similar to those published by Finlayson and Wilson (1961) who gave 91.1% possessing a toothbrush; by McHugh et al. (1964) who showed over 99% having a toothbrush and by Stephen and Sutherland (1971) showing 98.8% to possess a toothbrush.

Mansbridge (1960) correctly stated there was no way of verifying the accuracy of the children's statement and it is interesting to note that in the above Finlayson and Wilson (1961) study the answers given by the children and their parents did not agree in regard to the child owning a toothbrush, thus highlighting Mansbridge's (1960) observation on questionnaire answers. Although brushing twice per day was the most commonly stated practice, the result was due largely to the high percentage of girls brushing twice per day. The 44% for girls brushing twice a day in this survey is very close to both the 37% given by McHugh et al. (1964) and the 40% stated by Parfitt et al. (1958) for girls brushing twice daily. However, although the results agree with those of Stephen and Sutherland (1971) that brushing twice per day is most frequent the suspicion exists that a few different answers would have these survey figures agreeing with those of McHugh et al. (1964) that brushing once per day was the most frequent finding. Consequently it was not thought possible to state that thirteen year old Glasgow children were more toothbrushing conscious than thirteen year old Dundee children. Five hundred and seventeen (55%) children claimed to have brushed their teeth within the last twenty four hours. This figure is lower than the 70.7% stated by Stephen and Sutherland (1971) and the 73% stated by McHugh et al. (1964).

However, these differences may be due again to the many uncontrollable variables in question and answer.

Seven hundred and sixty three (82%) children stated that they had eaten since they last brushed their teeth and this result is very close to the 88.5% found by Stephen and Sutherland (1971).

The amount spent on sweets per week was higher than the amount found by Mansbridge (1960); McHugh et al. (1964) and Stephen and Sutherland (1971). This is probably due to both a higher standard of living and inflation.

Relationship to Dental Disease

Results

A comparison between the observed oral hygiene and the stated toothbrushing frequency was made and the results are shown in Table 44. The figures were subjected to a student 't' test and the results are shown in Table 45.

The children who brushed their teeth once, twice or three times per day had statistically significantly better oral hygiene than those who brushed rarely. The results are summarized in the text - Table D.

TABLE D

Comparison between observed oral hygiene and stated toothbrushing frequency

Frequency of brushing	Number in Sample	Mean Simplified Oral Hygiene Index
Thrice/day	48	1.01 ± 0.48 [1]
Twice/day	279	1.06 ± 0.43
Once/day	209	1.13 ± 0.42
5 - 6 times/week	20	1.18 ± 0.37
3 - 4 times/week	79	1.20 ± 0.47
1 - 2 times/week	94	1.23 ± 0.45
Rarely	173	1.35 ± 0.48

[1] Standard Deviation. Difference between groups brushing twice or more per day, once a day and less than once per day all highly significant ($p < 0.001$).

The relationship between the Simplified Oral Hygiene Index and the number of snacks eaten per day is shown in Table 46 with statistical analysis given in Table 47. There is no statistical difference between the number of snacks eaten per day and the oral hygiene score.

The effect of the number of snacks eaten per day on the D-M-F rate is shown in Table 48 with Table 49 giving the results of student 't' test. No significant correlation between the number of snacks eaten and the D-M-F rate was found.

The results of comparisons between the D-M-F rate and the number of times the children brushed their

teeth are shown in Table 50 with statistical results in Table 51. No consistant picture appears from the figures as children who brush their teeth twice a day have a statistically lower D-M-F rate than children who brush once or twice a week ($t = 2.7$: $p < 0.01$) whereas children who brush their teeth three times per day do not have any statistically significant difference in their D-M-F rate when compared to children who brush once or twice per week ($t = 1.1$: $p < 0.3$). These results will be referred to again in the discussion following.

The results of the effects of toothbrushing on gingivitis are given in Tables 52, 53 and 54. The results were statistically analysed and the results given in Tables 55, 56 and 57 show that children who brush their teeth twice or three times per day have significantly better gingival conditions than those who brush rarely for example Table 55 shows that children who brush their teeth twice per day have significantly lower papillary inflammation than children who brush rarely ($t = 5.72$: $p < 0.001$).

The effect of eating snacks on gingival condition was examined and results plus statistical examination are shown in Tables 58 to 63. The findings are too inconsistant to draw any positive conclusions.

The amount spent on sweets by the children and the relationship to the D-M-F rate is shown in Tables 64 and 65.

It can be seen (Table 64) that 545 children spent between 10 pence and 29 pence and that this was the most frequent amount spent per week, while the next most frequent was 45 pence and over (270 children) while 103 children spent 30 pence - 44 pence per week with the least frequent being 10 pence and under (10 children). Table 65 shows that children spending 10 pence and under per week have a significantly lower D-M-F rate than children spending above this amount. Tables 66 and 67 show the results of the amount spent on sweets per week by the children on the D-M-F rate excluding grade 1 cavities. No statistically significant relationship was found between the various amounts spent per week and the D-M-F rate excluding grade 1 cavities.

Discussion

The results show that children who brush their teeth regularly have better oral hygiene than children who brush rarely ($p < 0.001$) and this agrees with results published by McHugh et al. (1964); Mansbridge (1960) and Greene and Vermillion (1960) all of whom found a similar relationship between brushing frequency and oral hygiene. Although Mansbridge (1960) pointed out that 10% of children in the survey carried out by him, who brushed their teeth less than once per day had good oral hygiene, he suggested that:-

> "..... in these children natural self-cleansing may be due to the character of their usual diet".

However, in the present survey Tables 46 and 47 show no correlation between the number of snacks eaten per day and the Oral Hygiene Index. Perhaps finer detail in the present survey would be of value in respect to the effect of consumption of non-refined carbohydrate snacks on the Oral Hygiene Index. The fact that no correlation between snack eating and the D-M-F rate was found is a little surprising as children eating carbohydrate snacks would be expected to have a higher caries rate. However, as the D-M-F rate was compared to the total number of snacks eaten per day and not the type of snack, a further investigation into carbohydrates and non-carbohydrates snacks against D-M-F rate will be carried out in future. Tables 50 and 51 show that there was no significant tendency for children who brushed their teeth only rarely to have a higher D-M-F rate than children who brush twice or three times per day. Although children who brush their teeth frequently have better oral hygiene than children who brush rarely it was seen that no significant correlation between oral hygiene and D-M-F rate could be found. The relationship between oral hygiene and D-M-F rate has already been discussed in Chapter II. Hein (1954) has already said that toothbrushing is important for factors other than dental caries and results in this survey

would support that fact as do other published results (McHugh et al., 1964). However, Fosdick (1950); Weisenstein et al. (1954) and Mansbridge (1960) found that good oral hygiene significantly reduces the D-M-F rate while Miller and Hobson (1961) found on the contrary that good oral hygiene was associated with a higher D-M-F rate. However, direct comparison with other surveys is not possible with toothbrushing habits alone as Mansbridge (1960) correctly stated that difficulties exist in defining toothbrushing frequency and efficiency.

Following on from the point made by Hein (1954) that toothbrushing is important for reasons other than dental decay results from this survey show that more frequent toothbrushing leads to better gingival conditions - Tables 52 to 57.

The results show (Table 64) that the children who spend more than 10 pence per week on sweets have a higher D-M-F rate than those who spend 10 pence or less per week with the greatest significance being observed between children spending less than 10 pence and 30 - 44 pence per week ($t = 3.37$: $p < 0.001$). The other amounts 10 - 29 pence and over 45 pence are both significant at the 1% level ($t = 3.23$ and 2.96 respectively). The findings agree with those of Mansbridge (1960) who found a significant correlation between sweet consumption and D-M-F teeth in 426 twelve

to fourteen year old children living in Ayr. However, McHugh et al. (1964) did not find an obvious relationship between the numbers of D-M-F teeth and stated sweet consumption, although they did find a significant correlation between the number of decayed teeth and the amount of sweet consumption. Their correlation applied not only to the total number of decayed teeth but also was still significant when grade 1 cavities were excluded. The results of the present survey show no correlation between the number of decayed teeth excluding grade 1 cavities and the amount of sweet consumption. As the difference between the total number of teeth decayed and the number of teeth decayed excluding grade 1 cavities was greater in this survey than in that of McHugh et al. (1964), this may partly explain the difference in results.

The classical Vipeholm study (Gustafsson et al., 1954) demonstrated that between meal sweet consumption is a major factor in causing dental caries. The results published by Mansbridge (1960); McHugh et al. (1964) and the present survey all support this finding.

CHAPTER IV

CHAPTER IV

THE PREVALENCE OF EXTRINSIC TOOTH STAINS

The customary method used to describe acquired stains of teeth (Stones, 1966) is in two divisions - extrinsic and intrinsic. The definitions of these divisions may be given as:

a. Extrinsic Stains:-
are deposits of material upon the outer surface of the tooth and are usually removeable by instrumentation.

b. Intrinsic Stains:-
are deposits of material within the tooth substance from the dental pulp and are not usually removeable by instrumentation.

While these terms are useful in describing the situation of the stain they are of less value as a classification for the aetiological factors causing the stain. Aetiological factors acting externally on a newly erupted tooth may cause intrinsic stain to occur without the aetiological factor passing via the dental pulp (Ball and Ferguson, 1962; Ball, 1964).

The stains discussed in this survey are deposited upon the teeth, can be removed by instrumentation and are classified and generally accepted as extrinsic stains.

These coloured extrinsic stains and their relationship to dental disease have been investigated by dental

research workers with published results available from the late nineteenth century (Miller, 1894). The presence or absence of coloured extrinsic stains and especially black, brown and green plus the relationship of these stains to dental disease will be given in this Chapter. Comparisons with the results of other dental research workers in this field will be made in the discussion.

Results

Table 68 shows the number of children whose teeth had no stain, a single coloured stain or a combination of coloured stains present. Summarized results are shown in Table E.

TABLE E

Extrinsic Stain found on the teeth of 928 children aged thirteen years

		No Stain		Black Stain		Brown Stain		Green Stain	
	No.	No.	%	No.	%	No.	%	No.	%
Total	928	515	55.4	43	4.5	100	10.7	187	20.1
Boys	445	224	50.3	18	4.4	55	12.3	97	21.8
Girls	483	291	60.5	25	5.1	45	9.3	90	18.6

Discussion

The results of the study into the prevalence of coloured stains upon the teeth show that the stains in order of prevalence were:-

1.	Green	187 children	20.1%
2.	Brown	100 children	10.7%
3.	Black	43 children	4.5%
4.	Orange	36 children	3.8%

Five hundred and fifteen (55.4%) children had no coloured stain on their teeth and this was the most common finding.

The prevalence of extrinsic stains in deciduous teeth has been investigated and figures published by Mellanby and Coumoulos (1946) show a very similar situation to the figures for permanent teeth in this survey except for green stain which was approximately double.

Mellanby and Coumoulos (1946)

Percentage of teeth with no stain	51.6
Percentage of teeth with dark stain	10.6
Percentage of teeth with green stain	34.9

However, in an interesting survey carried out by Pedersen (1946) a higher incidence of both extrinsic black stain and no stain was shown in one to six year old children but a lower incidence of green stain.

Pedersen (1946). 1,157 Copenhagen children living at home.

Percentage of children with black stain	11.7
Percentage of children with no stain	76.0
Percentage of children with other stains	12.4

Pedersen (1946) also published figures to show that children of lower social class living in a Copenhagen Kindergarten had a higher incidence of black stain than those living at home. However, no reason could be found for these differences. A similar study to the present one but using deciduous teeth would be of value in adding further evidence on the distribution of the black material. In a survey carried out on permanent teeth by Shourie (1947) on 1,097 thirteen to sixteen year old Indian boys it was shown that approximately 14% had extrinsic black stain present. James (1963) showed that the prevalence of coloured extrinsic stains on the teeth of twelve year old children was 44% overall with 26% green, 6% orange and 12% black or brown. James (1964) in a survey on 2,070 children aged eleven and twelve years gave results for the prevalence of extrinsic stains as, coloured - 25.1% girls, 30.5% boys; dark - 12.1% girls, 17.1% boys; with no stain - 62.8% for girls and 52.4% for boys. These figures are very similar to those found in this survey and shown in Table E.

However, Sutcliffe (1967) found that brown was the most prevalent coloured extrinsic stain present in eleven to twelve year old children. The figures given by Sutcliffe (1967) were: black - 2.3% boys, 0.9% girls; brown - 22.5% boys, 16.7% girls; green - 8.8% boys, 10.9% girls; orange - 1.9% boys, 2.8% girls. The prevalence of extrinsic coloured stains upon the teeth of both children with deciduous and permanent dentition can be seen from the figures quoted to bear a fairly close relationship.

Relationship to Dental Disease

Results

The relationship of coloured extrinsic tooth stains to dental caries is shown in Table 69. The most important results from that Table are summarized below.

The mean D-M-F rate and Standard Deviation of children with stains and without tooth stains

Number	Stain	Mean D-M-F rate	Standard Deviation
43	Black	6.02	± 3.59
100	Brown	6.95	± 3.09
187	Green	8.87	± 3.98
36	Orange	7.83	± 4.72
515	None	8.75	± 4.82

The results of 't' tests carried out on Table 69 are shown in Table 70. The main points to emerge from the statistical analyses are detailed below under each coloured stain.

1. Black Stain

Comparison of the mean D-M-F rate of children whose teeth have extrinsic black stain with children whose teeth have no stain shows that the former have a statistically significantly lower D-M-F rate ($t = 4.65$: $p < 0.001$). Similarly when compared to children whose teeth have green stain the children with black stain on their teeth have significantly lower D-M-F rate ($t = 4.59$: $p < 0.001$). No statistically significant difference exists between the D-M-F rate of children whose teeth have black stain when compared to children whose teeth have either brown or orange stain present.

2. Brown Stain

A statistically significant difference is found between children whose teeth have extrinsic brown stain and those whose teeth have either no stain ($t = 4.61$: $p < 0.001$) or green stain ($t = 4.51$: $p < 0.001$) but no statistical difference is found between brown and orange stain.

3. Green Stain

No statistical difference exists between the D-M-F rate of children whose teeth have extrinsic green

stain and children whose teeth have either no stain (t = 0.05) or orange stain (t = 1.23). Comparisons with the other stains have already been detailed.

4. Orange Stain

No statistical difference exists between the D-M-F rate of children whose teeth have extrinsic orange stain when compared to children whose teeth have no stain (t = 1.13). Comparisons with other stains have already been given.

The relationship between the Simplified Oral Hygiene Index and the presence of extrinsic coloured stains upon the teeth is shown in Table 71. The main points are summarized in Table F.

TABLE F

The mean Simplified Oral Hygiene Index and Standard Deviation of children with stains and without tooth stains

Number	Stain	Mean Oral Hygiene Index	Standard Deviation
43	Black	1.09	± 0.28
100	Brown	1.14	± 0.37
187	Green	1.27	± 0.42
36	Orange	1.32	± 0.50
515	None	1.12	± 0.49

The results of statistical analyses tests carried out on the figures given in Table 71 are shown in Table 72.

The results show that children whose teeth have extrinsic black stain have a statistically significantly better Oral Hygiene Index than either children whose teeth have green stain ($t = 3.26$: $p < 0.01$) or orange stain ($t = 2.35$: $p < 0.02$). However, no statistical difference exists between children whose teeth have either brown stain ($t = 0.39$) or no stain ($t = 0.50$).

Children whose teeth have no stain have a statistically significantly better Oral Hygiene Index than children whose teeth have either extrinsic green stain ($t = 3.97$: $p < 0.001$) or orange stain ($t = 2.28$: $p < 0.05$) but no difference exists between no stain and extrinsic brown stain ($t = 0.39$). No statistical difference is found between the Oral Hygiene Indices of children whose teeth show green or orange stain. Children whose teeth have extrinsic brown stain have a statistically better Oral Hygiene Index than children who have green stain on their teeth ($t = 2.74$: $p < 0.01$).

The results of comparing the presence or absence of extrinsic coloured tooth stains with the P-M-A Index are given in Tables 73, 74 and 75. Statistical analyses carried out using these results show that no significant difference was found between the presence or absence of extrinsic coloured tooth stains and the P-M-A Index. Tables 76, 77 and 78 show the results of student 't' tests carried out on the tables of results.

Discussion

The relationship between extrinsic coloured tooth stains and dental disease, especially dental caries, has been and still is being subjected to the attention of dental research workers. In particular the relationship that exists between these coloured extrinsic tooth stains and dental caries is still controversial. Pickerill (1923); Bibby (1931); Mellanby and Coumoulos (1946); Pedersen (1946); Shourie (1947); Commerell (1955); Mellanby et al. (1957); James (1963); James (1964); Sutcliffe (1965); Sutcliffe (1967) all have shown that children whose teeth have extrinsic dark stain upon them have a statistically lower D-M-F rate while Bunting (1929) and Leung (1950) have shown no such relationship. In fact Bunting (1929) states:-

> "..... we have found many instances in which definite black lines similar to that which Pickerill describes, have been present in mouths in which caries is very active".

The author of the present survey would certainly agree with the statement made by Bunting (1929) as mouths which had both active caries and black stain present were examined. However, Bunting unfortunately does not give any statistical evidence to confirm that these cases are in the minority. Certainly the results of this survey show that children who have black stain upon their teeth have a lower D-M-F rate than children who have no stain ($t = 4.65$: $p < 0.001$).

Leung (1950) stated that institutionalized children did not show any statistical evidence that either green or brown stain was associated with caries experience. However, Pedersen (1946) in a survey including children at Kindergarten found a lower caries rate in children with black stain upon their teeth than in children with other or no stains. Further epidemiological survey work is needed before it can be said that institutionalized children have more or less coloured extrinsic tooth stains than do children living at home.

Results published by Sutcliffe (1967) showing the mean D-M-F rate for children with stains and without tooth stains when arranged in order of magnitude are exactly matched by the arrangement of stains in this survey.

Mean D-M-F rate for children with and without tooth stains

	Green	None	Orange	Brown	Black
Sutcliffe (1967)	6.06	5.67	4.91	4.83	3.06
Present Survey	8.87	8.75	7.83	6.95	6.02

Although no other surveys give exactly the same amount of detail those by Mellanby and Coumoulos (1946) and Pedersen (1946) do show the same tendency in magnitude of mean D-M-F rate against colour of stain for deciduous dentition.

James (1963) also gives coloured stains the highest D-M-F rate and then no stain and finally dark stain. It is very interesting to note that James (1963) in a survey of 1,273 children aged eleven to thirteen years living in high and low fluoride areas showed that although all the children living in the high fluoride area had a lower D-M-F rate than children living in the low fluoride area, within the high fluoride area children with black stain had the lowest D-M-F rate of all. This suggests strongly that the beneficial effect of fluoride is increased by the presence of black stain. Why this might be so will be discussed later in Chapter VIII.

Sutcliffe (1967) found with the exception of the first permanent molars children with dark stain had a smaller prevalence of D-M-F teeth for each tooth type and had previously found in an earlier study in 1965 on mandibular incisor caries that those with brown stain had a lower D-M-F rate.

The relationship between extrinsic tooth stains and oral cleanliness have been given in Tables 71 and 72 and are in close agreement with the only other published data comparing these parameters (Sutcliffe, 1967). Sutcliffe (1967) showed as in this survey that there was no difference in the standard of oral hygiene between children with black stain and those without stain.

However, in the present survey children whose teeth had black stain had a significantly better Oral Hygiene Index than those children who had green stain ($t = 3.26$: $p < 0.01$). Children who had green stain had significantly poorer oral cleanliness than those without stain as did children with orange stain.

The results published by Sutcliffe (1967) and borne out in this survey show that children with extrinsic tooth stains apart from dark extrinsic stains have poorer oral hygiene than children without stain. As there is no statistical difference between the standard of oral hygiene for children without stain and with dark stain it may be that the scoring system used by Greene and Vermillion (1964) which gives a Debris Index score to teeth having extrinsic stain present irrespective of colour may need rethinking as regards dark stain.

The fact that no statistical correlation could be demonstrated between the presence of extrinsic coloured tooth stains and the gingival condition using the P-M-A Index is interesting. It has already been shown that children with green stain upon their teeth have poorer oral hygiene than children without stain. The relationship between oral hygiene and gingivitis shows that poor oral hygiene is associated with increased gingivitis (McHugh et al., 1964) and so it might have been expected that children with green stain examined in this survey would have a higher P-M-A Index than children with no stain.

However, this is not the case and it is difficult to advance any reasons to explain these results.

CHAPTER V

CHAPTER V

BACTERIOLOGY SECTION

Description of Sample

Table 68 shows that of the 928 children examined in the present survey, 43 had only extrinsic black stain present on their teeth. A total of 24 children had extrinsic black stain present plus one or more other coloured extrinsic stain upon their teeth. These latter children were to be used if an insufficient number of children with extrinsic black stain only presented for examination. However, as 32 children from the black stain only group presented for examination no children from the black plus other stain group were included.

Control Group Selection

The control group was chosen from the children who had no stain upon their teeth. As the ages of the children in each group were already matched, three further criteria were employed to standardize the groups. These criteria were:-

1. D-M-F rate.
2. Simplified Oral Hygiene Index score.
3. P-M-A Index score.

The reasons for limiting the comparison parameters between the two groups to three were twofold.

1. The computer programmers stated that the inclusion of further parameters would provide greater complications.

2. As the above three entities were of most importance in this study it was felt that the inclusion of further subjects for comparison would not be necessary.

As the computer provided more names than were required, a further limiting of numbers accepted from the control group was carried out by using the D-M-F rate, for example:-
If three children with extrinsic black stain had a D-M-F rate of zero then appointments were sent to children from the control group who had a D-M-F rate of zero until three children presented for examination. This system was repeated for the other D-M-F rates until 32 children had been examined.

Methods of Examination

The children were examined at the Glasgow Dental Hospital and School in two consecutive days at weekly intervals. Three children were offered appointments in the mornings only at each weekly session. Examinations were carried out in a dental clinic with the patient seated in a standard dental chair and all examinations were carried out by the author. Standard, pre-sterilized, dental instruments were used during the examination.

Each examination took approximately twenty minutes and included recording the presence, or absence, of coloured extrinsic tooth stain and the distribution of coloured stain when present. The recording of oxygen tension investigations and the collection of gingival scrapings were made at the same visit.

The Collection of Gingival Scrapings

Methods of Collection

Gingival debris was removed, using sterile periodontal scalers and large excavators, from the buccal and lingual or palatal surface of all the posterior permanent teeth present. Material was also removed from the lingual surface of the lower permanent incisors. These areas were selected as almost all the extrinsic black tooth stain was found close to the gingival margin of the tooth crown in these sites. Occasionally the black stain was found in the fissure system of molar and premolar occlusal surfaces and was removed from these teeth. As the black stain material was firmly adherent to the surface of the crown of the tooth, instrumentation in its removal required forceful scraping of the tooth crown. The material thus collected would be a mixture of both gingival debris and the dental plaque as defined by Socransky et al. (1963) who stated:-

> "Gingival debris was considered to be material from the gingival crevice area or periodontal pocket, which was not firmly adherent to the tooth surface. The dental plaque, which was considered to be a non-calcified mass of bacteria tightly adhering to the surface of the clinical crown ..."

However, other workers (Bailit et al., 1964; Wilson C. de Araujo and MacDonald, 1964; Morhart et al., 1970; Mackler and Crawford, 1973) do not define exactly what material was removed.

No attempt was made to isolate the teeth from saliva when the gingival scrapings were obtained as Gibbons et al. (1964) have shown that the highest concentration of Bacteroides melaninogenicus is to be found in the gingival debris and that this site plus the tongue were primary ecological sites of these bacteria.

Nutrient Medium Employed

Prior to removal of the gingival debris one serum bottle containing 0.5 mls. of nutrient medium was weighed to the fourth decimal place on a Mettler Type H16 weighing balance. One serum bottle per patient was used and the nutrient medium into which the gingival scrapings were placed had the following formula:-

Tryptone (Oxoid)	10G
Sodium Chloride	5G
Beef Extract (Lab-Lemco Powder)	3G
Yeast Extract (Difco)	5G
Cysteine Hydrochloride	0.4G
Glucose	2G
Distilled Water	1,000 mls.
pH to	7.2 to 7.4

The above medium was sterilized by autoclaving then a Vitamin K and Haemin mixture sterilized by 0.45 u millipore filtration was added at 10 mls./litre. After collection of the gingival debris the serum bottle was again weighed and the amount of material collected ascertained.

Results

Tables 79 and 80 show the mean weight in grammes and the range of weights of material collected from the gingival scrapings.

Gingival Debris

Mean weight in Gms.

	Black Stain	No Stain	Total
Mean	0.011	0.011	0.011
Standard Deviation	0.007	0.005	0.006
Number of samples	32	32	64

Discussion

As Gibbons et al. (1964) have shown that the

distribution of bacteria in various sites of the oral cavity is different, clear definitions of the site and collection techniques involved would be desirous before comparisons are made with the results of other research workers.

The mean amount of gingival debris collected in this survey was exactly the same for both the control group and the black stain group. It may be tentatively suggested that the parameters employed when selecting the control group have proved to be the correct ones. This is of importance because although Socransky et al. (1963) did not find a significant difference in the mean number of Bacteroides melaninogenicus collected from periodontally diseased and "normal" individuals, they collected much more gingival debris from the former group. They went on to explain the results as:

> "This 'overall proliferation' of the flora of the gingival crevice area ....."

suggesting that greater numbers of bacteria were found in the periodontally diseased group than in the "normal" group. As the actual numbers of bacteria found in the group chosen in this survey are of very great import to the conclusions, the fact that the mean weight of material collected was the same for each group was satisfying.

Socransky et al. (1963) also stated that:

> "..... it was often difficult to remove 10 mg. of bacterial debris from the gingival crevice area of a 'normal' individual ....."

The results shown in Table 79 would agree with that statement.

The choice of the nutrient media used to collect the gingival debris will be discussed in Chapter VII.

CHAPTER VI

CHAPTER VI

OXYGEN TENSION INVESTIGATIONS

Methods of Investigation

Prior to the collection of gingival debris from both the children with extrinsic black stain and children with no tooth stains, readings of the levels of gaseous oxygen in various areas of the mouth were made. A Beckman Oxygen Analyser (Model 777) with a sensor probe was used to record the oxygen concentration in the gas atmosphere in contact with four specific areas of the mouth. These areas were the left and right maxillary and mandibular mucco-buccal fold region adjacent to the molar teeth. The sensor probe was prepared as per the manufacturer's instructions and left for approximately 20 minutes before use whence the reading was calibrated by a setting of approximately 21% with the oxygen probe operating in the room atmosphere.

The probe was recharged after every third child was examined as it was found that the conducting gel became exhausted after several readings. Care was taken to see that the probe end was not occluded by soft tissue or saliva as in the latter case the oxygen in the saliva was rapidly used up and a false reading obtained.

The patients were instructed to close their lips

tightly around the sensor probe and to breathe normally through the nose. After about 30 seconds the probe reached the level of ambient oxygen present in each site and these readings were recorded. The instrument was checked against the level of oxygen in the room before being used for the next child. Readings from all 64 children were obtained and are shown in the results below.

Results

The results are given in four quadrants relating to the readings obtained from the same quadrants of the mouth. Table 81 reproduced below shows the results for the total sample.

Mean Oxygen Tension for the Whole Group in Percentages

Mean	= 2.1	Mean	= 2.1
Standard Deviation	= 0.9	Standard Deviation	= 0.9
Range	= 0.6 to 4.2	Range	= 0.6 to 3.8
Number of readings	= 64	Number of readings	= 64
Mean	= 2.0	Mean	= 2.1
Standard Deviation	= 0.9	Standard Deviation	= 0.9
Range	= 0.6 to 4.2	Range	= 0.6 to 3.7
Number of readings	= 64	Number of readings	= 64

Tables 82, 83 and 84 give the results of oxygen tension

recordings for both the black stain group and the no stain group with statistical analysis (Table 84) showing that the former had significantly lower readings than the latter group ($p < 0.001$).

Discussion

The sensor probe of the Beckman Oxygen Analyser was not designed specifically for use in the mouth and certainly provided difficulties for the children. It would be fair to say that the children who became more adept in the use of, and tolerant of the discomfort caused by, the probe achieved lower readings. However, the average values for both groups were higher than those published by Eskow and Loesche (1971) who recorded readings on average of 0.4 ± 0.1 per cent and 0.3 ± 0.1 per cent in a group of 20 subjects. Certainly readings as low as these were obtained especially in the mouths of children of the black stain group but only if the children were instructed to suck while the sensor probe was in position.

Nonetheless in spite of these difficulties there was a statistically significant difference between the recordings of children from the black stain group and the no stain group. This result is of importance and will be discussed again in Chapter VII when the number of anaerobic bacteria from groups are compared.

However, it is worth stating at this point that the low levels of oxygen found in the mucco-buccal fold areas are likely to favour the establishment of a stable bacterial flora which is tolerant of low levels of oxygen. This would not necessarily exclude the presence of strict anaerobes such as Bacteroides melaninogenicus as facultative organisms could use the small amounts of oxygen entering the sulcus area.

CHAPTER VII

CHAPTER VII

BACTERIOLOGICAL INVESTIGATIONS

Bacteroides melaninogenicus, a member of the genus Bacteroides, was the organism under investigation from the gingival scrapings of both the non-stain group and the group with black tooth stain. A characteristic jet black pigment is produced when this organism is grown on blood agar, a property described by Oliver and Wherry (1921) in the original account of the organism. The production of black pigment by these organisms has been regarded as highly distinctive and useful in separating them from other organisms in mixed cultures (Loesche and Gibbons, 1965). Loesche and Gibbons (1965) confirmed the identity of these organisms in accordance with the findings of Sawyer et al. (1962) who investigated the biochemical characteristics of Bacteroides melaninogenicus. However, the latter group of workers used the technique of Gibbons and MacDonald (1960) to obtain and isolate these organisms.

Gibbons and MacDonald (1960) isolated Bacteroides melaninogenicus from human gingival scrapings stating that after five to seven days incubation:

> "Typical black colonies of the organism growing in association with, or adjacent to, other bacterial colonies were picked and streaked on the above medium to obtain pure cultures".

Thus it appears that the production of a black pigment by these organisms when grown on blood agar is the prime characteristic feature in their recognition as the biochemical investigations are carried out after selection of the black colonies. Tracy (1969) has shown that other members of the genus Bacteroides i.e. Bacteroides fragilis and Bacteroides necrophorus can also produce black pigment on blood agar media stating that:

> "If identification of B. melaninogenicus is attempted on the bases of chromogenesis alone, there may be confusion with other Bacteroides species that are chromogenic in mixed culture".

Tracy (1969) goes on to demonstrate the various chromogenic factors required by the three Bacteroides species studied, and shows that it is only Bacteroides melaninogenicus that does not require Vitamin K, for the production of black pigment. However, due to the volume of work involved in the present study it was not thought feasible to carry out differential media investigations into pigment production for the black colonies obtained on the mixed blood agar media. As no published work is available on the relative numbers of these organisms making up the total number of black colonies from gingival scrapings spread on blood agar media it is impossible to assess the degree of error involved in accepting all black colonies as

PLATE III

Black Colonies on
Blood Agar Enriched
Culture Medium

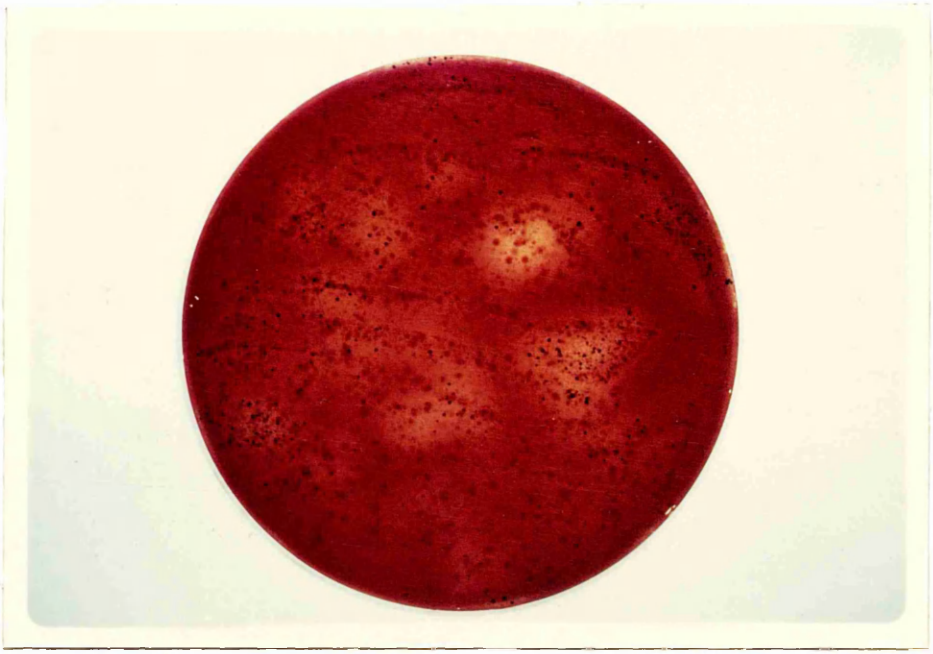
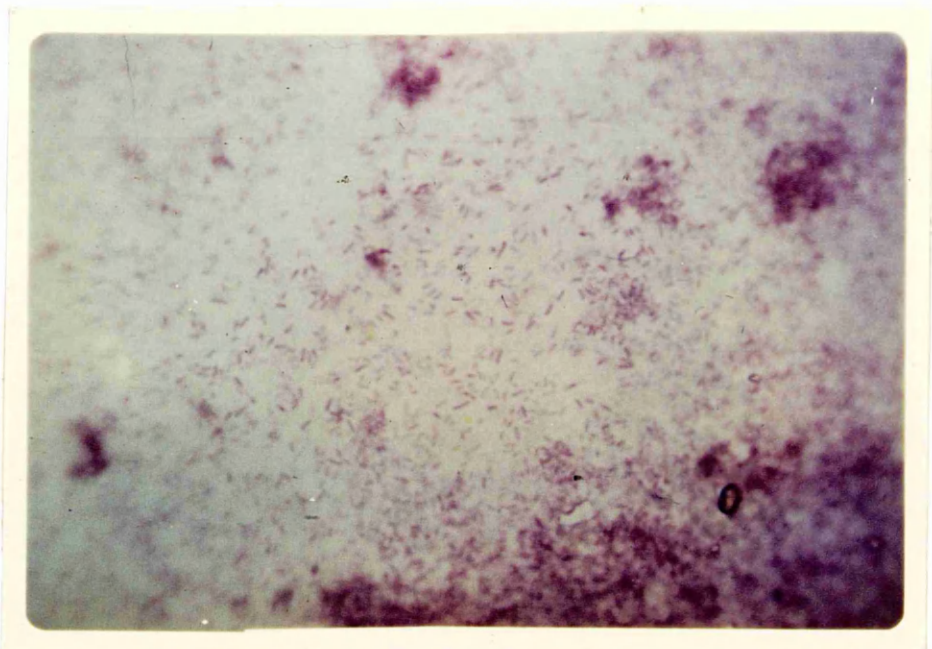


PLATE IV

Gram Stain of a Black
Colony shown in Plate III



Bacteroides melaninogenicus. However, all black colonies which proved to be gram negative coccobaccili on routine gram staining were accepted as Bacteroides melaninogenicus. Once again due to the numbers of black colonies involved it was not thought practical to try and differentiate the organisms on morphological grounds after routine gram staining (MacLennan, 1951). Plate III shows the presence of black colonies on a blood agar media culture plate. Plate IV shows the histological appearance of organisms, stained by grams method, from the colonies shown on Plate III. These two criteria were used in this study to identify the organism Bacteroides melaninogenicus.

Techniques Employed

All laboratory work, such as dilutions, platings, bacteriological counting and gram staining was carried out by the author.

Dispersion

Williams and Eickenberg (1952) stated that:

> "Quantitative estimates of the cultivable micro-organisms in pure cultures, water, milk, foodstuffs and the secretions and excretions of human beings are influenced by the physical arrangement and dispersion of the organisms in the samples".

They demonstrated that sonic vibration was better than mechanical shaking for dispersing bacteria in samples of human saliva.

Sonic oscillation has been used to disperse samples of gingival debris (Socransky et al., 1963), as has mechanical agitation (Morhart et al., 1970). An investigation into the efficiency of the various methods used to disperse gingival scrapings was carried out by Gilmour and Poole (1970). These workers showed that the most efficient method investigated by them was that of homogenization using ten down strokes of a tissue homogenizer.

Homogenization was used for dispersion of the gingival scrapings throughout this study.

The content of the serum bottles containing the liquid media plus gingival scrapings was transferred to a glass tube and using ten down strokes of a teflon covered pestle dispersion was achieved.

Immediately dispersion was completed serial tenfold dilutions were made using the same liquid media as was used for collecting the gingival scrapings from the children.

It was found that the serial dilutions made at 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were most useful for the quantitative investigations which follow. The effect on results of choosing only these dilutions will be discussed later in this Chapter.

Culture Media

The difficulties in growing Bacteroides melaninogenicus in mixed cultures but especially in pure culture

are already well known. It has been reported that human blood (Oliver and Wherry, 1921) and haematin (Evans, 1951) enhances growth as does the presence of other bacteria (Burdon, 1928). Rumen strains were shown to require Vitamin K as a growth factor (Lev, 1958). This latter finding was confirmed and extended to human oral strains (Gibbons and MacDonald, 1960) where 7 of 14 oral strains were shown to require a Vitamin K analogue as a growth factor. Recently, Lev et al. (1971) demonstrated that a rumen strain of Bacteroides melaninogenicus grown on a medium supplemented with both Vitamin K and Heme increased in growth rate when succinate was added.

The medium used for the quantitative analysis of Bacteroides melaninogenicus from the gingival scrapings is given below.

Tryptone	10G
Sodium Chloride	5G
Beef Extract (Lab-Lemco Powder) (Oxoid)	3G
Yeast Extract (Difco)	5G
Cysteine Hydrochloride	0.4G
Glucose	2G
Distilled Water	1,000 c.c.
Agar	12G
Horse Blood (Oxoid)	5 - 10%
pH to	7.2 - 7.4
Vitamin K/Haemin Mixture	10 mls./litre

0.1 mls. from each of the serial dilutions chosen was spread onto the above freshly prepared culture media.

The culture plates were then placed in a jar (Baird and Tatlock Ltd. (Btl.), Chadwell Heath, Essex) and anaerobic conditions achieved by using the Gaspak foil envelope supplied by Becton, Dickinson U.K. Limited. The efficiency of the Gaspak system was shown to be only slightly less than that of a standard technique involving the use of pump metering equipment and gas cylinders (Collee et al., 1972). However, greater efficiency, speed and simplicity were obtained. Two or three jars per week were used depending on the numbers of children who kept their appointments. The jars were then placed in an incubator at $37^{o}C$ for 7 days. After incubation the number of black colonies on each culture plate was noted and sample colonies were checked by routine gram stain. Several of the plates had such a heavy growth of Bacteroides melaninogenicus that it was not possible to ascertain a count. If all 4 culture plates had a countable number of black colonies then for each dilution the number of organisms in the original sample could be obtained. The average of all 4 calculations was worked out and from that the number of organisms per gram wet weight of material could be calculated. This procedure was carried out for both the control group and the black stain group so as comparisons could be made within the survey and with the results of other workers.

Results

Table 85 shows the results for the mean number of colonies counted for both the control group and the black stain group.

Mean Number of Black Colonies per gram wet weight of Gingival Scrapings

	No Stain Group	Black Stain Group
Mean	5.69×10^6	439.95×10^6
Standard Deviation	12.01	1,687.94
Number of Children	32	32

Statistical analysis shows that the black stain group did not have significantly more Bacteroides melaninogenicus organisms than did the no stain group ($t = 1.46$: $p > 0.2$).

As material from 5 patients in the black stain group and 12 in the no stain group did not have any black colonies after incubation, analyses into the comparison between the groups excluding these patients was undertaken. The results are shown in Table 86.

Mean Counts of Black Colonies in Gingival Crevice Material from no stain and black stain groups excluding negative culture plates

	No Stain Group	Black Stain Group
Mean	9.11×10^6	521.46×10^6
Standard Deviation	14.24	1,831.09
Number of Children	20	27

Statistical analysis shows that the black stain group

did not have significantly more black colonies than the no stain group ($t = 1.45$: $p > 0.2$).

The longest time delay between collecting the gingival scrapings and incubation was 4 hours.

Discussion

Although the statistical analysis of the numbers of Bacteroides melaninogenicus found in both groups was not significantly different the black stain group differ on two important points from the control group. Firstly, the average number of colonies counted for the black stain group is much higher than for the no stain group and secondly fewer children from the black stain group had culture plates without black colonies. In fact three of the five black stain cases came from one anaerobic jar and certainly the anaerobic conditions were very suspect. 73% of the children from the total number of 64 had positive cultures while 84% of the black stain group had Bacteroides melaninogenicus present in their mouths. These figures are very similar to those of Bailit et al. (1964) who found that over 80% of thirteen year old children examined by them had positive cultures, whereas Kelstrup (1966) found that the organism was present in only 20% of the thirteen year old children whom he examined.

Comparisons between the mean counts of Bacteroides melaninogenicus found in this survey and published figures from other research workers are detailed below.

Bacteroides Melaninogenicus

Comparison of Viable Counts from Published Results

	Age of Patients in Years	Counts/gm. wet weight
Wilson c. de Araujo and MacDonald, 1964	3 - 7	4×10^5 to 14×10^8
Present Survey	13	5×10^6 to 4×10^8
Socransky et al., 1963	Adults	1.3×10^7 to 5.5×10^9
Dale et al., 1961	Adults	9.5 to 10^8

These results suggest that adults tend to have a greater number of these organisms present in their mouths than do children. The weight of gingival debris collected was greater from patients with periodontal disease than from healthy mouths and this may influence the viable count of these organisms. However, Socransky et al. (1963) published information that it was often difficult to obtain 10 mgms. of gingival debris from healthy mouths. The average weight of gingival debris collected in the present survey was 11 mgms. and part of the reason for the higher viable organism counts found by Socransky et al. (1963) may be due to the inclusion of periodontally involved patients from whom it was fairly easy to obtain 100 mgms. of gingival debris.

The role of Bacteroides melaninogenicus in periodontal disease, mixed anaerobic infection throughout the body and its production of chemical substances has been extensively investigated.

Oliver and Wherry (1921) isolated the organism from infected surgical wounds while Socransky and Gibbons (1965); MacDonald and Gibbons (1962) and MacDonald et al. (1963) showed the importance of the organism in mixed anaerobic infections. An increase in the numbers of organisms isolated from patients with periodontal disease as compared to those with healthy mouths has been shown (Burdon, 1928; Hemmens and Harrison, 1942; Socransky et al., 1963). However, no significant difference in numbers was found nor was there any positive correlation between increased numbers of Bacteroides melaninogenicus and periodontal disease (Socransky et al., 1963; Morhart et al., 1970; Mackler and Crawford, 1973). Results in the present survey support the findings that there appears to be no correlation between the numbers of Bacteroides melaninogenicus present in the mouth and the severity of periodontal disease. The children in the control group did not have a significantly better or worse P-M-A Index and no significant difference was found in the number of organisms obtained from the children in each group. The mean weight of gingival debris was the same from each group although higher average numbers of organisms were obtained from the black stain group. Perhaps as Socransky et al. (1963) stated:

> "This 'overall proliferation' of the flora of the gingival crevice area could occur as a result of periodontal destruction, or it could be a prerequisite for the production of products essential for periodontal breakdown".

Certainly the organism has been shown to possess collagenolytic activity (Gibbons and MacDonald, 1961; Sawyer et al., 1962) while the pathogenic potential of the material in mixed anaerobic infection has been investigated (Kaufman et al., 1972).

Investigations into the chemical characteristics of the endotoxin of this organism have also been carried out (Mergenhagen et al., 1961; Hofstad, 1968 and 1970; Hofstad and Kristoffersen, 1971). A role in the initiation or development of human periodontal disease has been suggested from results using fluorescent antibody techniques (Takeuchi et al., 1970; Courant and Bader, 1966). The weight of evidence proves the importance of Bacteroides melaninogenicus in periodontal disease development but does not yet prove the organism to be the initiator.

CHAPTER VIII

CHAPTER VIII

FERMENTATION REACTIONS

Methods of Investigation

During the quantitative investigations for Bacteroides melaninogenicus grown from the gingival scrapings, black colonies were removed and spread on to fresh culture media in an attempt to obtain the organism in pure culture.

Unfortunately the attempts were unsuccessful until the last stages of the investigation. It was then that it was decided to add succinate to the culture medium so that it became as shown.

Medium Employed

Tryptone	10G
Sodium Chloride	5G
Beef Extract (Lab-Lemco Powder) (Oxoid)	3G
Yeast Extract (Difco)	5G
Cysteine Hydrochloride	0.4G
Glucose	2G
Distilled Water	1,000 mls.
pH to	7.2 to 7.4
Vitamin K/Haemin Mixture	10 mls./litre
Agar	12G
Horse Blood (Oxoid)	5 - 10%
Sodium Succinate	0.025 gms./litre

Using this media 4 oral strains were obtained in pure

culture and it was decided in spite of the small number to carry out fermentation reactions. Further attempts to grow these organisms were unsuccessful in spite of using a nutrient medium. Certainly Loesche et al. (1971) have shown that Kanamycin can aid in the isolation of these organisms from dental plaque and perhaps more strains would have been obtained had Kanamycin been added to the culture medium. Each of the 4 strains was introduced separately by stab inoculation into a culture medium containing the following constituents:-

Cysteine Hydrochloride	0.5G
Tryptone (Oxoid)	10.0G
Peptone	5.0G
Tryptone T	5.0G
Sodium Sulphite	0.5G
Sodium Chloride	5.0G
Agar	7.5G
Distilled Water	1,000 mls.
pH to	7.2
Phenol Red	50 mls.
Rabbit Serum	0.3%
Vitamin K/Haemin Mixture	10%

To this medium was added a sugar either glucose, sucrose or lactose to 0.75% of the final concentration.

The bacteria were added by stab inoculation and the universal containers were then incubated anaerobically using the Gaspak foil envelope inside a Baird and Tatlock Limited jar.

Some of the bacteria were spread onto fresh culture plates containing the routine medium and incubated with the sugar fermentation jars. This was to ensure that the bacteria were still viable.

Results

After 7 days incubation at 37°C no signs of fermentation had occurred in any of the sugars inoculated by the 4 oral strains under investigation. However, no growth had occurred on the routine material either.

Discussion

Difficulties in growing Bacteroides melaninogenicus in pure culture have presented problems in fermentation studies. That the organism does ferment certain carbohydrates has already been shown (Oliver and Wherry, 1921; Sawyer et al., 1962). Sawyer et al. (1962) also found that some oral strains were completely nonfermentative and this may have been the case with the 4 oral strains used in the present survey. However, due to lack of growth of these organisms on a routine culture medium it was felt that the organisms were nonviable rather than nonfermentative.

The importance of this investigation was that Sawyer et al. (1962) had shown that when these organisms fermented sugars in only three cases did the pH fall below 5.0.

Otherwise the pH ranged between 5.0 and 6.5. This is very important as the "Acidogenic Theory" of dental caries postulates that the initial attack on the tooth is decalcification of the inorganic portion caused by acids resulting from the action of micro-organisms on refined carbohydrates. The pH necessary to cause demineralization has been reviewed by Jenkins (1961) who pointed out the dangers in the idea of a "critical pH". Nonetheless the importance of acid production in the caries process was stressed (Jenkins, 1961). Further investigations will be required before the role of Bacteroides melaninogenicus in the caries progress can be understood as merely the organism's ability to ferment carbohydrates while maintaining the pH at 5.0 or above is not the total answer. Jenkins (1961) stated:

> "It is important to emphasise that enamel will dissolve, at least slightly, at any pH in water not saturated with calcium or phosphate ions, but only in saliva (or other solution), which is saturated above the critical pH is it necessary for the pH to fall below 5.5 before enamel can be dissolved".

The ability of Bacteroides melaninogenicus to ferment carbohydrates while maintaining the pH at 5.0 or above may partly explain the results obtained by James (1963) which have already been mentioned in Chapter IV. A combination of the protective action of fluoride on enamel and a higher pH value due to

Bacteroides melaninogenicus may explain the low D-M-F rate of the children in the high fluoride area (James, 1963).

CHAPTER IX

CHAPTER IX

INORGANIC CHEMICAL ANALYSIS

The black pigment produced by Bacteroides melaninogenicus was believed to be melanin (Oliver and Wherry, 1921). However, Schwabacher et al. (1947) investigated the nature of the pigment and identified it as a:-

> "..... haematin (not melanin) united in the cells with a bacterial protein to form a parahaematin".

The differences in the nature of the pigment suggested by both the above researchers may be due to the fact that the latter group of workers stated the pigment to be an intracellular material whereas the former stated:-

> "The pigment occurs as extracellular, amorphous masses, and apparently is a melanin".

Tracy (1969) further investigated the black pigment and found it to be extracellular and emphasised what was stated above that Schwabacher et al. (1947) investigated an intracellular material. The results given by Tracy (1969) showed the pigment to be ferrous sulphide.

It was decided to repeat, in modified form, the in vitro pigment investigations of Tracy (1969) and to chemically analyse the black pigment from the mouths of several children examined in this survey.

Collection of Gingival Scrapings

Gingival debris was collected and placed into nutrient broth as described in Chapter V. This material was to be used to grow Bacteroides melaninogenicus on

nutrient agar whence the pigment could be harvested and chemically analysed. This method was chosen in preference to using strains obtainable from the National Collection of Type Cultures. The results would still be comparable with those of Tracy (1969) and would prove valuable in comparing results using stock strains with those fresh from the mouth. Further samples were collected from both the black stain group and non-stain group, put into dry containers and stored in a deep freeze until they were required for chemical analysis.

Chemical Analysis of Gingival Debris

Samples of gingival debris from both the black stain group and the control group were subjected to analysis for iron and sulphide, the tests being performed according to the methods described in Spot Tests in Inorganic Analysis (Fiegl, 1958). The relevant tests are detailed below.

Test for Sulphide

Principle

Sulphides (and also thiosulphates and thiocyanates) catalyse the reaction between sodium azide and iodine:-

$$2NaN_3 + I_2 \longrightarrow 2NaI + 3N_2$$

This test is very sensitive for small samples and was

of particular value as under 10 mgms. of gingival debris were available for investigation.

Reagent

3% W/V sodium azide in 0.1 N-iodine.

Method

Place the solution in a microcentrifuge or capillary tube and invert the tube. The sample is introduced on the tip of a platinum wire whence in the presence of sulphides there is a rapid evolution of gas.

Results

Samples from the black stain group caused rapid evolution of gas, whereas samples from the control group caused a less vigorous evolution of gas and samples of saliva did not produce gas.

Conclusion

The samples contained sulphide, thiocyanate or thiosulphate.

Nitroprusside Test for Sulphides

Principle

The yellow alkaline solution of sodium nitroprusside, $Na_2[Fe(CN)_5NO]$, gives a red-violet colour

with soluble sulphides. The coloured product with sodium sulphide has the empirical formula $Na_4[Fe(CN)_5NOS]$. The colour is discharged on the addition of acid.

Reagent

1% (W/V) sodium nitroprusside.

Method

The gingival debris was macerated on a spotting tile in N-NaOH, and a drop of sodium nitroprusside was then added.

Limit of Sensitivity

1 γ sodium sulphide.

Results

There was no sign of any red-violet colour.

Conclusions

Although the results of this test were negative, it does not mean that sulphide was absent as this test detects only suluble sulphides and many sulphides are insoluble.

Thiosulphates and Thiocyanates

The reaction between sodium azide and iodine is catalysed by not only sulphides, as already shown, but

also by thiocyanates and thiosulphates. Tests which are specific for thiosulphates involve prior precipitation of sulphides as mercuric salts. Unfortunately in the case of substances like gingival debris and dental plaque this procedure is not technically possible. However, it is possible to test specifically for thiocyanates in the presence of sulphides.

Test for Thiocyanates with Cobalt Salts

Principle

Cobalt salts give a blue solution of $K_2[Co(CNS)_4]$ with thiocyanates in the presence of acetone.

Reagent

1% cobalt sulphate.

Method

A drop of the sample is placed in a vitreosil crucible, mixed with one drop of 1% cobalt sulphate and evaporated to dryness. The residue is violet. The colour fades on the addition of a few drops of acetone if no thiocyanate is present, whereas the acetone becomes blue-green in the presence of thiocyanates.

Results

Gingival debris samples from both the control group and the black stain group gave a blue-black colour on acetone treatment.

Conclusions

The results would suggest the presence of small amounts of thiocyanate but this may be salivary in origin.

Test for Ferrous Iron

Ferrous salts in solutions of mineral acids react with α,α'-bipyridyl to give a deep red complex. The limit of identification being 0.03 ug iron.

Reagent

2% (W/V) α,α'-bipyridyl in alcohol.

Method

Approximately 1 mg. of gingival debris was macerated on a spotting tile in one drop of 0.1 N or 5N - Hydrochloric acid and one drop of bipyridyl reagent added.

Result

No pink colour was formed.

Conclusions

The presence of ferrous iron was not indicated, however oxidation to ferric iron may have occurred or the content of iron be too low to detect by this test or the iron may have been very insoluble.

Test for Ferric Iron

In the presence of thioglycolic acid, ferric iron is reduced almost instantly to ferrous iron which can then be detected by the above test using 2% α,α'-bipyridyl in thioglycolic acid.

Results

A faint pink colour was formed.

Conclusions

A sample of gingival debris from the black stain group contained traces of ferric iron whereas the gingival debris from the non-stain group did not give this reaction.

From samples of gingival debris Bacteroides melaninogenicus were grown in pure culture using techniques and media already illustrated in Chapter V.

Studies on the Supernatant Samples

The black colonies were scraped from the blood agar plates and put into distilled water. The sample was then centrifuged at 3,000 r.p.m. for 20 minutes, the supernatant being retained. This process was repeated three times (Tracy, 1969) whence the supernatant was placed in a boiling water bath for 1 hour. Unfortunately the pigment did not coagulate and further centrifugation did not allow harvesting. However, two samples were sent for chemical test for iron and sulphide

and were stored in a deep freeze until studied.

Tests for Sulphides

The sodium nitroprusside test was negative for both samples while the iodine: sodium azide test produced a few tiny bubbles from both samples.

Tests for Iron

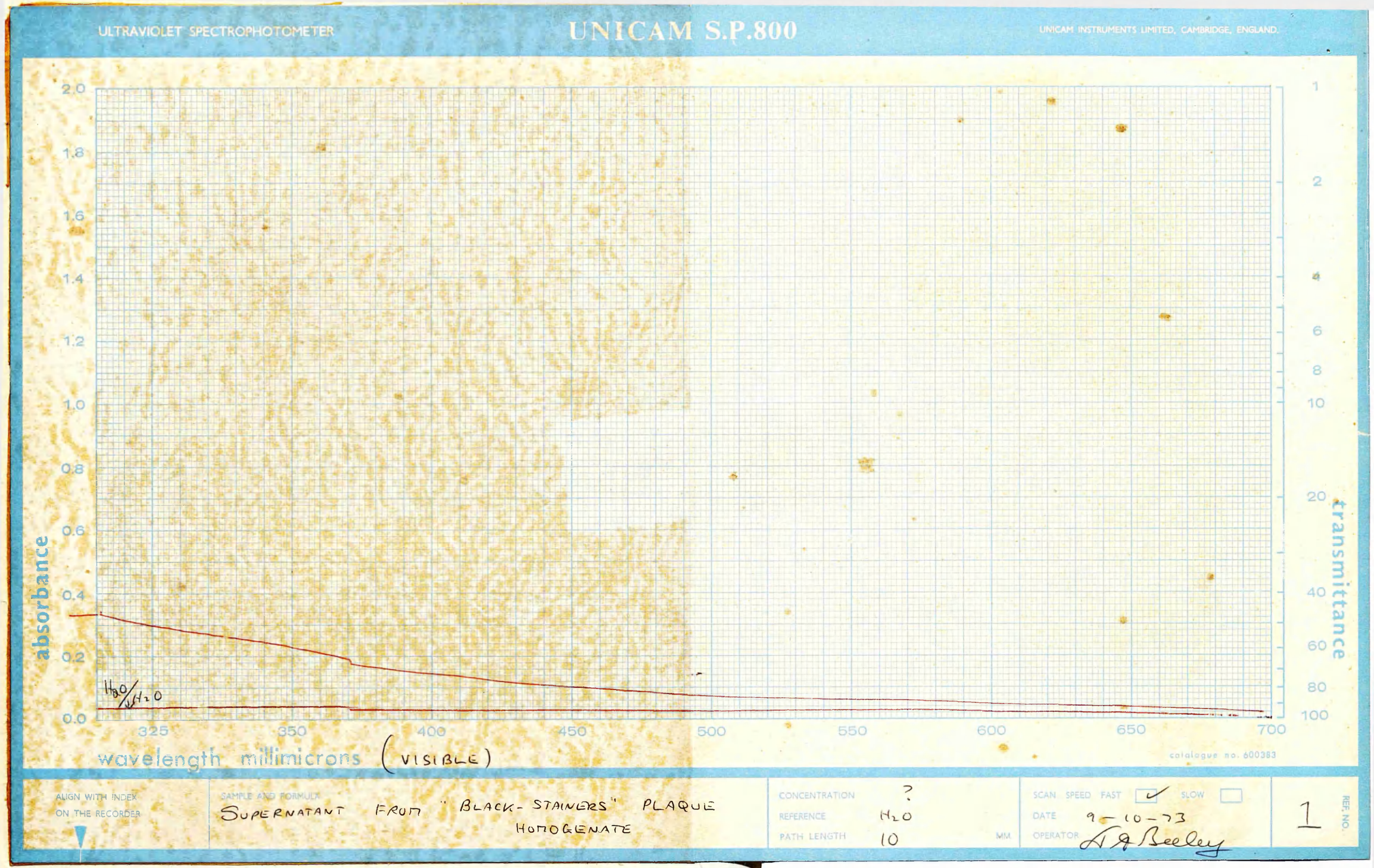
The α,α'-bipyridyl in alcohol test for ferrous iron was negative for both samples. The α,α'-bipyridyl in thioglycolic acid test for ferric iron produced a feint pink colour, indicating the presence of small amounts of ferric iron on both samples.

Test for Thiocyanates with Cobalt Salts

A black residue was formed in contrast to the blue-black residue formed from the tests on gingival debris. However, the black residue was due to charring of the sample. The presence of thiocyanate was not indicated.

Conclusions

The results could have occurred because the two solutions examined were too dilute. A further possibility for consideration was that the solutions might contain an iron sulphur protein (e.g. ferredoxin or rubredoxin) as the iron contained therin would be expected to give a ferrous/ferric iron reaction. However, in the case of rubredoxin none of the sulphur is acid labile and this compound would not be expected

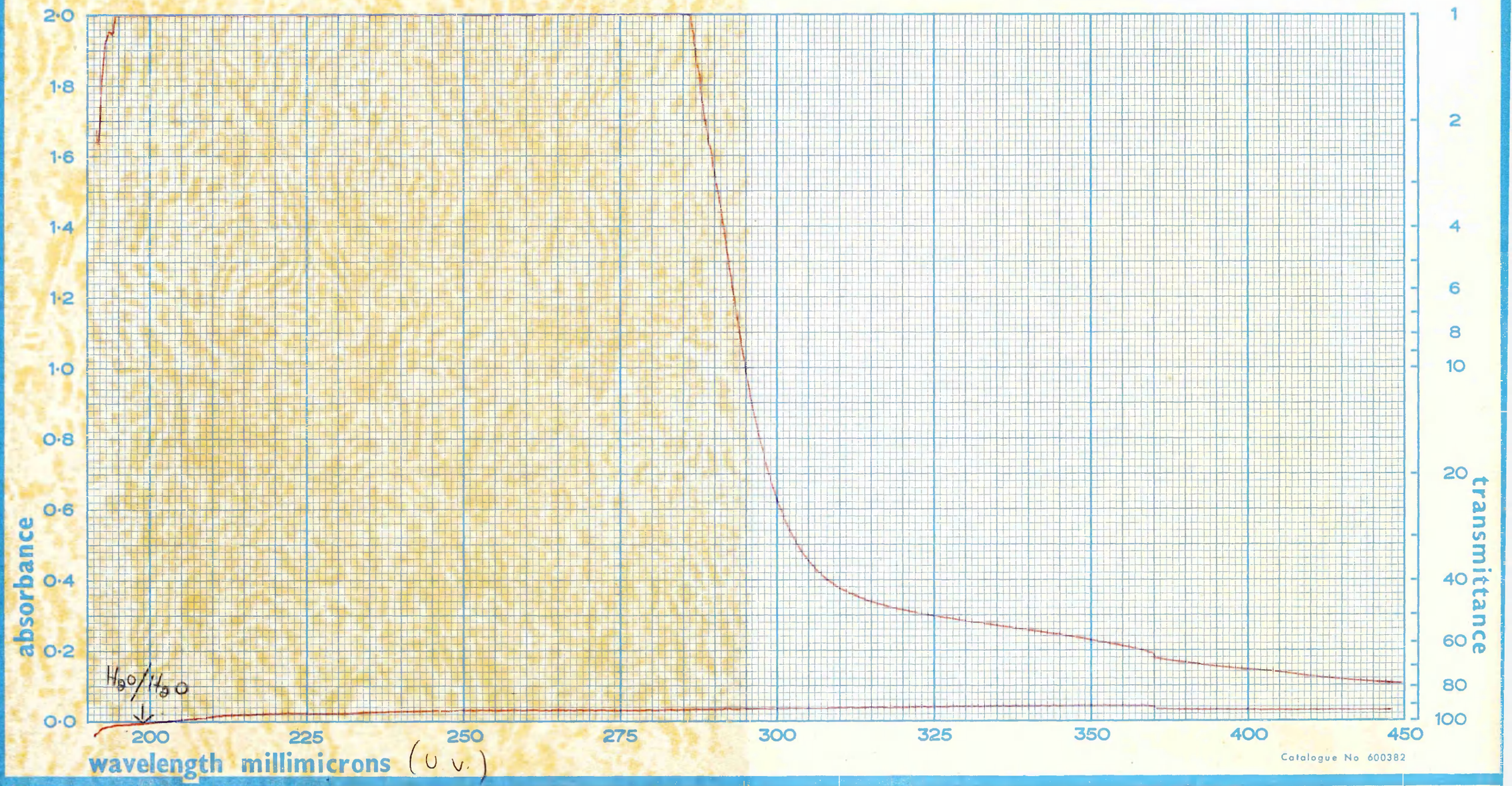

ULTRAVIOLET SPECTROPHOTOMETER
UNICAM S.P.800
UNICAM INSTRUMENTS LIMITED, CAMBRIDGE, ENGLAND.
absorbance
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
transmittance
1
2
4
6
8
10
20
40
60
80
100
325
350
400
450
500
550
600
650
700
wavelength millimicrons (VISIBLE)
catalogue no. 600383
H2O/H2O
ALIGN WITH INDEX ON THE RECORDER
SAMPLE AND FORMULA
SUPERNATANT FROM "BLACK-STAINERS" PLAQUE HOMOGENATE
CONCENTRATION ?
REFERENCE H2O
PATH LENGTH 10 MM
SCAN SPEED FAST ✓ SLOW
DATE 9-10-73
OPERATOR A R Beeley
1
REF. NO.

Spectra 1

ULTRAVIOLET SPECTROPHOTOMETER

UNICAM SP.800

UNICAM INSTRUMENTS LIMITED, CAMBRIDGE, ENGLAND.



Catalogue No 600382

ALIGN WITH INDEX ON THE RECORDER

SAMPLE AND FORMULA: SUPERNATANT FROM "BLACK-STAINERS" PLAQUE HOMOGENATE.

CONCENTRATION: ?

REFERENCE: H_2O

PATH LENGTH: 10 MM.

SCANSPEED FAST ☑ SLOW ☐

DATE: 9 – 10 – 73

OPERATOR: A A Beeley

REF. NO. 2.

Spectra 2

to give a sulphide reaction as the sulphur is present as cysteine. Furthermore the fact that it was possible to coagulate the pigment after boiling (Tracy, 1969) suggested that protein may be involved. It was therefore decided to carry out preliminary investigations into the possibility that the black pigment from both the mouths of the black stain group and from the supernatant recovered from bacteriological procedures contained iron-sulphur proteins. Spectral examinations were carried out using a Unicam SP 800 spectrophotomer.

Spectral Studies on the Gingival Debris from the Black Stain Group

Gingival debris (13 mgms. wet/weight) was homogenised in 1 ml. of ice-cold water, using an all glass hand micro-homogeniser. The homogenate was then centrifuged at 12,000 r.p.m. for 20 minutes to give a colourless supernatant and a dark grey precipitate. The supernatant was then subjected to spectral examination.

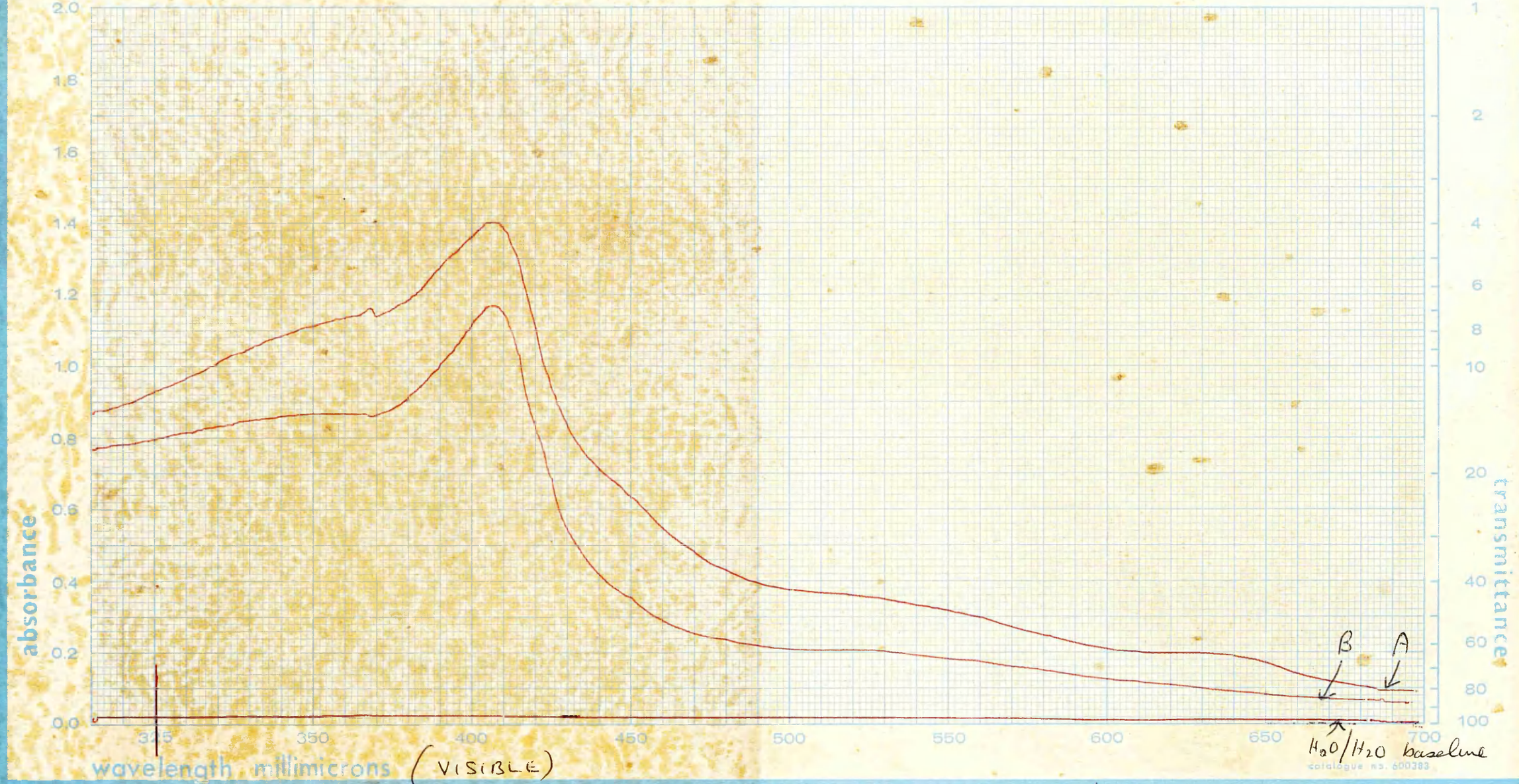
Results

Spectral examination did not indicate the presence of significant amounts of either iron-sulphur proteins or haem derivatives (Spectra 1 and 2). However, as the black pigment was insoluble in water it is hoped to carry out further spectral examinations once a satisfactory technique for dissolving the black stain has been found.

ULTRAVIOLET SPECTROPHOTOMETER

UNICAM S.P.800

UNICAM INSTRUMENTS LIMITED, CAMBRIDGE, ENGLAND.



ALIGN WITH INDEX ON THE RECORDER

SAMPLE AND FORMULA: A. Reed: Samples 'A' & 'B' from Bacteroides Melaninogenicus

CONCENTRATION: A: 0.23 mg/ml; B: 0.28 mg/ml

REFERENCE: H_2O

PATH LENGTH: 10 MM.

SCAN SPEED FAST ☐ SLOW ✓

DATE 1 — 10 — 73

OPERATOR A. M. Bailey

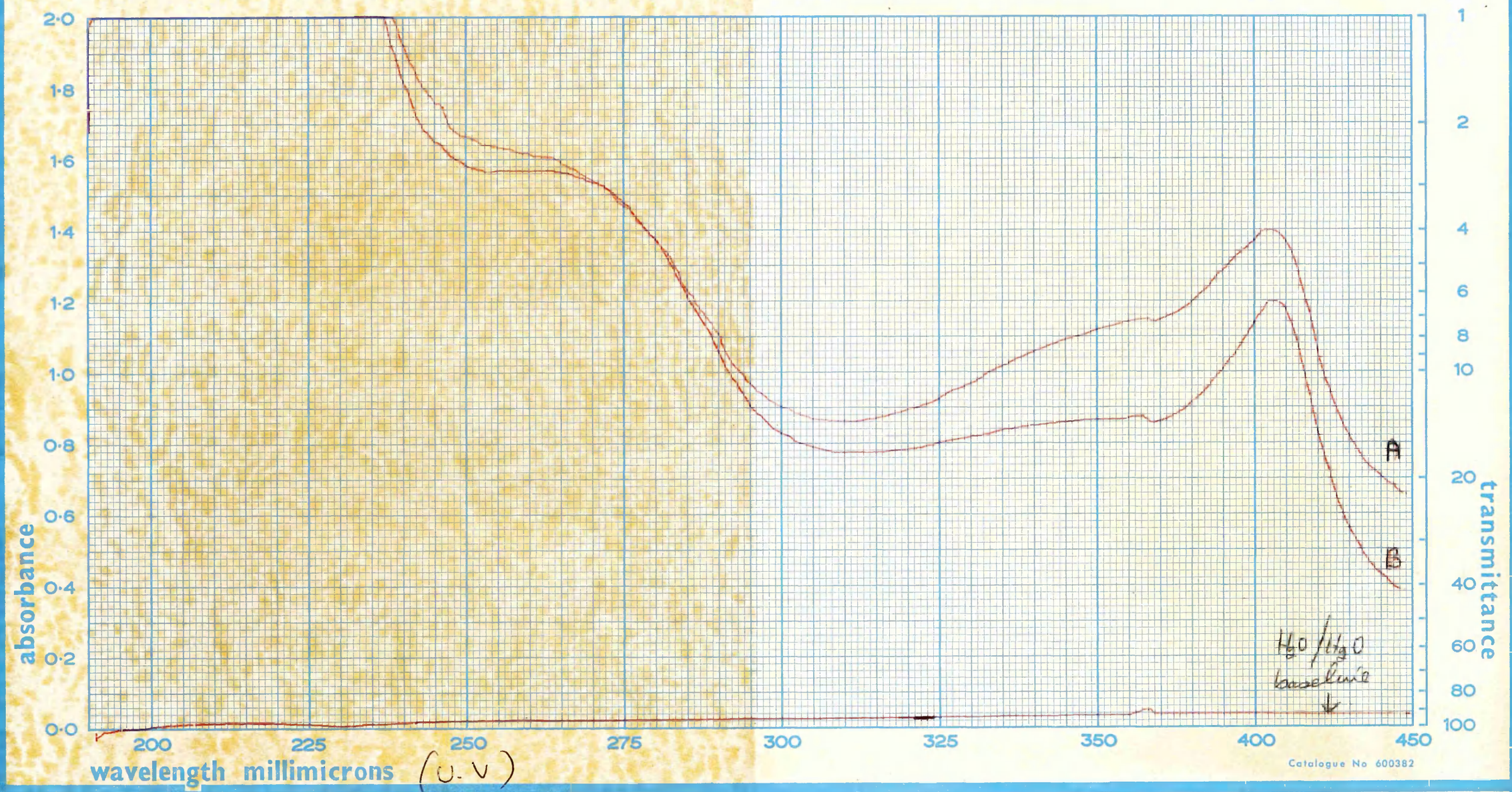
REF. NO. 3

Spectra 3

ULTRAVIOLET SPECTROPHOTOMETER

UNICAM SP.800

UNICAM INSTRUMENTS LIMITED, CAMBRIDGE, ENGLAND.



ALIGN WITH INDEX ON THE RECORDER

SAMPLE AND FORMULA J. Reid: Samples 'A' & 'B' from Bacteroides melaninogenicus

CONCENTRATION A: 0·23 mg/ml
B: 0·28 mg/ml

REFERENCE H_2O

PATH LENGTH 10 MM.

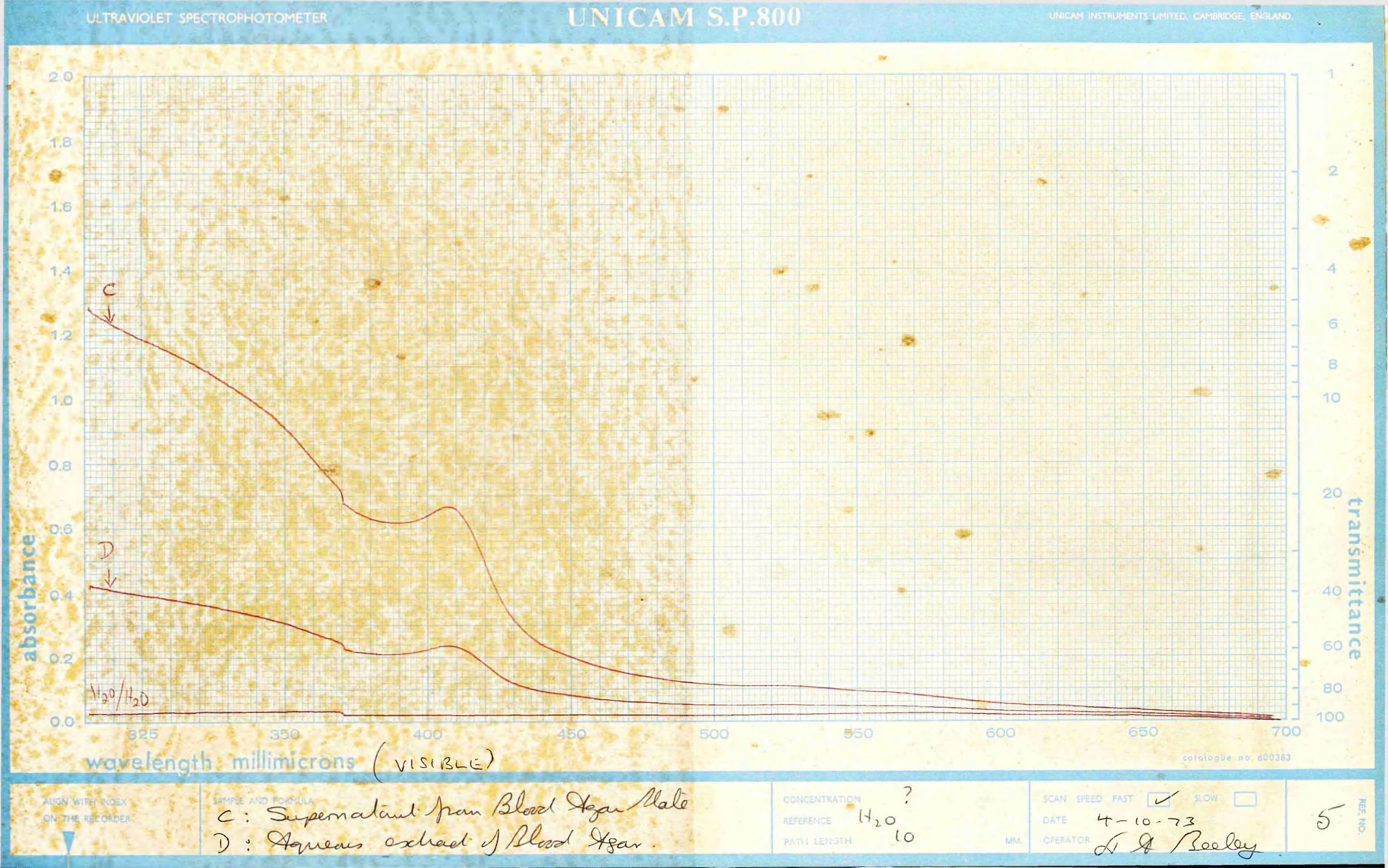
SCANSPEED FAST ☐ SLOW ☑

DATE 1 - 10 - 73

OPERATOR J A Beeley

REF. NO. 4

Spectra 4

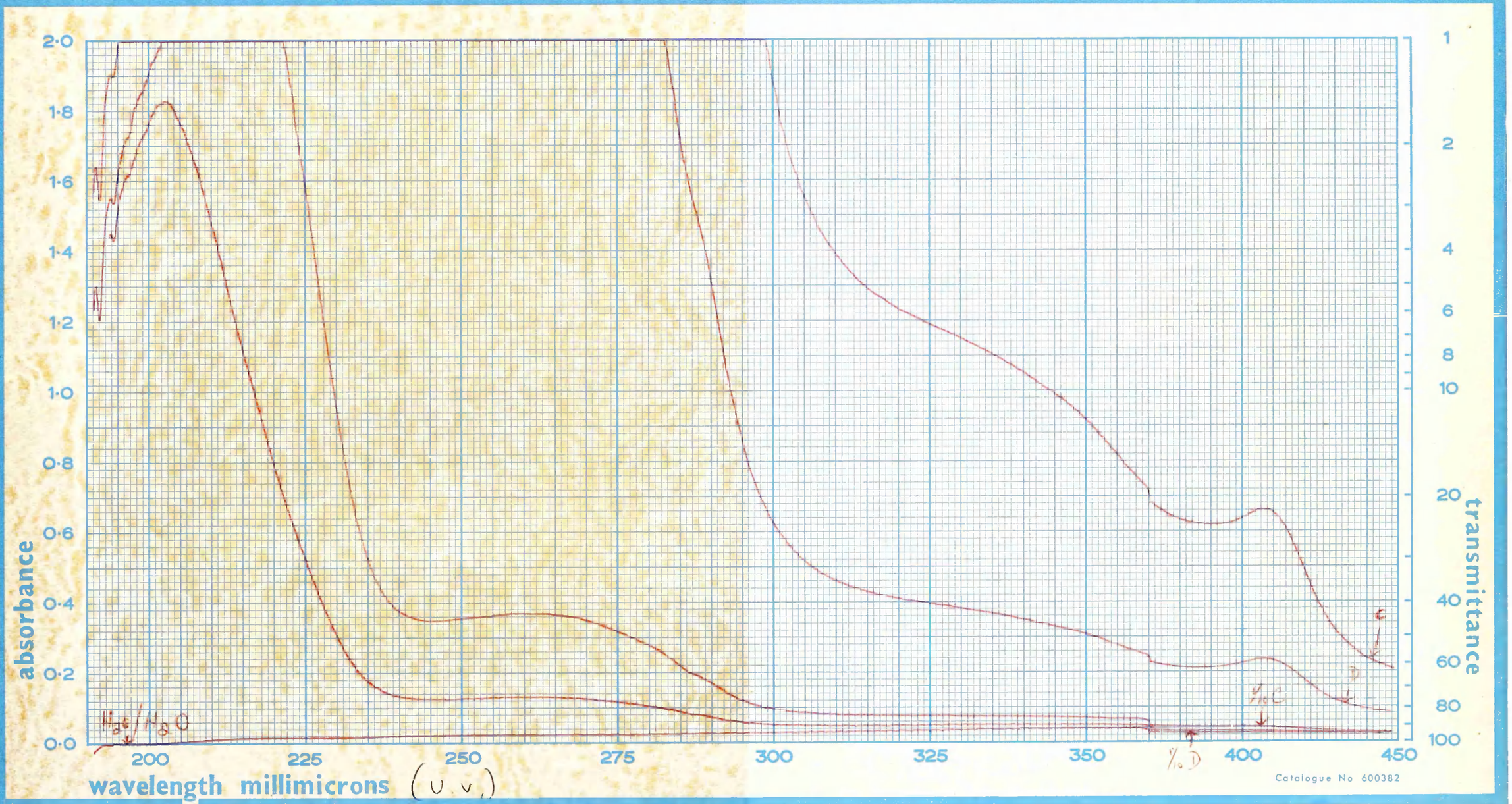

ULTRAVIOLET SPECTROPHOTOMETER
UNICAM S.P.800
UNICAM INSTRUMENTS LIMITED, CAMBRIDGE, ENGLAND.
absorbance
transmittance
wavelength millimicrons (VISIBLE)
C
D
H2O/H2O
catalogue no. 600383
ALIGN WITH INDEX ON THE RECORDER
SAMPLE AND FORMULA
C: Supernatant from Blood Agar Plate
D: Aqueous extract of Blood Agar.
CONCENTRATION ?
REFERENCE H2O
PATH LENGTH 10 MM.
SCAN SPEED FAST SLOW
DATE 4-10-73
OPERATOR L A Bailey
REF. NO. 5

Spectra 5

ULTRAVIOLET SPECTROPHOTOMETER

UNICAM SP.800

UNICAM INSTRUMENTS LIMITED, CAMBRIDGE, ENGLAND.



ALIGN WITH INDEX ON THE RECORDER

SAMPLE AND FORMULA
C : Supernatant from Blood Agar Plate.
D : Aqueous extract of Blood Agar.

CONCENTRATION ?
REFERENCE H_2O
PATH LENGTH 10 MM.

SCANSPEED FAST ☑ SLOW ☐
DATE 4-10-73.
OPERATOR N. A Bailey.

6 REF. NO.

Spectra 6

Spectral Studies on the Supernatant Liquid from the Bacteriological Investigations

Both solutions examined had peaks at 406 nm. (Spectra 3 and 4) which suggests the presence of a haem compound (e.g. Methaemoglobin, oxidized cytochromes) rather than an iron-sulphur protein. However, due to the method adopted for collecting the pigment from the culture plates it is most likely that the material came from the blood agar. To investigate whether or not the haem compound came from the blood agar medium or from the sample investigated, rinsings were taken from the surface of a blood agar culture plate and examined along with a sample of blood agar which had been macerated in water. Both samples were boiled whence the solution coagulated and turned brown. The precipitate was removed by centrifugation at 12,000 r.p.m. for 20 minutes and spectra were run on both samples (Spectra 5 and 6).

Both samples had a peak at about 407 nm. indicative of ferric ions in a haem ring. It also seems most likely that the results obtained on the supernatant mentioned previously were largely due to contamination of the supernatant with haem compound from the blood agar medium.

Conclusions

The spectral studies on the supernatant of an homogenate of gingival debris from the black stain group indicated that significant amounts of iron-sulphur

proteins or haem compounds were not present. The same was true for the supernatant from the microbiological culture plate scrapings. However, it is hoped to carry out further investigations into the black pigment in gingival scrapings once the problem of dissolving the material has been solved.

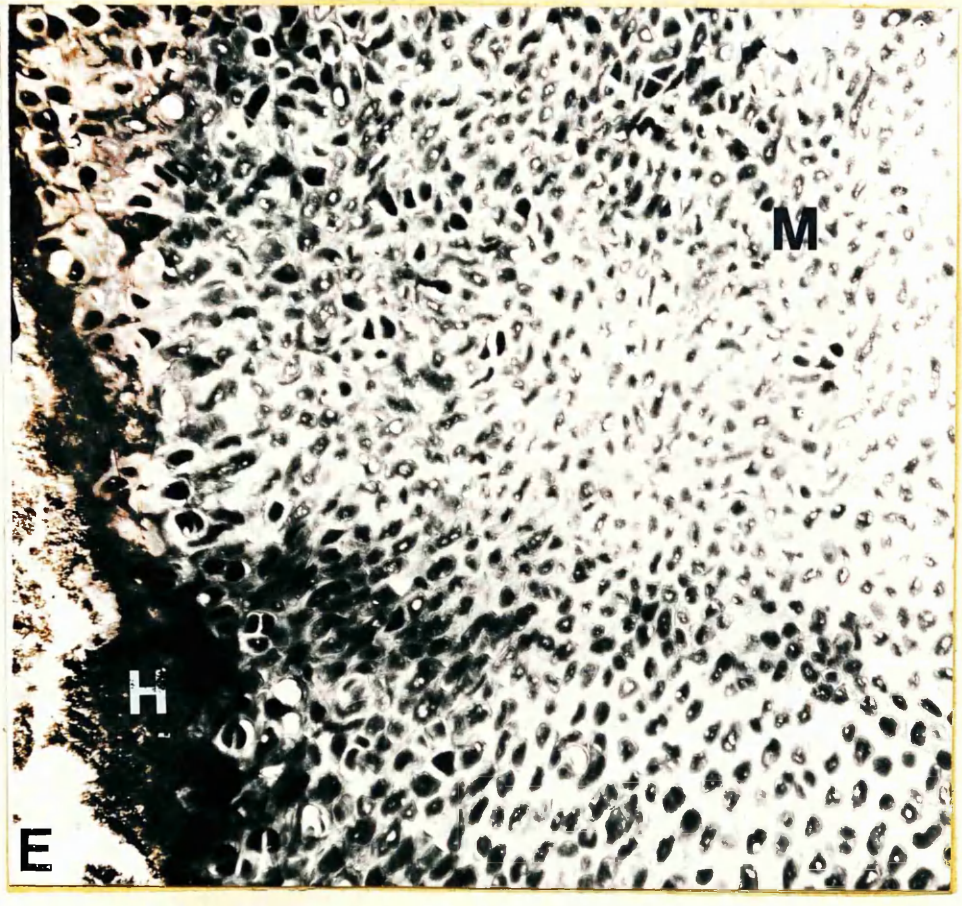
A few extracted teeth with extrinsic black stain upon them were obtained. These teeth were then prepared for examination using an electron microscope.

Method of Preparation

The teeth were fixed in 10% neutral buffered formalin and then placed in 0.1 M EDTA in 10% buffered formalin at pH 7.3. The solution was changed daily and the temperature kept at $4^{o}C$. Decalcification was obtained after 7 days using this method, whence under a dissecting microscope the pigmented area and superficial enamel was dissected off with a sharp scalpel. The material removed in this way was washed in phosphate buffer (pH 7.2) and post fixed for 30 minutes in 1% osmium tetroxide in phosphate buffer (pH 7.2) before routine processing in araldite.

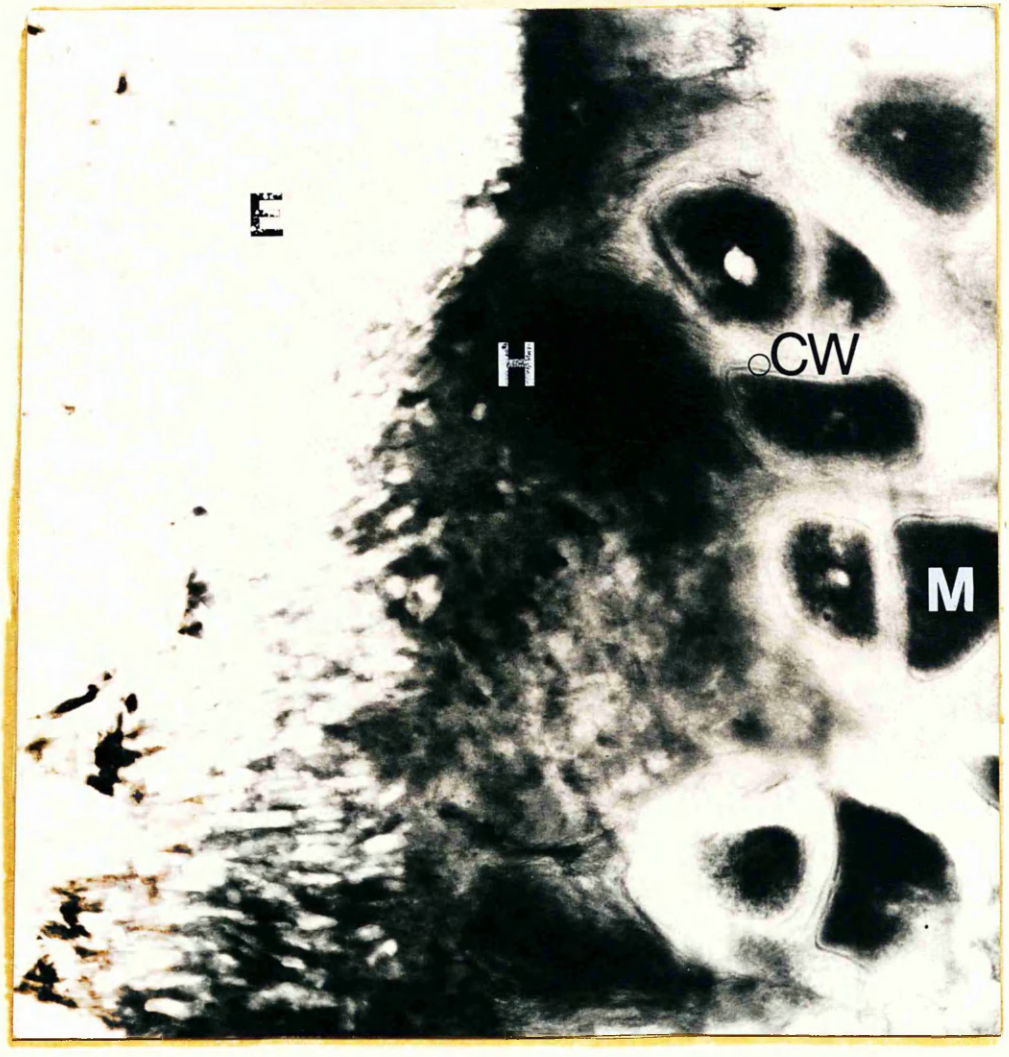
Thin sections were then prepared on a Cambridge-Huxley ultramicrotome and mounted on formvar and carbon coated grids. These sections were then stained with a saturated solution of uranyl acetate for 20 minutes and lead citrate for 5 minutes (Reynolds, 1963).

PLATE V



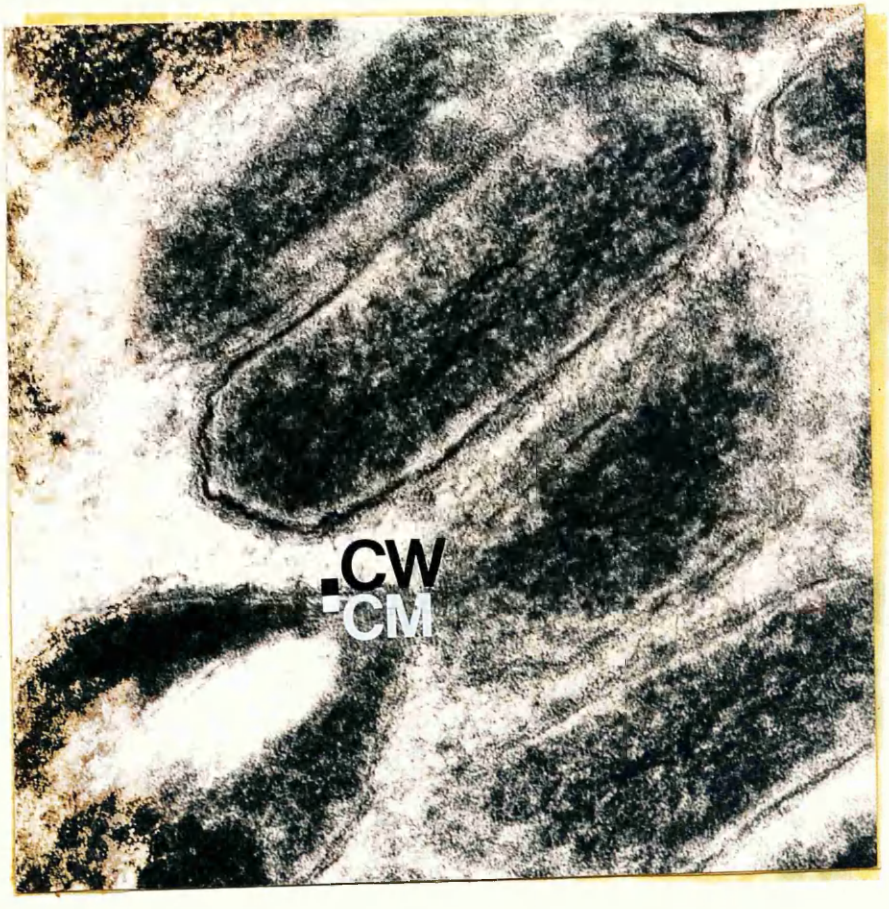
Dense aggregation of micro-organisms (M) forming a layer covering the enamel surface (E). Cocco-bacilli organisms within the bulk of the plaque are loosely packed. Homogeneous cell-free material (H) is interposed between the microbial layer and the enamel surface. Specimen stained with uranyl acetate and lead citrate. Magnification 5,600.

PLATE VI



Aggregation of gram negative cocco-bacilli micro-organisms (M) adjacent to homogeneous, cell-free material (H) interposed between the organisms and the enamel surface (E). A cell wall (CW) is visible. Specimen stained with uranyl acetate and lead citrate. Magnification 46,700.

PLATE VII



Aggregation of gram negative cocco-bacilli micro-organisms. A cell wall (CW) and a cytoplasmic membrane (CM) are visible. Specimen stained with uranyl acetate and lead citrate. Magnification 112,500.

The sections were examined on an AEI EM6B electron microscope.

Results

Plate V shows a dense aggregate of micro-organisms forming a layer covering the enamel. The dark, homogeneous, material which is interposed between the microbial layer and the enamel, is thought to be the extrinsic black tooth stain material. Plate VI which illustrates the same result but at a higher magnification shows the micro-organisms to be cocco-bacilli and in very close proximity to the cell-free, dark material on the surface of the enamel. A cell wall can be seen but cannot be differentiated into layers. No cytoplasmic membrane is visible. Plate VII shows a higher magnification of the organisms seen on Plate VI and demonstrates the presence of a cell wall and cytoplasmic membrane. However, neither cytoplasmic membranous structures nor small structures, referred to as bridges, running between the cell wall and cytoplasmic membrane, can be seen. Attempts to obtain yet higher magnification of the decalcified sections proved fruitless and perhaps the fixation and decalcifying techniques will need to be modified before this end is achieved. These organisms were shown to be gram negative cocco-bacilli.

A preliminary investigation into the ultrastructural morphology of a pure strain of Bacteroides melaninogenicus was undertaken with two main objectives in mind:-

1. To try to discover if these organisms have any characteristic features at the ultrastructural level.

2. To compare the ultrastructural morphology of the organisms grown from a known pure culture with the organisms shown on Plate VII.

A strain of Bacteroides melaninogenicus (N.C.T.C. 9338) was obtained from the National Collection of Type Cultures. The organism was grown on a nutrient medium under anaerobic conditions using techniques already described in Chapter VII and then prepared for examination using the electron microscope.

Method of Preparation

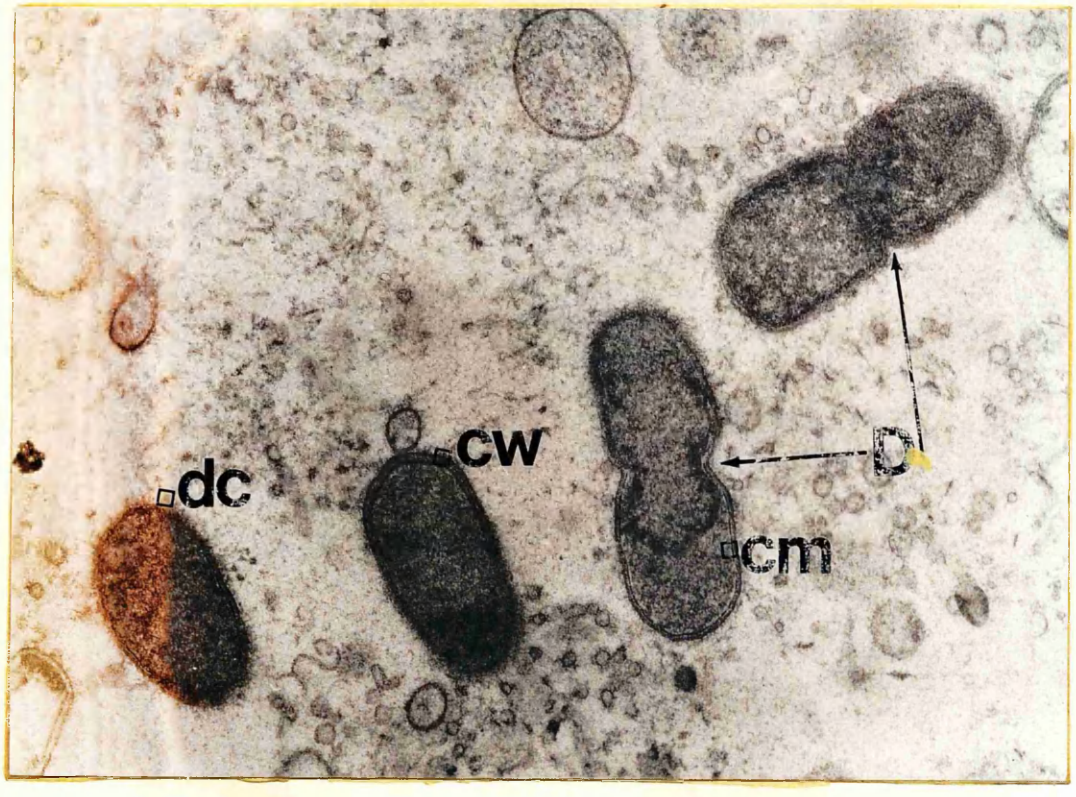
The black colonies were removed from the medium and placed in 1.3% glutaraldehyde in phosphate buffer at pH 7.2 for 2 hours. The osmotic pressure throughout was 348 milliosmoles.

The material was removed and post fixed for 30 minutes in osmium tetroxide in phosphate buffer (pH 7.2) before routine processing in araldite.

A Cambridge-Huxley ultramicrotome was used to prepare thin sections which were then mounted on formvar and carbon coated grids. These sections were then stained with a saturated solution of uranyl acetate for 20 minutes and lead citrate for 5 minutes (Reynolds, 1963).

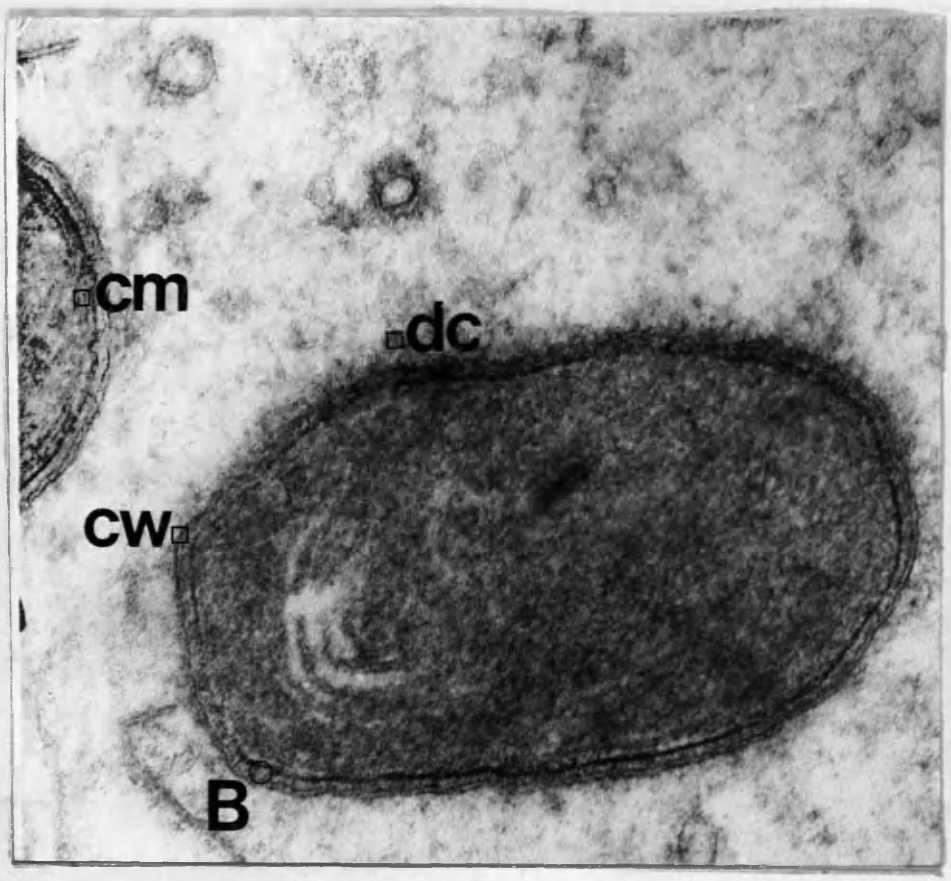
The sections were examined on an AEI EM6B electron microscope.

PLATE VIII



Aggregation of several Bacteroides melaninogenicus organisms (B), with two showing signs of cell division (D). A dense crust (DC) can be seen around the periphery of the cell. Both the cell wall (CW) and the cytoplasmic membrane (CM) are visible. Specimen stained with uranyl acetate and lead citrate. Magnification 50,000.

PLATE IX



BACTEROIDES MELANINOGENICUS

Cell wall (CW) seen as a triple layered structure separated by a space from the cytoplasmic membrane (CM) which appears to be triple layered; the outer dense layer of the cytoplasmic membrane can be seen in other areas as a double structure. Bridges (B) connecting the cell wall to the cytoplasmic membrane are visible. Specimen stained with uranyl acetate and lead citrate. Magnification 150,000.

Results

Plate VIII shows an aggregate of several Bacteroides melaninogenicus organisms two of which are at different stages of cell division. The appearance of these organisms at cell division would lend support to the method of cytokinesis suggested by Bladen and Waters (1963). These workers stated that during cytokinesis a centripetal growth of the cell wall-cytoplasmic membrane complex took place and terminated in complete fission of the organism. Cell-plate or transverse-septum formation involving inward growth of the cytoplasmic membrane ahead of the inward growth of the cell wall did not occur. Examination of the dividing organisms shown on Plate VIII support this latter statement.

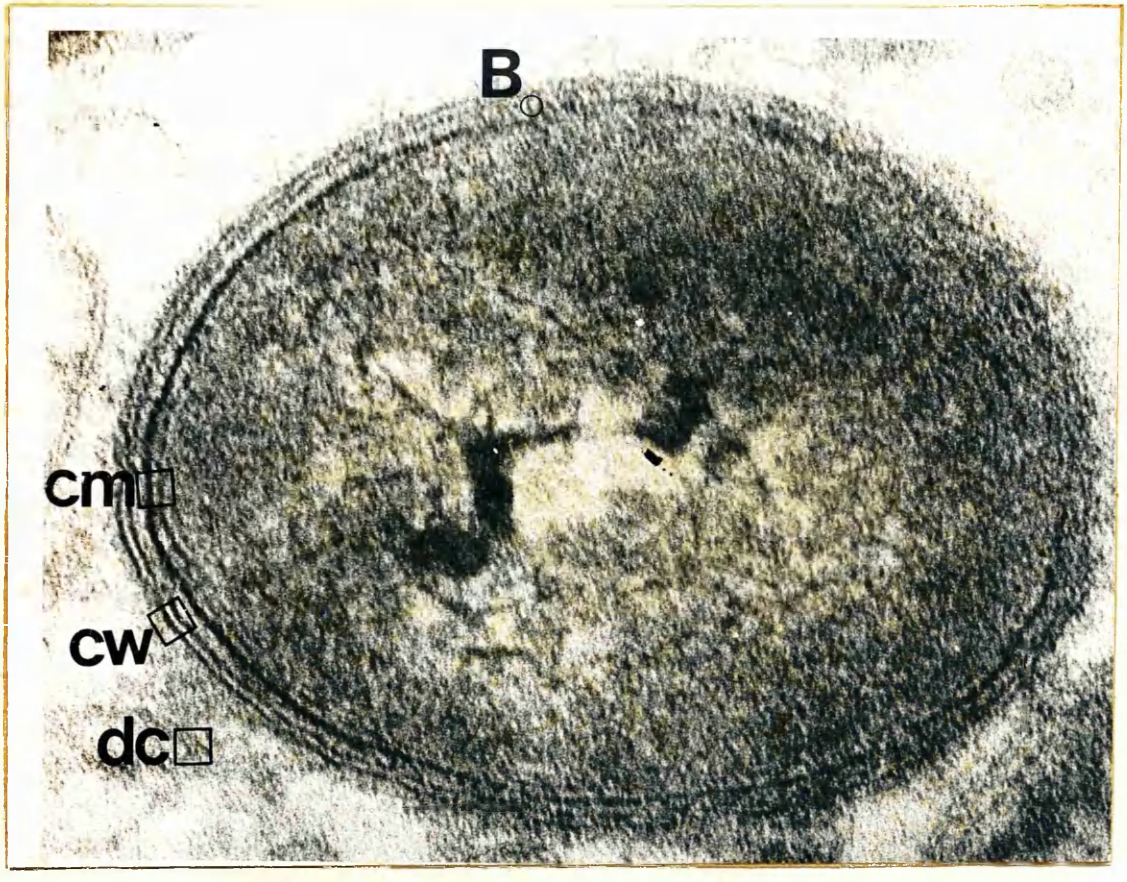
The cell wall can be seen as a three layered structure composed of two dense lines separated by a less dense layer.

The cytoplasmic membrane appears as two dense lines separated by a less dense layer.

An irregular coating, or dense crust, occassionally seen round the periphery of these organisms is thought to be due to adhering particles of the growth medium (Bladen and Waters, 1963).

Plate IX shows a higher magnification of organisms from the pure growth of Bacteroides melaninogenicus. The cell wall can be seen as a three layered structure

PLATE X



BACTEROIDES MELANINOGENICUM

The cell wall (CW) is visible as a triple layered structure separated by a space from a triple layered structure known as the cytoplasmic membrane (CM). Structures known as bridges (B) are seen connecting the cell wall to the cytoplasmic membrane. A dense crust (DC) can be seen round the periphery of the cell. Specimen stained with uranyl acetate and lead citrate. Magnification 270,000.

as can the cytoplasmic membrane. In certain areas the outer dense layer of the cytoplasmic membrane can be seen as a double structure. Small structures, referred to as bridges, can be seen running between the cell wall and the cytoplasmic membrane.

Plate X shows a high magnification of Bacteroides melaninogenicus and demonstrates the triple layered cell wall, separated from the triple layered cytoplasmic membrane by a space crossed by "bridges".

Conclusions

The ultrastructural morphology of the organisms examined from pure growth cultures of Bacteroides melaninogenicus have similar features to those described by Bladen and Waters (1963) for some unidentified strains of Bacteroides and may be summarized as follows:-

1. A triple layered cell wall completely encircling the organism. The cell wall was composed of two dense lines with the outer line more dense and broader than the inner line, and separated by a less dense layer.

2. A cytoplasmic membrane seen as two dense lines separated by a less dense layer. The outer dense layer was considerably thicker than the inner layer and usually more dense. Occassionally the thick outer dense layer could be seen as two thinner dense lines.

3. The space separating the cell wall from the cytoplasmic membrane had small structures, referred to as bridges, crossing it to connect both these structures.

4. A dense crust was seen around the periphery of the organism.

5. Cell division appeared to involve the centripetal growth of the cell wall-cytoplasmic membrane complex until complete fission of the organism occurred.

The organisms shown on Plate VII demonstrated only two of the above features, namely a cell wall and cytoplasmic membrane.

This fact plus the fact that the pure culture of Bacteroides melaninogenicus examined demonstrated no characteristic feature, allow only the statement that both organisms are similar in the features already mentioned.

Theilade et al. (1973) investigated the ultrastructure of black stain on human primary teeth and found the deposit to consist of mainly gram positive cocci or rods in an intermicrobial matrix. They observed calcification of the black stain. The results of the present investigations have shown the predominant micro-organisms to be gram negative coccobacilli which is not in agreement with the above results. However, further research may help to clarify the position.

It is therefore only tentatively suggested that the micro-organisms examined on the tooth section are Bacteroides melaninogenicus and that the homogeneous, cell-free layer is the pigment produced by these organisms.

Tracy (1969) stated:-

> "The function of the pigment remains obscure. However, since ferrous sulphide is a reducing agent, it is interesting to speculate that the pigment may facilitate the growth of the strictly anaerobic Bacteroides".

Therefore logically it might be expected that the micro-organisms would be surrounded by the pigment or that the pigment would be found nearer the plaque surface with the micro-organisms closer to the enamel. The pigment might then exert a protective function against oxygen reaching these micro-organisms.

However, two reasons may be advanced to explain the present findings:-

1. The pigment if placed between the micro-organisms and the outer surface of the plaque may prevent the access of nutrients.

2. As Schroeder and Boever (1969) stated:-

> "In studying microbial morphology of the dental plaque, one has to be aware of the fact, that in contrast to cells growing in a fluid medium, the micro-organisms growing on a tooth surface have no three-dimensional space to develop their 'characteristic morphology'".

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to assess the relationship between certain coloured extrinsic tooth stains and dental disease with particular emphasis on black extrinsic tooth stain. Bacteriological and chemical analyses were carried out into the aetiology and composition of the black material found upon the teeth of certain individuals.

An epidemiological survey was also carried out, in which dental caries, calculus, dental cleanliness and coloured extrinsic tooth stains were recorded on 928 children aged thirteen years. An analysis was made of the data to study possible relationships between dental caries and the other variables particularly black extrinsic tooth stain.

It was found that:-

1. Girls brushed their teeth more often than boys and had significantly cleaner mouths.

2. A significant correlation was found between oral hygiene and gingivitis in both boys and girls.

3. A mean number of 8.25 D-M-F teeth per child was found.

4. Children with dark extrinsic tooth stain had a significantly lower dental caries experience than those with either no stain or stain of other colours.

5. No significant correlation was found between the standard of oral hygiene in children with no tooth stain and children with extrinsic black stain.

6. A significant correlation between the stated sweet consumption and numbers of D-M-F teeth was found.

7. No significant correlation between oral hygiene and the D-M-F rate was found.

8. A significantly lower oxygen tension was found in children with extrinsic black tooth stain than those with no stain.

9. Greater numbers of Bacteroides melaninogenicus were found in children with black extrinsic tooth stain than those with no stain but the correlation between the groups was not significant.

10. Iron and sulphide was found in gingival scrapings from children with black stain upon their teeth but a less vigorous reaction for sulphide only was found in gingival scrapings from children with no coloured tooth stains.

11. Spectral studies on the supernatant of an homogenate of gingival debris from the black stain group indicated that significant amounts of iron-sulphur proteins or haem compounds were not present.

12. Iron was found in the black material recovered from culture plates on which Bacteroides melaninogenicus was grown. However, the test for sulphide was negative.

13. Spectrographic analysis of the supernatant from the microbiological culture plate did not indicate that significant amounts of iron-sulphur proteins or haem compounds were present.

14. Examination of the ultrastructure of Bacteroides melaninogenicus from a pure growth demonstrated the presence of a cell wall, cytoplasmic membrane, "bridges" and a dense crust. Both the cell wall and the cytoplasmic membrane were seen as triple layered structures.

15. Cell division of Bacteroides melaninogenicus appeared to be by inward growth of the cell wall-cytoplasmic membrane complex until fission was complete.

16. Electron microscopic studies of the organisms on the tooth section demonstrate both a cell wall and cytoplasmic membrane. These organisms were also shown to be gram negative cocco-bacilli.

17. An homogeneous cell-free dark material was demonstrated interposed between the microbial layer and the tooth surface

The results show that children with extrinsic black tooth stain had a lower D-M-F rate than children with no tooth stains. The numbers of Bacteroides melaninogenicus were greater in the black stain group although not sufficiently so to be statistically significant.

Chemical analysis of gingival scrapings revealed the presence of iron and sulphide in the black stain group but only thiocyanate in the gingival scrapings from the control group. Thus the black material from both in vivo and in vitro sources has iron with sulphide present in the former. These factors plus the lower oxygen tension found in the mouths of the black stain group suggest that Bacteroides melaninogenicus is the aetiological agent responsible for the extrinsic black stain found on the teeth of certain children in this study.

Certainly if black stain could be produced on the teeth of germ-free animals using Bacteroides melaninogenicus this would be further evidence to support the part played by these organisms in black stain production. This line of investigation would be interesting in spite of the fact that Bacteroides melaninogenicus would not

become established as a monocontaminant in mice (Gibbons et al., 1964). The part played by the organisms in the dental caries process could then be studied and it is hoped that further work into both these topics will be undertaken.

Perhaps the results of this study will strengthen the statement made by Bibby (1931):-

> "The principal value of this discussion seems to be its suggestion that a sweeping condemnation of all deposits on the surface of the teeth is unjustified, as at least, this particular plaque appears to exert nothing but a protective action".

ACKNOWLEDGEMENTS

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All the subjects and patients described in this thesis have been examined by me personally but I am indebted to my colleagues who have contributed information from other examinations and tests. I am grateful to Mr. M. Davies, Chief Dental Officer, City of Glasgow and his staff for making it possible to examine the children at school and to the Headmasters of all the schools I visited for the courtesy offered to me.

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I should also like to thank all of my colleagues who have given me encouragement and to Mr. A. Carmichael for helpful advice.

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TABLE 1

List of selected schools from which the chosen population was examined

District	School		Address	Grade	Denomination		Roll
North	Albert		Mansel St. Springburn	A	P		1,193
	Garrioch		Hotspur St. Springburn	C	P		507
	St. Augustine's	190	Liddlesdale Road	A		R.C.	1,846
	St. Columba of Iona	65	Callander Street	B		R.C.	619
South	Craigbank	36	Damshot Road	A	P		1,317
	St. Margaret Mary's	65	Dougrie Drive	A		R.C.	1,477
	St. Bonadventure's	83	Craigie Street	B		R.C.	773
East	Garthamlock	43	Craigievar Street	A	P		1,001
	Lochend	70	Lochend Road	B	P		740
	St. Gregory's	61	Crowlin Crescent	A		R.C.	1,594
	St. Andrew's	109	Torphin Crescent	B		R.C.	942
West	Kingsridge	41	Achamore Road	A	P		1,036
	Victoria Drive		Larchfield Avenue	B	P		1,120
	St. Pius'	45	Cally Avenue	A		R.C.	1,207
Central	Adelphi	12	Commercial Road	C	P		870
	Our Lady & St. Francis		Charlotte Street	A		R.C.	1,084
	St. Mungo's Academy	46	Parson Street	A		R.C.	1,715

P = Protestant
R.C. = Roman Catholic

TABLE 2

Number of children examined

MALE	FEMALE	TOTAL
445	483	928

TABLE 3

D-M-F rate of 928 thirteen year old Glasgow school children

D-M-F rate	Number of Males	Number of Females	Total
0	7	5	12
1	10	6	16
2	23	17	40
3	22	18	40
4	45	31	76
5	49	31	80
6	53	54	107
7	40	51	91
8	43	39	82
9	30	41	71
10	31	42	73
11	18	31	49
12	13	22	35
13	16	28	44
14	12	9	21
15	11	11	22
16	4	11	15
17	4	8	12
18	5	10	15
19	2	7	9
20	1	4	5
21	4	1	5
22	1	3	4
23	1	1	2
24	0	1	1
25	0	0	0
26	0	1	1
Total	445	483	928

TABLE 4

Mean D-M-F rate of the total sample

	MALES	FEMALES	TOTAL
Mean D-M-F rate	7.58	8.86	8.25
Standard Deviation	4.27	4.58	4.48
Number of children	445	483	928

TABLE 5

D-M-F rate $\overline{8 - 4}$ region - total sample

D-M-F rate	Number of MALES	Number of FEMALES	TOTAL
0	37	32	69
1	142	113	255
2	190	233	423
3	60	86	146
4	16	19	35
Total	445	483	928

Mean D-M-F rate $\overline{8 - 4}$ region

	MALES	FEMALES	TOTAL
Mean	1.721	1.890	1.809
Standard Deviation	0.924	0.908	0.919
Number of children	445	483	928

TABLE 6

D-M-F rate /4 - 8 region - total sample

D-M-F rate	Number of MALES	Number of FEMALES	TOTAL
0	29	27	56
1	163	135	298
2	172	208	380
3	61	82	143
4	20	31	51
Total	445	483	928

Mean D-M-F rate /4 - 8 region

	MALES	FEMALES	TOTAL
Mean	1.730	1.907	1.822
Standard Deviation	0.934	0.961	0.952
Number of children	445	483	928

TABLE 7

D-M-F rate /4 - 8 region - total sample

D-M-F rate	Number of MALES	Number of FEMALES	TOTAL
0	63	48	111
1	178	137	315
2	111	145	256
3	59	99	158
4	34	54	88
Total	445	483	928

Mean D-M-F rate /4 - 8 region

	MALES	FEMALES	TOTAL
Mean	1.602	1.946	1.781
Standard Deviation	1.118	1.155	1.149
Number of children	445	483	928

TABLE 8

D-M-F rate 8 - 4/ region - total sample

D-M-F rate	Number of MALES	Number of FEMALES	TOTAL
0	70	56	126
1	167	154	321
2	135	131	266
3	49	90	139
4	24	52	76
Total	445	483	928

Mean D-M-F rate 8 - 4/ region

	MALES	FEMALES	TOTAL
Mean	1.528	1.851	1.696
Standard Deviation	1.054	1.175	1.129
Number of children	445	483	928

TABLE 9

D-M-F rate 321/123 region - total sample

D-M-F rate	Number of MALES	Number of FEMALES	TOTAL
0	286	280	566
1	60	76	136
2	39	54	93
3	24	23	47
4	32	34	66
5	4	14	18
6	0	2	2
Total	445	483	928

Mean D-M-F rate 321/123 region

	MALES	FEMALES	TOTAL
Mean	0.804	0.975	0.893
Standard Deviation	1.305	1.444	1.381
Number of children	445	483	928

TABLE 10

D-M-F rate $\overline{321/123}$ region - total sample

D-M-F rate	Number of MALES	Number of FEMALES	TOTAL
0	404	426	830
1	18	19	37
2	8	16	24
3	7	8	15
4	7	9	16
5	1	3	4
6	0	2	2
Total	445	483	928

Mean D-M-F rate $\overline{321/123}$ region

	MALES	FEMALES	TOTAL
Mean	0.198	0.286	0.244
Standard Deviation	0.724	0.918	0.832
Number of children	445	483	928

TABLE 11

Number of teeth decayed in the total sample

Decayed teeth	Number of MALES	Number of FEMALES	TOTAL
0	42	50	92
1	51	51	102
2	61	68	129
3	56	63	119
4	54	48	102
5	46	58	104
6	43	39	82
7	19	21	40
8	22	22	44
9	18	19	37
10	8	13	21
11	9	16	25
12	6	8	14
13	3	1	4
14	3	0	3
15	1	2	3
16	0	1	1
17	1	1	2
18	1	0	1
19	0	1	1
20	1	0	1
21	0	1	1
Total	445	483	928

Mean number of teeth decayed

	MALES	FEMALES	TOTAL
Mean	4.310	4.356	4.334
Standard Deviation	3.348	3.432	3.390
Number of children	445	483	928

TABLE 12

Number of teeth missing in the total sample

Missing teeth	Number of MALES	Number of FEMALES	TOTAL
0	270	259	529
1	66	81	147
2	64	76	140
3	18	35	53
4	19	22	41
5	5	4	9
6	2	2	4
7	1	1	2
8	0	0	0
9	0	1	1
10	0	1	1
11	-	-	-
12	-	-	-
13	-	-	-
14	-	-	-
15	-	-	-
16	-	-	-
17	-	-	-
18	0	1	1
Total	445	483	928

Mean number of teeth missing

	MALES	FEMALES	TOTAL
Mean	0.827	1.039	0.938
Standard Deviation	1.282	1.614	1.468
Number of children	445	483	928

TABLE 13

Number of teeth filled in the total sample

Filled teeth	Number of MALES	Number of FEMALES	TOTAL
0	183	158	341
1	45	42	87
2	49	48	97
3	40	42	82
4	36	46	82
5	28	25	53
6	19	23	42
7	13	23	36
8	8	14	22
9	5	14	19
10	5	17	22
11	4	9	13
12	3	8	11
13	2	6	8
14	2	3	5
15	1	1	2
16	0	2	2
17	2	2	4
Total	445	483	928

Mean number of teeth filled

	MALES	FEMALES	TOTAL
Mean	2.458	3.470	2.845
Standard Deviation	3.139	3.811	3.415
Number of children	445	483	928

TABLE 14

Number of decayed teeth excluding grade 1 cavities

Decayed teeth excluding grade 1 cavities	Number of MALES	Number of FEMALES	TOTAL
0	101	120	221
1	83	80	163
2	62	59	121
3	60	55	115
4	48	50	98
5	36	35	71
6	15	31	46
7	13	16	29
8	8	14	22
9	8	9	17
10	2	6	8
11	2	0	2
12	3	2	5
13	0	0	0
14	0	3	3
15	2	1	3
16	0	0	0
17	2	1	3
18	0	1	1
Total	445	483	928

Mean number of decayed teeth excluding grade 1 cavities

	MALES	FEMALES	TOTAL
Mean	2.737	2.909	2.827
Standard Deviation	2.785	2.965	2.880
Number of children	445	483	928

TABLE 15

Mean number of grade 1 cavities

	MALES	FEMALES	TOTAL
Mean	1.582	1.427	1.501
Standard Deviation	1.548	1.370	1.459
Number of children	445	483	928

TABLE 16

Mean number of grade 2 cavities

	MALES	FEMALES	TOTAL
Mean	0.573	0.700	0.639
Standard Deviation	0.924	0.981	0.955
Number of children	445	483	928

TABLE 17

Mean number of grade 3 cavities

	MALES	FEMALES	TOTAL
Mean	1.804	1.913	1.861
Standard Deviation	2.074	2.150	2.114
Number of children	445	483	928

TABLE 18

Mean number of grade 4 cavities

	MALES	FEMALES	TOTAL
Mean	0.348	0.335	0.342
Standard Deviation	0.899	0.897	0.898
Number of children	445	483	928

TABLE 19

Mean number of teeth lost apart from caries - Orthodontic

	MALES	FEMALES	TOTAL
Mean	0.198	0.296	0.249
Standard Deviation	0.745	0.909	0.836
Number of children	445	483	928

TABLE 20

Mean number of teeth lost apart from caries - Congenitally absent

	MALES	FEMALES	TOTAL
Mean	0.002	0.006	0.004
Standard Deviation	0.047	0.102	0.080
Number of children	445	483	928

TABLE 21

Mean number of teeth lost apart from caries - Trauma

	MALES	FEMALES	TOTAL
Mean	0.038	0.017	0.027
Standard Deviation	0.203	0.157	0.181
Number of children	445	483	928

TABLE 22

Mean Debris Index Simplified

	MALES	FEMALES	TOTAL
Mean	1.14	0.94	1.04
Standard Deviation	0.36	0.34	0.36
Number of children	445	483	928

TABLE 23

Mean Calculus Index Simplified

	MALES	FEMALES	TOTAL
Mean	0.14	0.12	0.13
Standard Deviation	0.24	0.23	0.23
Number of children	445	483	928

TABLE 24

Mean Oral Hygiene Index Simplified

	MALES	FEMALES	TOTAL
Mean	1.27	1.07	1.17
Standard Deviation	0.47	0.45	0.45
Number of children	445	483	928

TABLE 25

The Oral Hygiene Index Simplified

Oral Hygiene Index Simplified	Number of MALES	Number of FEMALES	TOTAL
0.0 - 0.4	8	16	24
0.5 - 0.9	76	155	231
1.0 - 1.4	210	236	446
1.5 - 1.9	117	50	167
2.0 - 2.4	23	20	43
2.5 - 2.9	10	6	16
3.0 and over	1	0	1
Total	445	483	928

TABLE 26

Incidence of Calculus

	Number of MALES	Number of FEMALES	TOTAL
Calculus absent	283	344	627
Calculus present	162	139	301

TABLE 27

Mean Papillary Gingivitis Score+

	MALES	FEMALES	TOTAL
Mean	8.692	7.988	8.325
Standard Deviation	1.961	2.376	2.214
Number of children	445	483	928

TABLE 28

Mean Marginal Gingivitis Score+

	MALES	FEMALES	TOTAL
Mean	7.373	5.996	6.656
Standard Deviation	3.170	3.705	3.525
Number of children	445	483	928

TABLE 29

Mean Attached Gingivitis Score+

	MALES	FEMALES	TOTAL
Mean	0.265	0.230	0.247
Standard Deviation	1.049	1.217	1.139
Number of children	445	483	928

+ All three are components of the P-M-A Index.

TABLE 30

The P-M-A Index - total sample

P-M-A Index	Number of MALES	Number of FEMALES	TOTAL
0-0-0	1	2	3
1-0-0 - 2-1-0	4	11	15
3-0-0 - 5-4-3	34	71	105
6-0-0 - 8-7-6	113	143	256
9-0-0 and above	293	256	549
Total	445	483	928

Mean P-M-A Index

	MALES	FEMALES	TOTAL
Mean	8.68	7.99	8.32
Standard Deviation	1.96	2.38	2.21
Number of children	445	483	928

TABLE 31

Comparison between the Oral Hygiene Index Simplified and the D-M-F rate

D-M-F rate	Oral Hygiene Index Simplified							
	0	1 - 2	3	Total	0	1 - 2	Total	Grand Total
	Number of MALES				Number of FEMALES			
0	1	6	-	7	1	4	5	12
1	2	8	-	10	-	6	6	16
2	3	20	-	23	4	13	17	40
3	3	19	-	22	5	13	18	40
4	7	38	-	45	11	20	31	76
5	9	40	-	49	11	20	31	80
6	15	38	-	53	21	33	54	107
7	9	31	-	40	16	35	51	91
8	9	34	-	43	16	23	39	82
9	4	26	-	30	18	23	41	71
10	3	27	1	31	18	24	42	73
11	2	16	-	18	15	16	31	49
12	5	8	-	13	7	15	22	35
13	4	12	-	16	12	16	28	44
14	2	10	-	12	3	6	9	21
15	1	10	-	11	2	9	11	22
16	-	4	-	4	3	8	11	15
17	2	2	-	4	1	7	8	12
18	1	4	-	5	2	8	10	15
19	-	2	-	2	2	5	7	9
20	-	1	-	1	-	4	4	5
21	1	3	-	4	-	1	1	5
22	-	1	-	1	1	2	3	4
23	1	-	-	1	-	1	1	2
24	-	-	-	-	1	-	1	1
25	-	-	-	-	-	-	-	-
26	-	-	-	-	1	-	1	1
Total	84	360	1	445	171	312	483	928

TABLE 32

Comparison between the Oral Hygiene Index Simplified and the P-M-A Index

P-M-A Index	Oral Hygiene Index Simplified								Grand Total
	0	1 - 2	3	Total	0	1 - 2	3	Total	
	Number of MALES				Number of FEMALES				
0-0-0	1	-	-	1	1	1	-	2	3
1-0-0 - 2-1-0	2	2	-	4	9	2	-	11	15
3-0-0 - 5-4-3	13	21	-	34	36	35	-	71	105
6-0-0 - 8-7-6	27	85	1	113	51	92	-	143	256
9-0-0 and above	41	252	-	293	74	182	-	256	549
Total	84	360	1	445	171	312	-	483	928

Comparison between the Oral Hygiene Index Simplified and the mean P-M-A Index

Oral Hygiene Index Simplified	Mean P-M-A Index	MALES	FEMALES	TOTAL
0	Mean	7.79	7.34	7.48
	Standard Deviation	2.56	2.76	2.70
	Number of children	84	171	255
1 - 2	Mean	8.89	8.36	8.64
	Standard Deviation	1.85	2.19	2.03
	Number of children	360	312	672

TABLE 33

Comparison between the P-M-A Index and Calculus

P-M-A Index	Calculus absent			Calculus present			
	Number of MALES	Number of FEMALES	TOTAL	Number of MALES	Number of FEMALES	TOTAL	Grand Total
0-0-0	1	2	3	-	-	-	3
1-0-0 - 2-1-0	4	10	14	-	1	1	15
3-0-0 - 5-4-3	21	51	72	13	20	33	105
6-0-0 - 8-7-6	77	109	186	36	34	70	256
9-0-0 and above	180	172	352	113	84	197	549
Total	283	344	627	162	139	301	928

Mean number of children with calculus present or absent

		MALES	FEMALES	TOTAL
Calculus absent	Mean	8.58	7.85	8.18
	Standard Deviation	2.12	2.51	2.37
	Number of children	283	344	627
Calculus present	Mean	8.85	8.34	8.62
	Standard Deviation	1.90	2.28	2.09
	Number of children	162	139	301

TABLE 34

Do you have a toothbrush?

Number of children owning a toothbrush

	Number of MALES	Number of FEMALES	TOTAL
No	25	2	27
Yes	420	481	901
Total	445	483	928

TABLE 35

Toothbrushing frequency

Frequency	Number of MALES	Number of FEMALES	TOTAL
No Toothbrush	25	2	27
1/day	90	118	208
2/day	66	213	279
3/day	9	39	48
5 - 6/week	15	5	20
3 - 4/week	46	33	79
1 - 2/week	66	28	94
Rarely	128	45	173
Total	445	483	928

TABLE 36

Number of children who brushed their teeth in the past 24 hours

	Number of MALES	Number of FEMALES	TOTAL
No	230	155	385
Yes	191	326	517
+N/A	24	2	26
Total	445	483	928

+No Answer.

TABLE 37

Responses to the question "Have you eaten since last brushed teeth?"

	Number of MALES	Number of FEMALES	TOTAL
No	49	90	139
Yes	372	391	763
+N/A	24	2	26
Total	445	483	928

+No Answer.

TABLE 38

The amount spent on sweets per week

Amount spent	Number of MALES	Number of FEMALES	TOTAL
0	4	0	4
<5p	3	2	5
5p - 9p	1	0	1
10p - 14p	41	23	64
15p - 19p	51	77	128
20p - 24p	32	32	64
25p - 29p	140	149	289
30p - 34p	22	45	67
35p - 39p	7	8	15
40p - 44p	11	10	21
>45p	133	137	270
Total	445	483	928

TABLE 39

Responses to the question "Do you eat snacks?"

	Number of MALES	Number of FEMALES	TOTAL
No	169	144	313
Yes	276	339	615
Total	445	483	928

TABLE 40

The type of snacks eaten

	Number of MALES	Number of FEMALES	TOTAL
Non-Carbo-hydrate	123	167	290
Carbo-hydrate	153	172	325
+N/A	169	144	313
Total	445	483	928

+No Answer.

TABLE 41

The number of snacks eaten per day

Number of snacks	Number of MALES	Number of FEMALES	TOTAL
0	169	144	313
1	39	72	111
2	97	136	233
3	80	88	168
4	60	43	103
Total	445	483	928

TABLE 42

Responses to the question "Do you have a Dentist?"

	Number of MALES	Number of FEMALES	TOTAL
No	89	45	134
Yes	356	438	794
Total	445	483	928

TABLE 43

Responses to the question "Do you attend a Dentist regularly?"

	Number of MALES	Number of FEMALES	TOTAL
No	207	174	381
Yes	152	266	418
+N/A	86	43	129
Total	445	483	928

+No Answer.

TABLE 44

The Comparison between the mean Oral Hygiene Index Simplified and toothbrushing frequency

Toothbrushing frequency	Mean Oral Hygiene Index Simplified	MALES	FEMALES	TOTAL
No Toothbrush	Mean	1.346	1.300	1.342
	Standard Deviation	0.475	0.141	0.456
	Number of children	25	2	27
1/day	Mean	1.240	1.036	1.125
	Standard Deviation	0.437	0.383	0.419
	Number of children	90	118	208
2/day	Mean	1.132	1.043	1.064
	Standard Deviation	0.434	0.431	0.433
	Number of children	66	213	279
3/day	Mean	1.111	0.987	1.010
	Standard Deviation	0.454	0.484	0.476
	Number of children	9	39	48
5 - 6/week	Mean	1.220	1.040	1.175
	Standard Deviation	0.339	0.451	0.365
	Number of children	15	5	20
3 - 4/week	Mean	1.278	1.097	1.203
	Standard Deviation	0.465	0.460	0.469
	Number of children	46	33	79
1 - 2/week	Mean	1.294	1.082	1.231
	Standard Deviation	0.477	0.338	0.449
	Number of children	66	28	94
Rarely	Mean	1.363	1.327	1.353
	Standard Deviation	0.484	0.471	0.479
	Number of children	128	45	173

TABLE 45

Statistical Analyses

The relationship between the Oral Hygiene Index Simplified and toothbrushing frequency

Toothbrushing frequency used to illustrate the comparisons drawn between the above parameters using results shown in Table 44

Results of t-tests

Toothbrushing frequency		t value
No Toothbrush[+] v.	1/day	2.308
	2/day	2.986
	3/day	2.944
	5 - 6/week	1.379
	3 - 4/week	1.339
	1 - 2/week	1.102
	Rarely	0.114
1/day v.	2/day	1.569
	3/day	1.542
	5 - 6/week	0.577
	3 - 4/week	1.296
	1 - 2/week	1.940
	Rarely	4.899
2/day v.	3/day	0.735
	5 - 6/week	1.296
	3 - 4/week	2.364
	1 - 2/week	3.147
	Rarely	6.465
3/day v.	5 - 6/week	1.547
	3 - 4/week	2.228
	1 - 2/week	2.667
	Rarely	4.411
5 - 6/week v.	3 - 4/week	0.288
	1 - 2/week	0.597
	Rarely	1.992
3 - 4/week v.	1 - 2/week	0.399
	Rarely	2.340
1 - 2/week v.	Rarely	2.071

[+]v. = compared to.

TABLE 46

Comparison between the mean Oral Hygiene Index Simplified and the number of snacks eaten per day

Number of snacks	Mean Oral Hygiene Index Simplified	MALES	FEMALES	TOTAL
0	Mean	1.272	1.111	1.198
	Standard Deviation	0.458	0.414	0.445
	Number of children	169	144	313
1	Mean	1.223	1.032	1.099
	Standard Deviation	0.340	0.419	0.402
	Number of children	39	72	111
2	Mean	1.299	1.080	1.171
	Standard Deviation	0.495	0.416	0.462
	Number of children	97	136	233
3	Mean	1.209	1.068	1.135
	Standard Deviation	0.497	0.507	0.506
	Number of children	80	88	168
4	Mean	1.357	0.970	1.195
	Standard Deviation	0.439	0.378	0.455
	Number of children	60	43	103

TABLE 47

Statistical Analyses

The relationship between the Oral Hygiene Index Simplified and the number of snacks eaten per day

The number of snacks eaten per day are used to illustrate the comparisons drawn between the above parameters using results shown in Table 46

Results of t-tests

Number of snacks/day			t value
0	+v.	1	2.166
		2	0.686
		3	1.357
		4	0.058
1	v.	2	1.478
		3	0.659
		4	1.631
2	v.	3	0.729
		4	0.444
3	v.	4	1.009

+v. = compared to.

TABLE 48

Comparison between the mean D-M-F rate and the number of snacks eaten per day

Number of snacks		Mean D-M-F rate		
		MALES	FEMALES	TOTAL
0	Mean	7.361	8.444	7.859
	Standard Deviation	4.270	4.363	4.340
	Number of children	169	144	313
1	Mean	7.410	9.319	8.649
	Standard Deviation	4.011	4.465	4.389
	Number of children	39	72	111
2	Mean	7.814	8.912	8.455
	Standard Deviation	4.447	4.712	4.626
	Number of children	97	136	233
3	Mean	7.662	9.534	8.643
	Standard Deviation	4.050	5.097	4.709
	Number of children	80	88	168
4	Mean	7.817	7.953	7.874
	Standard Deviation	4.549	3.748	4.214
	Number of children	60	43	103

TABLE 49

Statistical Analyses

The relationship between the $\overset{mean}{\wedge}$D-M-F rate and number of snacks eaten per day

The number of snacks eaten per day are used to illustrate the comparisons drawn between the above parameters using results shown in Table 48

Results of t-tests

Number of snacks/day			t value
0[+]	v.	1	1.634
		2	1.529
		3	1.788
		4	0.031
1	v.	2	0.377
		3	0.011
		4	1.318
2	v.	3	0.397
		4	1.130
3	v.	4	1.394

[+]v. = compared to.

TABLE 50

The Comparison between the mean D-M-F rate and the stated toothbrushing frequency

Toothbrushing frequency		Mean D-M-F rate		
		MALES	FEMALES	TOTAL
No Toothbrush	Mean	6.542	3.500	6.308
	Standard Deviation	4.681	0.707	4.567
	Number of children	25	2	27
1/day	Mean	7.769	8.441	8.148
	Standard Deviation	4.313	4.555	4.453
	Number of children	90	118	208
2/day	Mean	8.273	9.183	8.968
	Standard Deviation	4.391	4.448	4.443
	Number of children	66	213	279
3/day	Mean	5.333	9.128	8.417
	Standard Deviation	2.398	4.175	4.161
	Number of children	9	39	48
5 - 6/week	Mean	7.533	5.200	6.950
	Standard Deviation	4.257	1.924	3.900
	Number of children	15	5	20
3 - 4/week	Mean	6.630	8.697	7.494
	Standard Deviation	3.574	4.660	4.163
	Number of children	46	33	79
1 - 2/week	Mean	7.409	8.214	7.649
	Standard Deviation	3.934	4.193	4.007
	Number of children	66	28	94
Rarely	Mean	7.875	9.378	8.266
	Standard Deviation	4.556	5.714	4.911
	Number of children	128	45	173

TABLE 51

Statistical Analyses

The relationship between the $\overset{mean}{D}$-M-F rate and stated toothbrushing frequency

Toothbrushing frequency used to illustrate the comparisons drawn between the above parameters using results shown in Table 50

Results of t-tests

Toothbrushing frequency			t value
No Toothbrush[+]	v.	1/day	1.943
		2/day	2.847
		3/day	1.956
		5 - 6/week	0.514
		3 - 4/week	1.173
		1 - 2/week	1.359
		Rarely	2.018
1/day	v.	2/day	2.015
		3/day	0.399
		5 - 6/week	1.295
		3 - 4/week	1.167
		1 - 2/week	0.968
		Rarely	0.244
2/day	v.	3/day	0.839
		5 - 6/week	2.213
		3 - 4/week	2.737
		1 - 2/week	2.684
		Rarely	1.531
3/day	v.	5 - 6/week	1.385
		3 - 4/week	1.212
		1 - 2/week	1.053
		Rarely	0.214
5 - 6/week	v.	3 - 4/week	0.550
		1 - 2/week	0.724
		Rarely	1.387
3 - 4/week		1 - 2/week	0.248
		Rarely	1.289
1 - 2/week	v.	Rarely	1.108

[+] v. = compared to.

TABLE 52

Comparison between the mean Papillary Gingivitis Score[+] and stated toothbrushing frequency

Toothbrushing frequency	Mean Papillary Gingivitis Score	MALES	FEMALES	TOTAL
No Toothbrush	Mean	8.833	8.500	8.808
	Standard Deviation	1.736	2.121	1.721
	Number of children	25	2	27
1/day	Mean	8.846	7.932	8.330
	Standard Deviation	2.071	2.507	2.366
	Number of children	90	118	208
2/day	Mean	8.076	7.793	7.860
	Standard Deviation	2.276	2.291	2.287
	Number of children	66	213	279
3/day	Mean	8.000	7.410	7.521
	Standard Deviation	2.784	2.989	2.932
	Number of children	9	39	48
5 - 6/week	Mean	8.467	8.000	8.350
	Standard Deviation	1.767	1.581	1.694
	Number of children	15	5	20
3 - 4/week	Mean	8.457	8.970	8.671
	Standard Deviation	2.084	1.862	1.998
	Number of children	46	33	79
1 - 2/week	Mean	8.742	8.071	8.543
	Standard Deviation	2.018	2.176	2.077
	Number of children	66	28	94
Rarely	Mean	9.008	8.756	8.942
	Standard Deviation	1.539	2.134	1.711
	Number of children	128	45	173

[+]Part of the P-M-A Index.

TABLE 53

Comparison between the mean Marginal Gingivitis Score[+] and stated toothbrushing frequency

Toothbrushing frequency	Mean Marginal Gingivitis Score	MALES	FEMALES	TOTAL
No Toothbrush	Mean	7.208	9.500	7.385
	Standard Deviation	3.050	0.707	2.994
	Number of children	25	2	27
1/day	Mean	7.538	5.932	6.632
	Standard Deviation	3.304	3.681	3.603
	Number of children	90	118	208
2/day	Mean	6.530	5.742	5.928
	Standard Deviation	3.659	3.619	3.638
	Number of children	66	213	279
3/day	Mean	6.333	5.718	5.833
	Standard Deviation	3.742	4.180	4.070
	Number of children	9	39	48
5 - 6/week	Mean	6.933	5.600	6.600
	Standard Deviation	3.369	2.966	3.251
	Number of children	15	5	20
3 - 4/week	Mean	7.587	7.000	7.342
	Standard Deviation	2.963	3.473	3.178
	Number of children	46	33	79
1 - 2/week	Mean	7.394	5.929	6.957
	Standard Deviation	3.323	3.999	3.580
	Number of children	66	28	94
Rarely	Mean	7.758	6.800	7.509
	Standard Deviation	2.702	3.788	3.040
	Number of children	128	45	173

[+]Part of the P-M-A Index.

TABLE 54

Comparison between the mean Attached Gingivitis Score[+] and stated toothbrushing frequency

Toothbrushing frequency	Mean Attached Gingivitis Score	MALES	FEMALES	TOTAL
No Toothbrush	Mean	0.833	---	0.769
	Standard Deviation	2.278	---	2.196
	Number of children	25	2	27
1/day	Mean	0.242	0.212	0.225
	Standard Deviation	1.078	0.959	1.011
	Number of children	90	118	208
2/day	Mean	0.121	0.225	0.201
	Standard Deviation	0.448	1.250	1.114
	Number of children	66	213	279
3/day	Mean	---	0.077	0.063
	Standard Deviation	---	0.480	0.433
	Number of children	9	39	48
5 - 6/week	Mean	0.200	---	0.150
	Standard Deviation	0.775	---	0.671
	Number of children	15	5	20
3 - 4/week	Mean	0.152	0.303	0.215
	Standard Deviation	0.788	1.741	1.268
	Number of children	46	33	79
1 - 2/week	Mean	0.409	0.179	0.340
	Standard Deviation	1.312	0.945	1.214
	Number of children	66	28	94
Rarely	Mean	0.242	0.444	0.295
	Standard Deviation	0.849	1.791	1.166
	Number of children	128	45	173

[+]Part of the P-M-A Index.

TABLE 55

Statistical Analyses

The relationship between the Papillary Gingivitis Score and stated toothbrushing frequency

Toothbrushing frequency used to illustrate the comparisons drawn between the above parameters using results shown in Table 52

Results of t-tests

Toothbrushing frequency			t value
No Toothbrush[+]	v.	1/day	1.28
		2/day	2.61
		3/day	2.38
		5 - 6/week	0.91
		3 - 4/week	0.35
		1 - 2/week	0.68
		Rarely	0.36
1/day	v.	2/day	2.20
		3/day	1.79
		5 - 6/week	0.05
		3 - 4/week	1.22
		1 - 2/week	0.78
		Rarely	2.92
2/day	v.	3/day	0.76
		5 - 6/week	1.22
		3 - 4/week	3.07
		1 - 2/week	2.67
		Rarely	5.72
3/day	v.	5 - 6/week	1.46
		3 - 4/week	2.40
		1 - 2/week	2.15
		Rarely	3.21
5 - 6/week	v.	3 - 4/week	0.73
		1 - 2/week	0.44
		Rarely	1.48
3 - 4/week	v.	1 - 2/week	0.42
		Rarely	1.04
1 - 2/week	v.	Rarely	1.59

[+]v. = compared to.

TABLE 56

Statistical Analyses

The relationship between the Marginal Gingivitis Score and stated toothbrushing frequency

Toothbrushing frequency used to illustrate the comparisons drawn between the above parameters using results shown in Table 53

Results of t-tests

Toothbrushing frequency			t value
No Toothbrush[+]	v.	1/day	1.19
		2/day	2.33
		3/day	1.88
		5 - 6/week	0.85
		3 - 4/week	0.06
		1 - 2/week	0.62
		Rarely	0.19
1/day	v.	2/day	2.12
		3/day	1.25
		5 - 6/week	0.04
		3 - 4/week	1.63
		1 - 2/week	0.74
		Rarely	2.59
2/day	v.	3/day	0.16
		5 - 6/week	0.88
		3 - 4/week	3.37
		1 - 2/week	2.40
		Rarely	4.97
3/day	v.	5 - 6/week	0.82
		3 - 4/week	2.20
		1 - 2/week	1.63
		Rarely	2.66
5 - 6/week	v.	3 - 4/week	0.91
		1 - 2/week	0.44
		Rarely	1.19
3 - 4/week	v.	1 - 2/week	0.74
		Rarely	0.40
1 - 2/week	v.	Rarely	1.26

[+]v. = compared to.

TABLE 57

Statistical Analyses

The relationship between the Attached Gingivitis Score and stated toothbrushing frequency

Toothbrushing frequency used to illustrate the comparisons drawn between the above parameters using results shown in Table 54

Results of t-tests

Toothbrushing frequency			t value
No Toothbrush[+]	v.	1/day	1.24
		2/day	1.31
		3/day	1.63
		5 - 6/week	1.36
		3 - 4/week	1.21
		1 - 2/week	0.96
		Rarely	1.07
1/day	v.	2/day	0.31
		3/day	1.82
		5 - 6/week	0.48
		3 - 4/week	0.06
		1 - 2/week	0.77
		Rarely	0.62
2/day	v.	3/day	1.54
		5 - 6/week	0.31
		3 - 4/week	0.13
		1 - 2/week	0.99
		Rarely	0.90
3/day	v.	5 - 6/week	0.55
		3 - 4/week	1.03
		1 - 2/week	2.01
		Rarely	2.21
5 - 6/week	v.	3 - 4/week	0.34
		1 - 2/week	0.97
		Rarely	0.86
3 - 4/week	v.	1 - 2/week	0.63
		Rarely	0.48
1 - 2/week	v.	Rarely	0.26

[+]v. = compared to.

TABLE 58

Comparison between the mean Papillary Gingivitis Score[+] and the number of snacks eaten per day

Number of snacks		Mean Papillary Gingivitis Score		
		MALES	FEMALES	TOTAL
0	Mean	8.615	8.028	8.345
	Standard Deviation	2.018	2.299	2.168
	Number of children	169	144	313
1	Mean	8.897	7.472	7.973
	Standard Deviation	1.651	2.415	2.274
	Number of children	39	72	111
2	Mean	8.619	7.993	8.253
	Standard Deviation	2.059	2.393	2.276
	Number of children	97	136	233
3	Mean	8.550	8.375	8.458
	Standard Deviation	2.056	2.286	2.175
	Number of children	80	88	168
4	Mean	9.083	7.907	8.592
	Standard Deviation	1.670	2.635	2.194
	Number of children	60	43	103

[+]Part of the P-M-A Index.

TABLE 59

Statistical Analyses

The relationship between the Papillary Gingivitis Score and the number of snacks eaten per day

The number of snacks eaten per day are used to illustrate the comparisons drawn between the above parameters using results shown in Table 58

Results of t-tests

Number of snacks			t value
0	+v.	1	1.53
		2	0.52
		3	0.53
		4	0.97
1	v.	2	1.07
		3	1.79
		4	2.03
2	v.	3	0.93
		4	1.30
3	v.	4	0.48

+v. = compared to.

TABLE 60

Comparison between the mean Marginal Gingivitis Score[+] and the number of snacks eaten per day

Number of snacks		Mean Marginal Gingivitis Score		
		MALES	FEMALES	TOTAL
0	Mean	7.219	6.174	6.738
	Standard Deviation	3.174	3.708	3.464
	Number of children	169	144	313
1	Mean	6.769	4.764	5.468
	Standard Deviation	3.731	3.747	3.847
	Number of children	39	72	111
2	Mean	7.124	5.985	6.459
	Standard Deviation	3.324	3.561	3.502
	Number of children	97	136	233
3	Mean	7.637	6.773	7.185
	Standard Deviation	2.965	3.587	3.324
	Number of children	80	88	168
4	Mean	8.250	5.907	7.272
	Standard Deviation	2.634	3.945	3.431
	Number of children	60	43	103

[+]Part of the P-M-A Index.

TABLE 61

Statistical Analyses

The relationship between the Marginal Gingivitis Score and the number of snacks eaten per day

The number of snacks eaten per day are used to illustrate the comparisons drawn between the above parameters using results shown in Table 60

Results of t-tests

Number of snacks			t value
0	+v.	1	3.06
		2	0.93
		3	1.40
		4	1.36
1	v.	2	2.29
		3	3.86
		4	3.62
2	v.	3	2.12
		4	1.98
3	v.	4	0.19

+v. = compared to.

TABLE 62

Comparison between the mean Attached Gingivitis Score[+] and the number of snacks eaten per day

Number of snacks		Mean Attached Gingivitis Score		
		MALES	FEMALES	TOTAL
0	Mean	0.331	0.146	0.246
	Standard Deviation	1.247	0.802	1.068
	Number of children	169	144	313
1	Mean	0.077	0.111	0.099
	Standard Deviation	0.354	0.742	0.632
	Number of children	39	72	111
2	Mean	0.330	0.199	0.253
	Standard Deviation	1.048	1.010	1.026
	Number of children	97	136	233
3	Mean	0.150	0.511	0.339
	Standard Deviation	0.748	1.971	1.524
	Number of children	80	88	168
4	Mean	0.250	0.233	0.243
	Standard Deviation	1.083	1.525	1.279
	Number of children	60	43	103

[+]Part of the P-M-A Index.

TABLE 63

Statistical Analyses

The relationship between the Attached Gingivitis Score and the number of snacks eaten per day

The number of snacks eaten per day are used to illustrate the comparisons drawn between the above parameters using results shown in Table 62

Results of t-tests

Number of snacks			t value
0	+v.	1	1.88
		2	0.00
		3	0.68
		4	0.07
1	v.	2	1.77
		3	1.90
		4	1.07
2	v.	3	0.67
		4	0.07
3	v.	4	0.58

+v. = compared to.

TABLE 64

Comparison between the D-M-F rate and the amount spent on sweets

D-M-F rate	Amount spent on sweets										
	<10p	10-29p	30-44p	>45p	Total	<10p	10-29p	30-44p	>45p	Total	Grand Total
	Number of MALES					Number of FEMALES					
0	-	5	1	1	7	-	3	-	2	5	12
1	1	4	2	3	10	-	3	1	2	6	16
2	-	13	-	10	23	-	14	-	3	17	40
3	2	14	2	4	22	1	5	4	8	18	40
4	1	30	4	10	45	-	17	6	8	31	76
5	-	28	2	19	49	-	20	3	8	31	80
6	1	32	4	16	53	1	30	8	15	54	107
7	1	20	4	15	40	-	29	6	16	51	91
8	1	30	1	11	43	-	24	3	12	39	82
9	-	16	5	9	30	-	25	6	10	41	71
10	-	15	2	14	31	-	28	4	10	42	73
11	1	8	6	3	18	-	16	6	9	31	49
12	-	8	2	3	13	-	12	4	6	22	35
13	-	10	1	5	16	-	15	-	13	28	44
14	-	9	1	2	12	-	5	3	1	9	21
15	-	7	1	3	11	-	9	1	1	11	22
16	-	1	1	2	4	-	7	2	2	11	15
17	-	2	1	1	4	-	3	3	2	8	12
18	-	4	-	1	5	-	6	1	3	10	15
19	-	1	-	1	2	-	4	2	1	7	9
20	-	1	-	-	1	-	3	-	1	4	5
21	-	4	-	-	4	-	-	-	1	1	5
22	-	1	-	-	1	-	2	-	1	3	4
23	-	1	-	-	1	-	-	-	1	1	2
24	-	-	-	-	-	-	1	-	-	1	1
25	-	-	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-	1	1	1
Total	8	264	40	133	145	2	281	63	137	483	928

TABLE 64 (continued)

Comparison between the mean D-M-F rate and the amount spent on sweets

Amount spent on sweets		Mean D-M-F rate		
		MALES	FEMALES	TOTAL
<10p	Mean	5.38	4.50	5.20
	Standard Deviation	3.25	2.12	2.97
	Number of children	8	2	10
10p - 29p	Mean	7.70	8.86	8.30
	Standard Deviation	4.52	4.51	4.55
	Number of children	264	281	545
30p - 44p	Mean	8.10	9.05	8.68
	Standard Deviation	4.14	4.49	4.36
	Number of children	40	63	103
45p and over	Mean	7.32	8.85	8.09
	Standard Deviation	3.83	4.79	4.40
	Number of children	133	137	270

TABLE 65

Statistical Analyses

The relationship between the $\overset{mean}{\wedge}$D-M-F rate and amount spent on sweets

The amount spent on sweets is used to illustrate the comparisons drawn between the above parameters using results shown in Table 64

Results of t-tests

Amount spent on sweets		t value
<10p $^{+}$v.	10p - 29p	3.23
	30p - 44p	3.37
	>45p	2.96
10p - 29p v.	30p - 44p	0.81
	>45p	0.63
30p - 44p v.	>45p	1.17

$^{+}$v. = compared to.

TABLE 66

Comparison between the amount spent on sweets and the number of teeth decayed excluding grade 1 cavities

Decayed teeth excluding grade 1 cavities	Amount spent on sweets				
	<10p	10-29p	30-44p	>45p	Total
	Number of children				
0	2	135	21	63	221
1	2	95	18	48	163
2	2	72	10	37	121
3	0	72	15	28	115
4	2	58	12	26	98
5	2	41	7	21	71
6	0	21	8	17	46
7	0	18	4	7	29
8	0	12	3	7	22
9	0	10	1	6	17
10	0	3	1	4	8
11	0	1	1	0	2
12	0	2	1	2	5
13	0	0	0	0	0
14	0	0	1	2	3
15	0	3	0	0	3
16	0	0	0	0	0
17	0	2	0	1	3
18	0	0	0	1	1
Total	10	545	103	270	928

TABLE 67

Statistical Analyses

The relationship between the amount spent on sweets and the number of teeth decayed excluding grade 1 cavities

The amount spent on sweets is used to illustrate the comparisons drawn between the above parameters using results shown in Table 66

Results of t-tests

Amount spent on sweets		t value
<10p [+]v.	10p - 29p	0.48
	30p - 44p	1.07
	>45p	0.88
10p - 29p v.	30p - 44p	1.38
	>45p	1.21
30p - 44p v.	>45p	0.47

[+]v. = compared to.

TABLE 68

The prevalence of extrinsic tooth stain

Stain	Number of MALES	Number of FEMALES	TOTAL
None	224	291	515
Black	18	25	43
Brown	55	45	100
Orange	23	13	36
Green	97	90	187
Black/ Green	4	6	10
Brown/ Green	12	7	19
Brown/ Orange	-	1	1
Brown/ Orange/ Green	1	-	1
Black/ Brown	9	3	12
Black/ Brown/ Green	1	1	2
Orange/ Green	1	1	2
Total	445	483	928

TABLE 69

Comparison between the mean D-M-F rate and extrinsic tooth stain

Stain	Mean D-M-F rate	MALES	FEMALES	TOTAL
None	Mean	8.107	9.251	8.753
	Standard Deviation	4.660	4.882	4.816
	Number of children	224	291	515
Black	Mean	4.167	7.360	6.023
	Standard Deviation	2.749	3.569	3.589
	Number of children	18	25	43
Brown	Mean	6.491	7.511	6.950
	Standard Deviation	2.943	3.202	3.089
	Number of children	55	45	100
Orange	Mean	6.043	11.000	7.833
	Standard Deviation	3.111	5.508	4.724
	Number of children	23	13	36
Green	Mean	8.629	9.122	8.866
	Standard Deviation	4.076	3.880	3.980
	Number of children	97	90	187
Black/ Green	Mean	4.500	4.167	4.300
	Standard Deviation	3.697	3.545	3.401
	Number of children	4	6	10
Brown/ Green	Mean	5.667	6.571	6.000
	Standard Deviation	2.934	5.028	3.727
	Number of children	12	7	19
Brown/ Orange	Mean	---	5.000	5.000
	Standard Deviation	---	---	---
	Number of children	---	1	1
Brown/ Orange/ Green	Mean	4.000	---	4.000
	Standard Deviation	---	---	---
	Number of children	1	---	1
Black/ Brown	Mean	5.556	5.667	5.583
	Standard Deviation	3.087	0.577	2.644
	Number of children	9	3	12
Black/ Brown/ Green	Mean	4.000	7.000	5.500
	Standard Deviation	---	---	2.121
	Number of children	1	1	2
Orange/ Green	Mean	5.000	2.000	3.500
	Standard Deviation	---	---	2.121
	Number of children	1	1	2

TABLE 70

Statistical Analyses

The relationship between the $\overset{mean}{\wedge}$D-M-F rate and extrinsic tooth stain

Extrinsic tooth stain is used to illustrate the comparisons drawn between the above parameters using the results shown in Table 69

Results of t-tests

Stain		t value
None $^{+}$v.	Black	4.651
	Brown	4.608
	Orange	1.128
	Green	0.045
Black v.	Brown	1.475
	Orange	1.861
	Green	4.586
Brown v.	Orange	1.044
	Green	4.514
Orange v.	Green	1.231

$^{+}$v. = compared to.

TABLE 71

Comparison between the mean Oral Hygiene Index Simplified and extrinsic tooth stain

Stain	Mean Oral Hygiene Index Simplified	MALES	FEMALES	TOTAL
None	Mean	1.216	1.045	1.119
	Standard Deviation	0.499	0.462	0.485
	Number of children	224	291	515
Black	Mean	1.222	1.004	1.095
	Standard Deviation	0.246	0.276	0.283
	Number of children	18	25	43
Brown	Mean	1.193	1.067	1.136
	Standard Deviation	0.336	0.401	0.370
	Number of children	55	45	100
Orange	Mean	1.465	1.054	1.317
	Standard Deviation	5.096	3.777	5.023
	Number of children	23	13	36
Green	Mean	1.367	1.160	1.267
	Standard Deviation	0.420	0.391	0.418
	Number of children	97	90	187
Black/ Green	Mean	1.400	1.250	1.310
	Standard Deviation	0.216	0.409	0.338
	Number of children	4	6	10
Brown/ Green	Mean	1.592	1.086	1.405
	Standard Deviation	0.523	0.308	0.512
	Number of children	12	7	19
Brown/ Orange	Mean	---	0.800	0.800
	Standard Deviation	---	---	---
	Number of children	---	1	1
Brown/ Orange/ Green	Mean	1.000	---	1.000
	Standard Deviation	---	---	---
	Number of children	1	---	1
Black/ Brown	Mean	1.244	1.067	1.200
	Standard Deviation	0.475	0.404	0.447
	Number of children	9	3	12
Black/ Brown/ Green	Mean	1.800	0.800	1.300
	Standard Deviation	---	---	0.707
	Number of children	1	1	2
Orange/ Green	Mean	1.800	1.600	1.700
	Standard Deviation	---	---	0.141
	Number of children	1	1	2

TABLE 72

Statistical Analyses

The relationship between the Oral Hygiene Index Simplified and extrinsic tooth stain

Extrinsic tooth stain is used to illustrate the comparisons drawn between the above parameters using the results shown in Table 71

Results of t-tests

Stain		t value
None [+]v.	Black	0.501
	Brown	0.388
	Orange	2.283
	Green	3.966
Black v.	Brown	0.716
	Orange	2.351
	Green	3.255
Brown v.	Orange	1.974
	Green	2.738
Orange v.	Green	0.553

[+]v. = compared to.

TABLE 73

Comparison between the mean Papillary Gingivitis Score[+] and extrinsic tooth stain

Stain		Mean Papillary Gingivitis Score		
		MALES	FEMALES	TOTAL
None	Mean	8.589	8.000	8.256
	Standard Deviation	2.031	2.387	2.256
	Number of children	224	291	515
Black	Mean	8.833	7.960	8.326
	Standard Deviation	2.455	2.806	2.670
	Number of children	18	25	43
Brown	Mean	8.873	7.711	8.350
	Standard Deviation	1.428	2.437	2.022
	Number of children	55	45	100
Orange	Mean	8.174	7.769	8.028
	Standard Deviation	2.329	2.862	2.501
	Number of children	23	13	36
Green	Mean	9.031	8.111	8.588
	Standard Deviation	1.674	2.175	1.980
	Number of children	97	90	187
Black/ Green	Mean	9.000	7.833	8.300
	Standard Deviation	2.000	2.563	2.312
	Number of children	4	6	10
Brown/ Green	Mean	8.333	7.857	8.158
	Standard Deviation	2.270	2.734	2.387
	Number of children	12	7	19
Brown/ Orange	Mean	---	6.000	6.000
	Standard Deviation	---	---	---
	Number of children	---	1	1
Brown/ Orange/ Green	Mean	10.000	---	10.000
	Standard Deviation	---	---	---
	Number of children	1	---	1
Black/ Brown	Mean	7.444	9.000	7.833
	Standard Deviation	3.046	1.732	2.791
	Number of children	9	3	12
Black/ Brown/ Green	Mean	10.000	10.000	10.000
	Standard Deviation	---	---	---
	Number of children	1	1	2
Orange/ Green	Mean	10.000	8.000	9.000
	Standard Deviation	---	---	1.414
	Number of children	1	1	2

[+]Part of the P-M-A Index.

TABLE 74

Comparison between the mean Marginal Gingivitis Score[+] and extrinsic tooth stain

Stain		Mean Marginal Gingivitis Score		
		MALES	FEMALES	TOTAL
None	Mean	7.397	6.234	6.740
	Standard Deviation	3.261	3.613	3.509
	Number of children	224	291	515
Black	Mean	8.056	6.400	7.093
	Standard Deviation	2.920	3.775	3.504
	Number of children	18	25	43
Brown	Mean	6.927	5.533	6.300
	Standard Deviation	2.834	3.865	3.392
	Number of children	55	45	100
Orange	Mean	7.478	5.231	6.667
	Standard Deviation	2.998	4.086	3.546
	Number of children	23	13	36
Green	Mean	7.691	5.600	6.684
	Standard Deviation	3.015	3.836	3.582
	Number of children	97	90	187
Black/ Green	Mean	7.000	4.333	5.400
	Standard Deviation	3.559	3.933	3.836
	Number of children	4	6	10
Brown/ Green	Mean	6.000	5.000	5.632
	Standard Deviation	3.908	4.041	3.876
	Number of children	12	7	19
Brown/ Orange	Mean	---	0.0	0.0
	Standard Deviation	---	---	---
	Number of children	---	1	1
Brown/ Orange/ Green	Mean	10.000	---	10.000
	Standard Deviation	---	---	---
	Number of children	1	---	1
Black/ Brown	Mean	6.000	8.000	6.500
	Standard Deviation	4.330	1.732	3.873
	Number of children	9	3	12
Black/ Brown/ Green	Mean	6.000	10.000	8.000
	Standard Deviation	---	---	2.828
	Number of children	1	1	2
Orange/ Green	Mean	10.000	6.000	8.000
	Standard Deviation	---	---	2.828
	Number of children	1	1	2

[+]Part of the P-M-A Index.

TABLE 75

Comparison between the mean Attached Gingivitis Score[+] and extrinsic tooth stain

Stain	Mean Attached Gingivitis Score	MALES	FEMALES	TOTAL
None	Mean	0.263	0.247	0.254
	Standard Deviation	1.091	1.218	1.163
	Number of children	224	291	515
Black	Mean	0.556	0.200	0.349
	Standard Deviation	1.294	1.000	1.131
	Number of children	18	25	43
Brown	Mean	0.200	---	0.110
	Standard Deviation	1.007	---	0.751
	Number of children	55	45	100
Orange	Mean	0.304	---	0.194
	Standard Deviation	1.105	---	0.889
	Number of children	23	13	36
Green	Mean	0.299	0.378	0.337
	Standard Deviation	1.052	1.687	1.391
	Number of children	97	90	187
Black/ Brown	Mean	0.222	---	0.167
	Standard Deviation	0.667	---	0.577
	Number of children	9	3	12

[+]Part of the P-M-A Index.

TABLE 76

Statistical Analyses

The relationship between the Papillary Gingivitis Score and extrinsic tooth stain

Extrinsic tooth stain is used to illustrate the comparisons drawn between the above parameters using the results shown in Table 73

Results of t-tests

Stain		t value
None +v.	Black	0.167
	Brown	0.417
	Orange	0.532
	Green	1.890
Black v.	Brown	0.053
	Orange	0.511
	Green	0.606
Brown v.	Orange	0.695
	Green	0.957
Orange v.	Green	1.269

+v. = compared to.

TABLE 77

Statistical Analyses

The relationship between the Marginal Gingivitis Score and extrinsic tooth stain

Extrinsic tooth stain is used to illustrate the comparisons drawn between the above parameters using the results shown in Table 74

Results of t-tests

Stain		t value
None +v.	Black	0.635
	Brown	1.180
	Orange	0.119
	Green	0.184
Black v.	Brown	1.253
	Orange	0.535
	Green	0.687
Brown v.	Orange	0.625
	Green	0.954
Orange v.	Green	0.026

+v. = compared to.

TABLE 79

Mean weight in grames of material collected from gingival scrapings - total sample

Stain	Number of children	Mean weight in grames	Standard Deviation
Black	32	0.011	± 0.007
Non	32	0.011	± 0.005

TABLE 80

Range of weights in grames of gingival scrapings collected from the black and non-stain groups

Stain	Number of children	Range of weights in grames
Black	32	0.0040 - 0.0290
Non	32	0.0030 - 0.0220

TABLE 81

Mean oxygen tension recordings in percentages relating to the four quadrants of the mouth - total sample

Mean	2.100	Mean	2.149
Standard Deviation	0.996	Standard Deviation	0.919
Number of children	64	Number of children	64
Range of recordings	0.63 - 4.20	Range of recordings	0.63 - 3.78
Mean	2.041	Mean	2.120
Standard Deviation	0.954	Standard Deviation	0.950
Number of children	64	Number of children	64
Range of recordings	0.63 - 4.20	Range of recordings	0.63 - 3.78

TABLE 82

Mean oxygen tension recordings in percentages relating to the four quadrants of the mouth - black stain group

Mean	1.378	Mean	1.470
Standard Deviation	0.500	Standard Deviation	0.437
Number of children	32	Number of children	32
Range of recordings	0.63 - 2.10	Range of recordings	0.84 - 2.10
Mean	1.352	Mean	1.352
Standard Deviation	0.456	Standard Deviation	0.416
Number of children	32	Number of children	32
Range of recordings	0.63 - 2.10	Range of recordings	0.63 - 2.1

TABLE 83

Mean oxygen tension recordings in percentages relating to the four quadrants of the mouth - non stain group

Mean	2.822	Mean	2.828
Standard Deviation	0.830	Standard Deviation	0.758
Number of children	32	Number of children	32
Range of recordings	0.63 - 4.20	Range of recordings	0.63 - 3.78
Mean	2.730	Mean	2.888
Standard Deviation	0.814	Standard Deviation	0.666
Number of children	32	Number of children	32
Range of recordings	0.84 - 4.20	Range of recordings	0.84 - 3.78

TABLE 84

Comparison of the oxygen tension recordings between the black stain and non stain groups relating to the four quadrants of the mouth

Results of t-tests

t = 8.43	t = 8.78
t = 8.36	t =11.07

TABLE 85

Mean number of black colonies recorded for the black stain and non stain groups

Stain	Number of children	Mean number of black colonies	Standard Deviation
Black	32	439.95×10^6	$\pm$ 1687.94
Non	32	5.69×10^6	$\pm$ 12.01

TABLE 86

Mean number of black colonies recorded on positive culture plates for both the black and non stain groups

Stain	Number of children	Mean number of black colonies	Standard Deviation
Black	27	521.46×10^6	$\pm$ 1831.09
Non	20	9.11×10^6	$\pm$ 14.24