HISTOMORPHOMETRIC ANALYSIS OF CHANGES IN ORAL STRUCTURES ASSOCIATED WITH CARIOGENIC PROCESS IN THE RAT

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DEDICATION

This work is dedicated to

my darling wife, Dr. (Mrs.) M. B. Allen and

my son, Olukunmi Allen Jr.

CERTIFICATION

This is to certify that the thesis titled

"Histomorphometric analysis of changes in Oral Structures Associated with Cariogenic process in the Rat"

Submitted to the School of Postgraduate Studies, University of Lagos for the degree of Ph.D in Anatomy is a record of original research carried out by:

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DISSERTATION ABSTRACT

HISTOMORPHOMETRIC ANALYSIS OF CHANGES IN ORAL STRUCTURES ASSOCIATED WITH CARIOGENIC PROCESS IN THE RAT

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Experiments were undertaken to develop a model which could be used to investigate the effect of various local environmental factors, dietary components from various food groups such as carbohydrate, proteins and minerals on the macroscopic and microscopic changes involved in the initiation and progression of dental caries and also to quantitate the changes in dental caries and oral tissue structures using stereological methods.

The experimental groups consisting of weaner Sprague Dawley rats (80 - 100g) were innoculated with a mixture of 0.2 mls of oral bacteria (Actinomyces viscosus, Streptococcus mutans and Lactobacilli casei). They were fed with cariogenic diet and various Nigerian foodstuffs, 5 days a week for a maximum of 24 weeks for each

experiment. Four hundred and eighty maxillary and mandibular teeth were examined; a total of 2,400 teeth were examined and scored for caries using modified Keyes Method (Keyes, 1958).

The results showed that the etiology of dental caries is multifactorial which includes microbial, dietary and host factor, it is not sufficient that the three etiological factors be present in the right proportions, but also they must have enough virulence or degree of intensity to induce the dental caries.

To determine the effect of maternal nutrition on caries susceptibility of the rats born of adequately nourished and malnourished mothers, it was found that the incidence and severity of dental caries between offsprings of adequately nourished and malnourished mothers were highly statistically significantly different ($p < 0.001$). Low birth weight was also observed in offsprings of malnourished mothers.

Stereological methods were used to quantitate morphological changes in the salivary glands of rats placed on cariogenic diet (sugar), cassava and control rats fed normal laboratory chow. Quantitative differences were observed between rats placed on cassava and sugar/control rats. Histological (qualitative) differences were also observed between these groups of experimental rats and controls.

Experiments were also carried out to determine whether in the created model, aqueous extracts of the Nigeria chewing sticks can inhibit the caries process. It was found that there is significant reduction in the incidence and severity of dental caries in the group treated with aqueous extracts of Serindeia warneckei. The results of this study showed that aqueous extracts of S. warneckei inhibited the caries process in Sprague Dawley rat.

INTRODUCTION

About a century ago Koch, working with his tubercule baccillus established that a single type of microorganism was the sole cause of tuberculosis. Subsequent findings by others that single types of bacteria caused other diseases led to a strong belief that there are specific bacteria for specific diseases. People then anticipated that one day a bacterial species would be identified for the aetiology of dental caries. However, Miller (1890), an American dentist who had worked in Koch's laboratory claimed that several types of microorganisms were involved in the process of caries.

Some thought that nutritional supplements would solve the problem as in beriberi, pellagra and rickets which responded to treatment with vitamins (Bibby, 1982). Sixty years ago it was confidently expected that nutritional supplements of some sort would soon be found to bring the remaining unconquered ills of mankind under control. People then thought nutritional approach would soon make the dentist's drill useless except as a museum exhibit, but that day has not yet come. The explanation for the location of caries lesions that appealed to dentist can be traced back to Aristotle (Guerini, 1909). This concept was given experimental support 160 years ago by Robertson (1835), who concluded that the reactions that activated caries occurred in food at the tooth surface and did not involve systemic responses of any sort. Except for reservations that some systemic influences may have an indirect effect on caries, Robertson's conclusion holds today. Since the time of Miller, many types of experiments have reinforced the belief that caries begins with the breakdown of foods at the tooth surface. No serious investigator questioned this basic fact. However, there is a question that one must first consider before others. Has the use of fluorides shown that caries can be controlled and

has it been turned into a problem of little importance? of course, even if this is true there will be a continuing need to improve the effectiveness of existing preventive procedures. Beyond this, scientific research requires answers to questions regarding the nature of bacterial involvement, the significance, if any, of the nutritional or systemic factors, tooth resistance, and which variables in the diet, as distinct from nutrition, might modify caries activity. These are the areas where ideas are still conflicting.

STATUS OF CARIES CONTROL

Since neither nature nor man can make a tooth that will resist the highest level of caries attack, attention must be focused on how best to control the strength of the caries attack or at least to keep it from becoming so powerful that it will negate the beneficial effects of fluoride and other preventive regimes. This means doing something about the food factor that determines the severity of the caries attack.

Under the impact of fluoride, recent years have seen dramatic reductions of caries in several Western communities. In the United States (Brunelle, Miller and Carlos, 1982), where about three-quarters of the population now use fluoridated water, caries prevalence has fallen by almost half. Such finding has raised a question as to whether caries should any longer be called the most prevalent and expensive of childhood diseases. However, there are two reasons why caries may still be able to hold that doubtful honour (Bibby, 1982). The first reason is that fluoride therapy does not eliminate dental caries, it reduces its incidence but it cannot provide protection against the severest levels of caries attack. The second reason is that what has been described as a "caries explosion" is taking place in some of the developing

countries of the world, where populations are abandoning their native diets and incresingly turning to western foods (Bibby, 1981).

The prospect of fluorides providing significant benefits in the developing countries is somewhat dismal. The absence of adequate water systems stands in the way of water fluoridation, and the shortage of trained personnel and public funds reduces the chances of putting fluorides to use by other means (Bibby, 1981). Neither educational nor legislative action on cariogenic foods can be advocated to solve the growing caries problem.

THE ROLE OF BACTERIA IN DENTAL CARIES

The past 25 to 30 years have seen much discussion about the bacterial cause of caries. Much of this has dealt with the developemt of dental plaques. For a while it almost seemed that claims were going to be made that Streptococcus mutans was the single and specific cause of plaques and dental caries, and that if this microorganism could be controlled caries would disappear. As happened with similar enthusiasm for the lactobacilli 50 years ago, bacteriologist later found reasons to doubt the claim of bacterial specificity and gave greater credence to the belief that the establishment of S. mutans in the mouth, particularly in animals, was largely dependent on the types of foods eaten and that, as with the lactobacilli, changes in the numbers of S. mutans in the mouth could be the result as well as the cause of caries initiation (Bibby. 1979).

The observed increases in the numbers of S. mutans found with caries probably happen because the other more numerous types of acidogenic organisms produce plaque-laden sites isolated from saliva that gave an acid environment which provides S. mutans with a more competitively favourable growth situation.

This occurs because S. mutans is more aciduric than other oral Streptococci and also because it is more susceptible to the growth inhibiting effects of the oral fluids (Berenie and Bibby, 1981). If this reasoning is correct, the greatest importance of S. mutans in caries would not be in the initiation of caries but in the expansion of lesions.

In the 1920s two species were isolated from carious mouths and were suggested as the causal organisms. Lactobacillus acidophilus or odontolyticus (McIntosh et al. 1922; 1924; 1925) and Streptococcus mutans (Clarke, 1924). Lactobacilli was being focussed on as the causative organism. It produces acid more slowly than Streptococci but can survive and continue to produce acid at pH below 4.0, an acidity which inhibits most acid producing organism. In the early 1960s when scientist were talking about dextrans in plaque, the emphasis changed to streptococci, which represent up to 80% of the plaque organisms. S. mutans, the organism associated with caries in 1924, is now considered the most likely causative organism. The evidence is as follows:

1. S. mutans is a rapid acid-producer and synthesizes copious dextran from sucrose.

2. When gnotobiotic animals are infected with S. mutans, very extensive caries is rapidly produced, especially on smooth surfaces; other bacteria (Lactobacilli and Actinomyces) also produce caries but apparently not so vigorously, although no experiment appears to have been done in which the cariogenicity of many bacteria have been directly compared under identical conditions.

3. A correlation has been reported between the number or the proportion of S. mutans in plaque (expressed in various ways) and the presence or absence of a high DMFT[1] score in human subjects. Even within individual mouths, the proportion of S. mutans on surfaces with caries has been found to be higher than on sound surfaces (Bibby, 1982). On the other hand, a study of the initiation of caries in various sites along with the investigation of the presence of S. mutans in children showed little correlation and gave no support to the belief that this organism was essential for caries (Hardie, 1977). Also Huxley et al. (1975) found in rats that the initiation of caries neither required S. mutans nor was accelerated by a higher proportion of these organisms in plaque. Therefore, there is circumstantial evidence that S. mutans dominates the plaque bacteria and plays an important part in caries in the normal mouth. It does not necessarily follow, however, that this organism is essential for caries or that caries would be prevented if S. mutans were inhibited. If this dominant organism were removed, other acid-producing species which are normally unable to compete successfully with it might then assume a leading cariogenic role. In considering the relationship between lactobacilli and S. mutans, it is well established that in the gnotobiotic animal infection with S. mutans alone can produce caries. A combination of Streptococci and lactobacilli would be expected to be more effective in producing a pH sufficiently low to dissolve enamel in the environment of the plaque. Streptococci cease to produce acid at pH values of about 4.3 whereas lactobacilli may continue , but at a reduced rate (although the optimum pH for both species is about 6.0), to below 4.0.

[1]DMFT: Diseased Missing and Filled Teeth.

The joint effect of the two species would, therefore, be expected to attack the enamel more intensively than streptococci alone.

Although recent years have produced much excellent research on oral bacteriology it can be questioned whether it has brought us much closer to practical ways of preventing caries. Two considerations gave rise to this somewhat negative assessment. One is the knowledge that several different types of organisms are capable of producing caries. The second is that even if one bacterial type after another is therapeutically inactivated, bacterial variants can be expected to evolve to take their place. These could be microorganisms with increased saccharolytic capacity, or potentially pathogenic types like those that emerge when the natural bacterial population of the gut is suppressed or eliminated, as with antibiotics. Since this modified bacterial population in the mouth may be less desirable than the one that normally thrives therin, it is probably safer not to try to change it but find a pragmatic solution to the effect of oral bacteria on dental caries.

BACTERIAL COMPONENTS AND PLAQUE FORMATION

Plaque is a complex ecological system and our knowledge of the various stages in its maturation is far from complete. However, a clearer picture is gradually emerging. The first stage in the formation of plaque on a "smooth" surface is the absorption of salivary components including glycoproteins to form pellicle. Bacteria then adhere to pellicle by means of their surface components. There is considerable discussion on the relative contributions of the different bacterial surface components. There are those who argue for the importance of ionic or hydrophobic interactions

(physico-chemical properties) while there is increasing evidence for a specific interaction based on lectin-like molecules, probably surface proteins. It is likely that both systems contribute.

Bacterial accumulation to form plaque is the result of the growth of adherent organisms and their interaction with other bacteria that find an ecological niche. An important (though not essential or exclusive) feature is the formation from sucrose of polysaccharides, particularly glucans, by organism such as S. mutans. Although these were originally nominated as playing a major role in the adherence of S. mutans to the tooth surface, the concensus now is that they bind to the bacterial cell surfacce to form bridges between cells and thus 'glue' the plaque together (Staat, Langley and Doyle, 1980).

DIET

Over the years various types of food have been named as the main cause of caries. "Sweetmeats" and sugary foods by the early English and French dentists, the manner of food preparation by Robertson, and flour and potatoes by Miller. Since the begining of this century refined sugar, sucrose, has received most of the blame (Bibby, 1982).

There is good reason to believe that the modern food usages of the Western world are particularly conducive to caries. It is now well documnented that the great increase in caries noted by the early dentists accompanied the dietary changes that took place in Europe as sugar became plentiful, as wheat flours were more finely milled, and potatoes became a staple food. This belief is supported by the dramatic increases in tooth decay that have occurred in primitive populations in the Arctic, and the Pacific

regions, and in Africa as they began using the refined food of the West (Bibby, 1982). Perhaps the most dramatic demonstration of the dominant role of foods in caries was seen in relation to the two World Wars. In the First War dietary restrictions in England, Denmark and Germany produced dramatic drops in caries followed in the succeding peace by a return to previous levels, as restricted items, sugar and refined flour became more plentiful. The same pattern was seen again in England and the Scandinavian countries during and after the Second World War. Caries benefits were independent of findings on growth and health and were greatest where dietary restrictions were harshest. Whether the reduction in sugar intake, the elimination of more highly refined flour or reduction in the frequency of eating, played the most important part has been the subject of debate. Probably all were involved.

In the matter of the cariogenicity of diets, it is not enough to agree that a substance such as sucrose is the most destructive food ingredient, for we know from animal tests (Haldi et al. 1953) and the Viperholm study (Gustafssom et al, 1952) that sucrose used in liquid form (its principal use by man) is only fractionally as destructive to the teeth as when eaten in solid form. Further, sucrose is almost always used in mixtures of various sorts, some of which will reduce and others increase its destructiveness. Therefore, to identify the most caries facilitating items in our diet it is necessary to consider first the complete foods, and then their individual ingredients. Only in this way will information that could be used to prescribe non-cariogenic diets be obtained, or be utilized by manufacturers who want to make their products less cariogenic.

CARIOGENICITY OF DIFFERENT FOODS

Different sources of information can indicate whether some types of foods are more cariogenic or less cariogenic than others. These are:

- caries prevalence in relation to the use of a specific food or group of foods
- tests on animals
- the production of experimental caries in human teeth
- the measurements of the caries associated reactions of foods, such as acid production and enamel demineralization on fermentation of foods by oral bacteria, acid production in plaques.

BAKED FOODS

Earlier, reasons were given for believing that the sugar component of diets was of major importance in determining their cariogenicity. Epidemiological evidence of a somewhat different kind has been reported in a recent survey. This concludes that "in addition to sugar one other refined carbohydrate, wheat, may be positively related to the prevalence of dental caries in nations throughout the world" (Screenby, 1982). This statement supports the fact that baked foods that combine wheat flour and sugar may be peculiarly cariogenic.

FREQUENCY OF EATING

Observations on caries activity in children and adults have led to the conclusion that the frequency of eating is of great importance in determining the severity of the caries attack. Most dietary studies show that the severity of caries increases with the frequency of eating (Trithart and Weiss 1957; Gustarfsson et al, 1952).

The following inferences can be drawn from the available clinical or epidemiological evidence:

- foods can differ in their ability to produce caries.
- these differences can result from variation other than their sugar content.
- the frequency of eating is a significant determinant of caries activity.
- liquid foods seem to be less destructive to the teeth than solid foods (Bibby, 1982).

FACTORS WHICH ENHANCE CARIES PROGRESSION

Acid Production From Foods

Although the formation of acid from carbohydrate foods is the fundamental starting point of caries, it is difficult to believe that in-vitro (or in-vivo) measurements of this parameter can cast much light on the relative cariogenicity of foods.

Among reasons for this opinion is that the amount of acid formed differs in different tooth sites. Several studies have shown that the amount of acid formed does not parallel the amount of enamel that will be demineralized (Bibby and Mundorff, 1975; Bibby, 1981). Therefore, measurements of the amount of enamel dissovled is a preferable index of the action food will have on the teeth. However, it is worth recording that foods differ widely in the amounts and speed of acid production. Fermentation is slowed down by sugar concentrations higher than 5 or 10 per cent and inhibited by acid foods. More acid is produced from some flour and sugar mixtures than from corresponding sugar concentrations (Frostell, 1964).

Demineralization of Enamel

The dissolution of enamel by pure chemicals has been the subject of much study. The fact that solubility work of equal reliability cannot be done using biological variables, such as saliva, oral bacteria or foods, seem to have discouraged investigators from trying

to establish differences in the amounts of enamel demineralized by fermenting foodstuffs.

One group (Osborn, Noriskin and Statz, 1937) found that the unrefined corn and sugar used by native people demineralized teeth less than did the refined counterpart used by the white people in the area. Jenkins et al. (1944) confirmed this finding and showed that there were enamel protective substances in unrefined cereal and unrefined sugar. Over the years, different methods have been used to compare the enamel demineralizing power of about 200 foods, including varieties of cookies, cakes, candies, vegetable, grains, desserts, and beverages (Bibby and Mundorff, 1975; Bibby, 1981). From these findings it can be concluded that the amount of enamel dissolved does not parallel the amounts of acid produced from foods, nor does it follow the sugar content of the substrates (Bibby and Weiss, 1970). Other pieces of information of interest to come from such studies are that sugar and flour mixtures can be more destructive to enamel than the sugar alone (Bibby, 1981); milk in foods seems to offer protection to the enamel; and phosphate added to candies and other foods makes them less destructive.

Acid Formation in Plaques.

About 50 years ago, Stephan (1944) showed that the pH of dental plaques on the teeth of caries-active patients was lower than that of plaques from persons with little or no caries. The significance of plaque pH findings is open to question since different measurement procedures produce different results. The pH curves given by indwelling electrodes (Graf and Muhlemann, 1966) located between the teeth differ in shape from those

given by probe electrodes or by the harvesting method (Bibby, 1982). Claims that the results given by the indwelling electrodes are most meaningful because they register what is happening at the principal site of caries attack have been questioned recently. Reasons for this are doubts about the placement of the electrode and the nature of the plaque accumulations on the electrodes surface, together with the finding that almost any kind of sugar-containing food or cooked starch give essentially the same pH depressions to levels of between pH 4 and 4.5 (Miller, McLaughlin and Mullen, 1982).

Stephan (1940) used fine antimony electrodes to measure the pH of plaque which had been allowed to accumulate over 4 days. He found that within 2 or 3 minutes of rinsing the mouth with sugar solution (between 10% and 50% of glucose or sucrose were used), the pH of the plaque fell from an average of about 6.5 to about 5 and took up to 40 minutes to return to the original figure. (see 'Stephan Curve', Fig. 1.1) shows this.

FIG. 1.1

The pH of plaque after rinsing the mouth with 10% glucose solution. The dotted line represents a typical value for the pH below which decalcification of enamel begins (the "critical pH)"

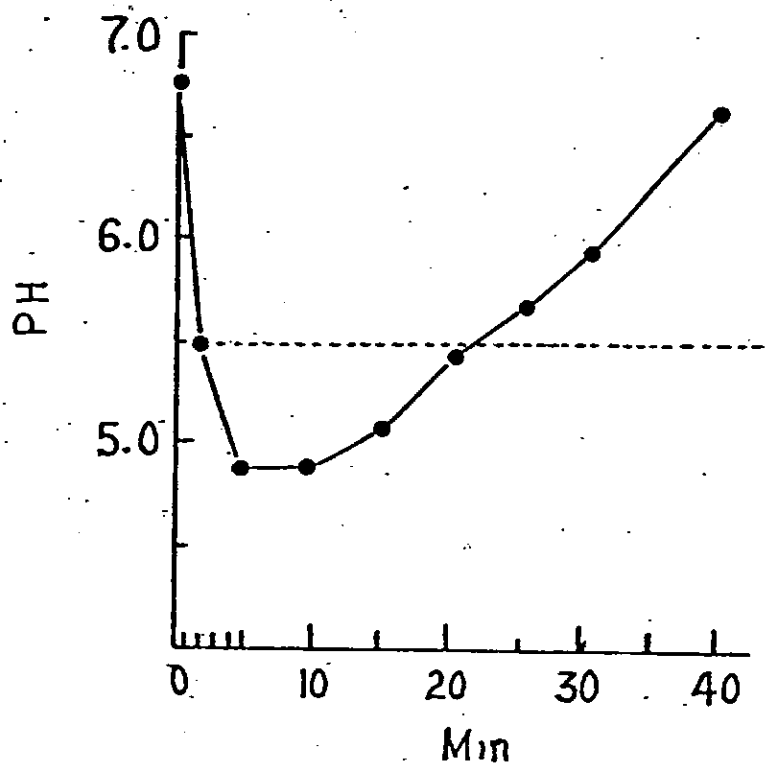


Fig. 1.1

When the teeth were thoroughly brushed after the completion of such an experiment, a second sugar rinse produced a much smaller change in pH since the plaque containing the acid-producing bacteria had been largely removed. The main results have been confirmed in plaque near the contact points (i.e. potential sites of caries) in preference to the smooth surfaces originally tested, and with normal carbohydrate foods as opposed to the sugar rinses originally used (Jenkins and Kleinberg, 1956; Ludwig and Bibby, 1957).

Factors Involved in the Rise of pH

When sucrose reaches the plaque some are converted into extracellular polysaccharides and some, after hydrolysis into glucose and fructose, polymerize into intracellular polysaccharides and the remainder undergoes glycolysis to lactic acid. The pH rises, partly by loss of acid by outward diffusion and partly by conversion of lactic acid into the less highly ionized acetic and propionic acids. After eating, the concentration of sugar in saliva and plaque falls quite rapidly so that further acid production from free sugar ceases but continues from the levans and intracellular polysaccharides (and, to a smaller extent) from the dextrans also.

When Lilienthal (1955) carried out tests on the buffering power of plaque removed from the teeth of people who had abstained from tooth-brushing for some days, bicarbonate was absent and he concluded that bicarbonate was unimportant in buffering plaque but this

conclusion is clearly wrong. The evidence that saliva (whose chief buffer is bicarbonate) plays an important part in reducing the drop in pH which occurs after carbohydrate and in accelerating the rise in pH is as follows:

1. Englander et al. (1959) compared the drop in plaque pH after sugar rinses in subjects when normal access of saliva to the plaque was allowed and when saliva was prevented from reaching the plaque.

2. In some subjects who are very caries-prone, the pH of the plaque may remain between 5 and 6 for some hours after meals. It is also known that if such subjects suck a sweet, thus increasing saliva flow as well as its pH and buffering power, the pH of the plaque rises (Jenkins and Kleinberg, 1956).

3. The incidence of caries greatly increases in animals after removal of the salivary glands. Evidently saliva has an important influence in reducing caries mainly by its buffering power and partly by its antibacteria actions (Radden, 1962).

ANIMAL MODELS FOR DENTAL CARIES RESEARCH: GENERAL CONSIDERATIONS ON ETIOLOGICAL FACTORS.

Caries is a disease found in humans throughout the world, particularly in technically developed countries. It is less frequently observed in humans living in remote areas far from urban centers. Some animals are also susceptible to the disease in the wild or in the captive or domesticated state, but less so in the former (Bowen et al, 1966). Rodents, such as the rat and the hamster, and some nonhuman primates show considerable susceptibility to dental caries. The variation in the prevalence of this disease is greatly influenced by the

dietary habits of the animal which may in turn determine the implantation and activity of the tooth flora. Hereditary or systemic factors, however, could also influence the expression of the disease. Monkeys, such as Macaca irus, have been found useful in experimental dental caries studies (Bowen et al, 1966; Bowen, 1967; Bowen and Cormick, 1967). Macaca mulata has been used by Lehner et al. (1975a; 1975b) as an experimental model for immunological studies of dental caries. Other monkeys such as the squirrel monkey have potential to be used as models to study caries.

Dogs and cats have not been used in dental caries studies as they do not seem to develop the disease readily. No systematic studies has been done to understand the reasons for such a lack of susceptibility to the disease, but it could be speculated that diet, salivary composition, morphology of teeth, and characteristic eating and occlusal habits may contribute to their resistance to the disease. Although dogs are not caries prone,they accumulate large amounts of calculus and bacterial deposits around their teeth (Chauncey et al, 1963).

Herbivores such as the cow or the sheep may have carious-like lesions, but do not develop the rampant form of the disease seen in humans and in some experimental animals such as the rat. Because of their size and their special dietary and ruminant habits, they do not constitute useful models for caries studies (Bowen, 1967). Most rat and hamsters species are highly susceptible to caries, provided they are fed a sugar containing diet and injected with a cariogenic oral flora. Little use has been made of mice as animal models in caries research. Recently, a black fat mouse (PBB) has been described (Navia and Hunt, 1972). When infected with the appropriate cariogenic organism presents extensive decay of the permanent molars. Their small size, which allows the use of large number in

confined quarters, and the relative speed with which lesions are formed are desirable characteristics .

Other animals, such as the guinea pig and the rabbit, are not useful in dental caries research as they do not develop carious lesions. The special morphology of their molars and the fact that they are continously erupting (just as the incisors) are probably the reasons for their lack of susceptibility to dental caries. Apart from availability and the large amount of background information on these animals, the reason for their wide use in caries investigations is that the gross and histopathological appearance of the carious lesions are essentially similar to those found in human teeth.

Caries can be produced in a number of different laboratory animals, but recently the albino rat has been the animal of choice. Most of the caries related work used pure carbohydrates, and only a few investigators have examined the effects of human foodstuffs. Most of this work has limited value because they were done before it became possible to regulate the frequency of feeding and the amount of food eaten by each animal. Accordingly, the results were largely determined by whether the animals liked the food and ate it frequently or in small or large amounts. On feeding 53 human foodstuffs to rats, one investigator (Stephan, 1966) found the highest caries "score" was given by a sandwich cookie, with the next highest given by sucrose, apples, bananas and raisins. No caries was produced by peanuts and very little by potato chips. Others (Konig and Grenby, 1965; Konig, 1967;) showed that honey and sugar additions to the bread increased caries but that it was reduced by cheese, and that 70 per cent and 80 per cent extraction wheat flours were equally cariogenic. In contrast, McDonald (1972) produced significantly less caries with brown bread than with white bread, although the difference did not appear when the wheat

flours were fed to the rats. Hartles (1967) concluded that "biscuit diets were morecariogenic than might have been expected from their comparative low sugar content (9.9 and 22.3%).

It is possible that the manufacturing processes may have influenced (their) composition and consistency". Of particular interest is the finding (Frostell and Baer, 1971) that gelatinized starches were up to ten times more cariogenic than the untreated counterparts. (Table 1.1)

In the past, regulated amounts of some human foodstuffs have been fed to rats by the Konig Hoffer rat feeding machine (Konig, Schmid and Schmid, 1968), which controls the frequency of eating. An early finding was that the amount of caries increased with the frequency of eating. A further modification of animal testing of food cariogenicity requires that the essential nutrients be fed to rats by stomach tube, the test foods alone being allowed in the mouth. Using this procedure, Bowen et al. (1980) found that a sandwich cookie was most cariogenic, followed in order by an unsweetened breakfast cereal, chocolate candy, potato chip, caramel and chocolate. Navia (1980) obtained more caries with corn flakes than with milk chocolate, which was more cariogenic than potato chips. It is worth noting that with the foods used in Navia's study the results obtained in tests made without gastric intubation were essentially the same as when it was used. Sintes et al. (1982) using the Bowen procedure, compared several fruits. They found bananas most cariogenic, followed in order by oranges, apples, freeze-dried apples, then sucrose. In view of the inconsistencies in the findings in animal models it does not seem that they give a true indication of the caries producing potentials of foods in man.

TABLE 1.1

60-DAY CARIES IN RATS FED REGULAR AND GELATINIZED STARCHES

(data from Frostell and Baer, 1971).

		No of Animals	Caries "score"
Wheat	regular	24	2.5
	gelatinized	24	6.42
Potato	regular	18	16.60
	gelatinized	23	33.52
Arrowroot	regular	23	2.87
	gelatinized	12	24.58
Tapioca	regular	21	4.14
	gelatinized	24	32.55

THE IMMUNOLOGY OF CARIES AND THE POSSIBILITY OF PREVENTION BY A VACCINE

The use of vaccines has proved a very effective means of preventing a number of diseases, and studies by several groups over the past 20 years have aimed at establishing whether a vaccine against dental caries is feasible. Dental caries is the net result of the interplay of a number of factors, and therefore it is not suprising that there are a variety of means that could be effective in prevention. The basic cause of caries is the production of acid from dietary carbohydrate by plaque bacteria, so if accumulation of these bacteria can be minimized, then a reduction in caries should result. The first indication that vaccination might be feasible came from the work of Bowen (1969) who showed that monkeys injected with killed cells of Streptococcus mutans developed less caries than contol animals. Between 1969 and the mid-1970s there were a series of papers on the reduction in caries following vaccination of monkeys or rodents with cells or cell walls of S. mutans. These prompted an International Symposium in 1977 on "Secretory Immunity and the Immune Sytem", at which developments and prospects were discussed (McGhee, Mestecky and Babb, 1978). Comparisons in saliva and serum of caries-high and caries-low subjects showed that the concentrations of IgA and IgG were higher in the serum of the caries-high, but lower in the saliva of the caries-low group, the differences being significant for IgA (Kennedy et al., 1968).

Possible Mechanism of Vaccine Action

The classical mechanism of action of a vaccine is for the circulating antibodies in combination with complement and other components of the humoral system to cause the death of the target organism. Secretory antibodies lack this capacity and accordingly this has led to some workers expressing sceptism about the effectiveness of a vaccine. However, a number of functions for secretory immunoglobulins are now known. Although the prime target function in caries vaccination has not yet been identified, it is reasonable to implicate adherence, growth and/or acid production by cariogenic bacteria (McGhee and Michalek, 1981).

Bacterial components that could be invoked as playing a role in adherence and bacterial accumulation are:

- the type-specific polysaccharides and teichoic acids,
  including lipteichoic acids;
- surface protein antigens
- dextran binding proteins

Antibodies may also inhibit enzymic activities and hence the interest in antibodies against glucosyltransferase, which sythesizes glucans from sucrose. Another possibility is to have antibodies against enzymes required for sugar uptake and metabolism. All of these proposals have their protagonist but the best results have been obtained by immunizing rodents with glucosyltranferase and monkeys with antibodies against purified surface

proteins from S. mutans (McGhee and Michalek, 1981). However, immunization against dental caries may not work because it is against one bacterium (Streptococcus mutans) whereas studies of smears suggest that that plaque may contain as many as 400 million organisms, the major ones being Streptococcus, Staphylococcus, Actinomyces, Veillonella (Stralfors, 1950)

RATIONALE

Over the past several decades, workers have examined the composition of dental plaque and tried to relate their findings to its metabolism and the pathological processes that result from it. However, a major obstacle to this study has been the lack of sufficient material for performing in-vitro and in-vivo investigations. In order to carry out such experiments at the present time, it is necessary to have volunteers who would stop brushing their teeth for several days to allow plaque to accumulate enough for analysis of various biochemical events and/or shifts in microbial populations. In most cases plaque sampling would be required at regular intervals and may be for a long duration of time. Obviously, experiments involving such plaque sampling is severely limiting in man. Because of this and the difficulties involved in working with humans, researchers have turned to model systems designed to allow the study of bacterial growth, plaque metabolism and its sequellae as affected by different variables.

One of such models is the animal model which has been used extensively for the study of oral diseases such as oral cancer, dental caries and periodontal disease. Most of the studies however, on dental caries have been done by innoculating the animals with single cariogenic bacterium in order to initiate the disease.

However, in-vitro bacteria combination experiments (Stephans and Hemmens, 1946) showed that combining bacteria gave different pH responses with glucose than those seen with the individual bacterium and that the differences in the acid producing capabilities of

plaque from different individuals may reflect variation in the properties of microorganisms having different acid production potentials.

Therefore, such experiments done with single organisms may not reflect accurately what operates in the human dental plaque.

It has also been estimated by Stralfors (1950) that plaque contained 400 million organism as counted on smears, but the major microbial constituents are Streptococcus, Staphylococcus, Actinomyces, Veillnella, Neisseria and Fusobacteria. Others such as Lactobacillus, Yeasts, Bacteroides and Vibros are generally present at lower levels (Gibbons et al, 1964; Loesche, Hardie and Syed, 1972; Hardie and Bowden, 1974; Socransky et al, 1977; Denepitiya and Kleinberg, 1982; Wijeyeweera et al, 1984).

AN OUTLINE OF THE STUDIES:

OBJECTIVE

The overall objective of this project is to identify and characterize the effect of various local enviromental factors on the initiation and progression of dental caries. It would also investigate the effect of dietary components from various food groups such as carbohydrates, proteins and minerals on the macroscopic and microscopic changes involved in the initiation, progression and/or initiation of dental caries.

SPECIFIC AIMS

1. To create a model for caries initiation using a multibacterial oral innoculation procedure on the rat dentition.
2. To investigate the effects of maternal nutrition on the development of dental caries.
3. To investigate the potential of Nigerian foodstuffs as cariogenic and periopathic agents.
4. To study the effect of local chewing stick extracts on caries process and periodontal tissue.
5. To quantitate the changes in dental caries and oral tissue structures (at a practical level) using Stereological methods.

CHAPTER TWO

TO CREATE A MODEL FOR CARIES INITIATION USING A MULTIBACTERIA ORAL INNOCULATION PROCEDURE ON THE RAT DENTITION.

SUMMARY

Experiments were carried out to determine the role played by multi-innoculation of 0.5mls aliquot concentration of major prominent oral bacteria (Lactobacilli casei, Streptococcus mutans, and Actinomyces viscosus) and 56% sugar diet on the pathogenesis of dental caries. 40 weaner Sprague Dawley rats weighing between 80 - 100gms at 17 days were divided into the following treatment groups consisting of 10 animals per group. Group 1 - water, food, no bacteria innoculation (BI), Gp. II - water, food + sugar and no BI; Gp. III - water + Bacteria, food and BI; Gp. IV - water + Bacteria, food + sugar, and BI. Each animal was sacrificed at 16 weeks, the upper and lower jaws were defleshed, dried and scored for caries observation using modified Keyes method (1958). Dental caries was initiated in all the experimental groups, i.e. Group II, III, IV. The incidence of caries in all the experimental groups was compared with the control group (Gp. I) and was found to be significantly different ($p < 0.01$). The severity of caries was greater in the experimental groups when compared to the control ($p < 0.001$). The incidence and severity of caries between experimental groups show no significant difference.

The data suggest that the cariogenic diet (56% granulated sugar) in the presence of multibacteria innoculation is responsible for the cavitations seen on the molar surfaces of the teeth. A model for cariogenesis is hereby created using Sprague Dawley rat.

INTRODUCTION

Dental Caries have been known to be the most prevalent dental disease and is related to dental plaque. Dental plaque is the term used to refer to the microbial deposits found on the surfaces of the teeth and adjacent soft tissues. The composition of this plaque depends on many factors which includes the types of bacteria found elsewhere in the mouth (Liljemark and Gibbons, 1971; Hardie and Bowden, 1974; Socransky et al, 1977; Killan et al, 1979), components of the diet especially the frequency of ingestion and retention of fermentable carbohydrates (Stahn, 1966; Hargreaves, 1968; Littleton et al, 1967a, 1967b) and the composition and flow of saliva (Salako and Kleinberg, 1988).

Lack of sufficient materials for performing in-vitro and in-vivo investigations has been a major obstacle in the study of dental caries in man. In order to conduct these studies in the present time one would require volunteers who would stop brushing their teeth for several days to allow plaque accumulation. In most cases this is for a long duration of time. Obviously this is quite difficult to achieve in humans. Therefore researchers turned to laboratory animal model systems designed to allow the study of bacterial growth, plaque metabolism and their interaction with the oral cavity milieu and its sequellae as affected by different variables.

Most of the studies, however, on dental caries have been done by innoculating the animals with a single cariogenic or periopathic bacterium in order to initiate the diseases (Krasse and Carlsson, 1970).

Stephans and Hemman, (1946), in test-tube bacteria combination experiments however showed that combined bacteria gave different pH responses with glucose than those

seen with the individual bacterium and that the differences may reflect variations in the properties of microorganisms having different acid production potentials. Therefore such experiments done with single organisms may not reflect accurately what operates in the human detal plaque. It has been estimated by Stralfor (1950) that plaque contained 400 million organisms as counted on smears, but the major microbial constituents are streptococcus, staphylococcus, actinomyces, veillonella, neisseria and vibrios are generally present at lower levels (Gibbons et al 1964; Loesche, Hardie and Syed, 1972; Hardie and Bowden, 1974; Socransky et al, 1977; Denepitiya and Kleinberg, 1982; Wijeyeweera et al, 1984). Because of this problem of not having a suitable model to work with, a cariogenic diet (56% granulated sugar) and multibacteria oral innoculation was fed to rat and this created cavitations on the molar surfaces of the teeth.

MATERIALS AND METHODS

40 weaner Sprague-Dawley rats weighing between 80 - 100gms at 17 days old were purchased from the Laboratory Animal Centre, College of Medicine, University of Lagos.

The animals were left to acclamatise for one week in the departmental rat room (25 - 28 oC) with light from 0600 to 1900h daily.

Animals were innoculated with 0.5mls of S. mutans and a combination of Actinomyces viscosus + Lactobacilli casei of the same volume. The innoculations were done for 5 days consecutively using a sterile needle, syringe and swab. 1 ml of bacteria broth was added to 100mls of water taken ad libitum. Animals were randomly divided into 4 main groups consisting of 10 animals per group. Group I: water, food (Laboratory chow) no bacteria innoculation (BI): Group II: water, food + sugar no BI; Group III: water + bacteria, food and BI: Group IV: water + bacteria, food + sugar and BI.

Animals were sacrificed after ether anaesthesia at 16 weeks. The upper and lower jaws were defleshed, 480 maxillary and mandibular teeth were scored for caries using the modified Keyes method (Keyes, 1958). (Fig. 2.8)

To score the teeth for caries: the jaw was fixed in 10% formol saline for 24 to 48 hours. The soft tissue was defleshed as above. The boundaries of the lesion were noted and recorded on the Charts. All scoring was done under the Stereomicroscope at 20 to 40X magnification.

INCIDENCE:

Represents a simple enumeration of the number of teeth affected by caries in one quadrant of the jaws. The minimum being 0 and maximum 3 for any of the quadrant.

SEVERITY:

Represents the size/area of the carious lesion on any particular tooth. The severity for the tooth is assigned values ranging from 1 to 4 according to their measurement in "mm".

a.)	*0*	*no lesion*
b.)	*1*	*small lesion (measuring between 1 - 2.5mm)*
c.)	*2*	*medium lesion (measuring between 2.5 - 4mm)*
d.)	*3*	*large lesion (measuring between 4 - 5.5mm)*
e.)	*4*	*complete destruction (measuring > 5.5mm or covering more than 80% of the occulusal surface).*

The scores of various groups were then analysed statistically using one-way analysis of variance (ANOVA).

RESULTS

From the results we were able to initiate caries formation in all the experimental groups i.e. Group II, III and IV. Scores were analysed using the modified Keyes method (Keyes, 1958). The incidence of caries in all the experimental groups were compared with the control group (Group I). The means of incidence of caries between Group I and the three groups were significantly different ($p < 0.01$), (Figures 2.2 - 2.5). Means of incidence of caries between Group II, III and IV showed no significant difference as shown in Fig. 2.1.

Also the severity of caries was greater in the experimental groups when compared to the control ($p < 0.001$). (Figs. 2.2 - 2.5).

Although there were no statistically significant differences between the three experimental groups, Group IV still showed more severe incidence of caries as compared to the other groups as shown in Table 2.2 and 2.3.

TABLE 2.1

INCIDENCE AND SEVERITY OF CARIES IN RATS TREATED WITH MULTIBACTERIAL ORAL INNOCULATION AND SUGAR DIET

GROUP I		GROUP II		GROUP III		GROUP IV	
INCD.	SEV.	INCD.	SEV.	INCID.	SEV.	INCD.	SEV.
7	7	11	11	10	11	11	11
8	8	12	14	12	13	13	10
7	7	10	10	10	10	10	10
8	8	12	17	9	9	9	12
$\bar{X}$ 7.50	7.50	11.25	13.00	10.25	10.75	10.75	14.75
S.D 0.577	0.577	0.957	3.162	1.258	1.708	0.957	3.594

TABLE 2.2

INCIDENCE OF CARIES IN RATS TREATED WITH MULTIBACTERIAL ORAL INNOCULATION AND SUGAR DIET

CONTROL	EXPERIMENTAL		
GROUP I	GROUP II	GROUP III	GROUP IV
7.50 ± 0.58^a	11.25 ± 0.96^b	10.25 ± 1.26^b	10.75 ± 0.957^b

a = means $\pm$ S.D

b = $p < 0.01$

TABLE 2.3

SEVERITY OF CARIES IN THE RATS TREATED WITH MULTIBACTERIAL ORAL INNOCULATION AND SUGAR DIET

CONTROL	EXPERIMENTAL		
GROUP I	GROUP II	GROUP III	GROUP IV
7.50 ± 0.58^{a}	13.00 ± 3.16^{c}	10.75 ± 1.71^{c}	14.75 ± 3.594^{c}

a = means $\pm$ S.D

c = $p < 0.001$

FIGURE 2.1

BAR CHART SHOWING INCIDENCE AND SEVERITY OF

DENTAL CARIES IN THE EXPERIMENTAL AND CONTROL GROUPS

Fig.2-1

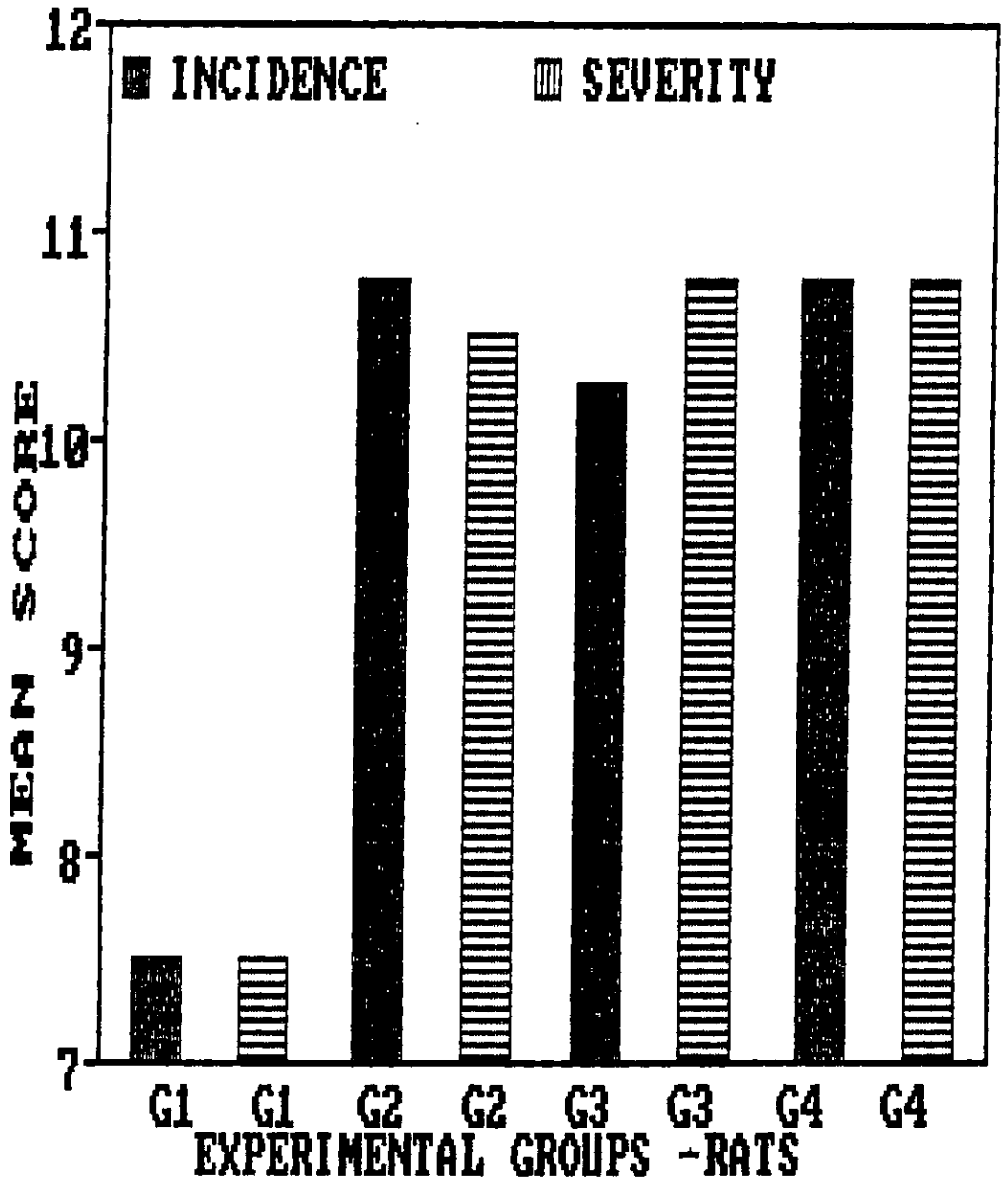

12
11
10
9
8
7
INCIDENCE
SEVERITY
MEAN SCORE
G1 G1 G2 G2 G3 G3 G4 G4
EXPERIMENTAL GROUPS -RATS

FIGURE 2.6

KEYES (1958) SCORING KEY

1. Surface mark or crack
2. Small perforation
3. Medium lesion and perforation
4. Large lesion and perforation
5. Quadrant obliteration

SCORING KEY

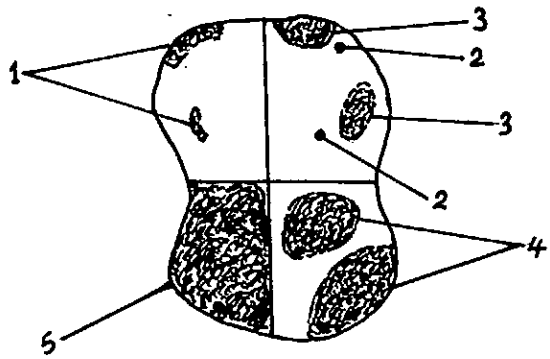


Fig. 2.6

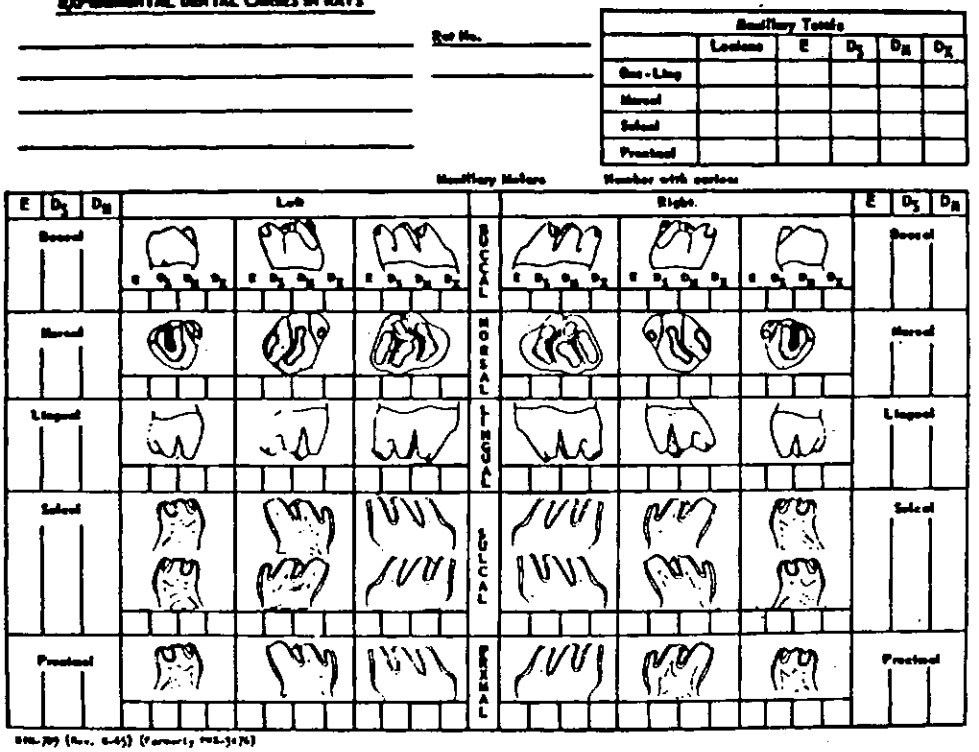
FIGURE 2.7

CARIES SCORE CARDS (Keyes, J. of Dental Research, 1958,

Amer. Dental Assoc.

(A.) Maxillary molar card. (B) Mandibular molar card.

A



B

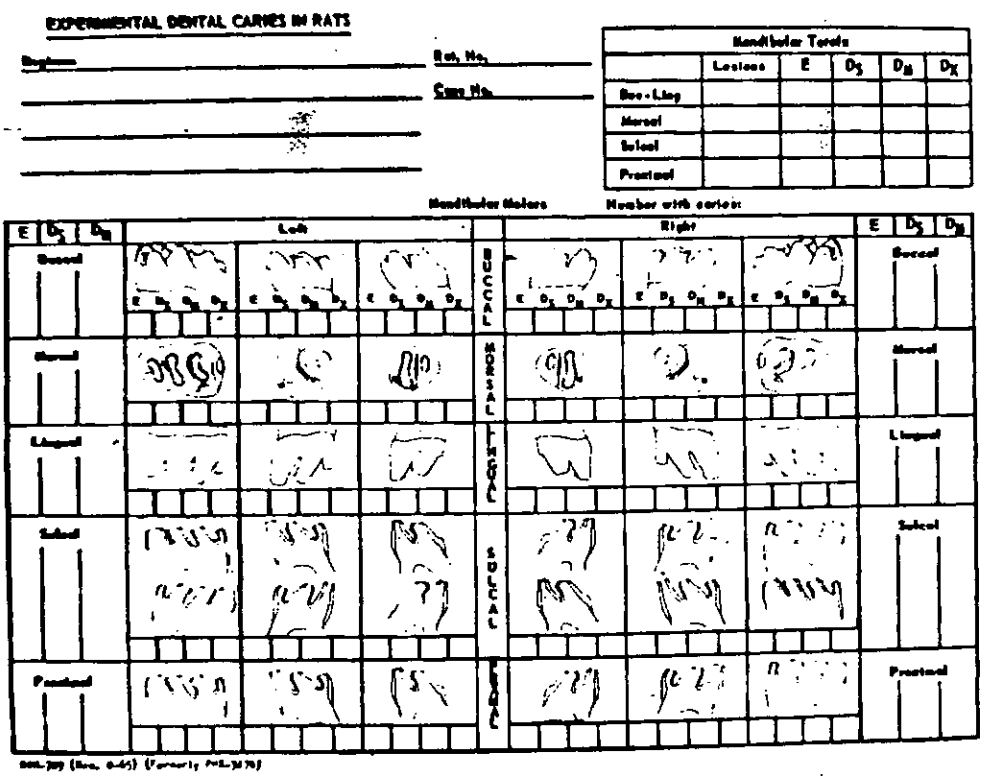


Fig. 2-7 Caries Score Cards (Keyes, J. of Dental Research, 1958, Amer. Dental Assoc.). (A) Maxillary molar card. (B) Mandibular molar card.

FIGURE 2.8

MODIFIED CARIES SCORE CHART FOR SPRAGUE-DAWLEY RAT

Fig. 2.8

SCORE CHART FOR SPRAGUE DAWLEYS RAT

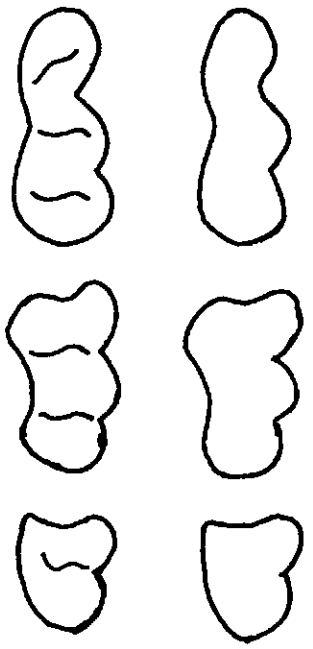
TREATMENT

SCORE CHART FOR SPRAGUE DAWLEYS RAT

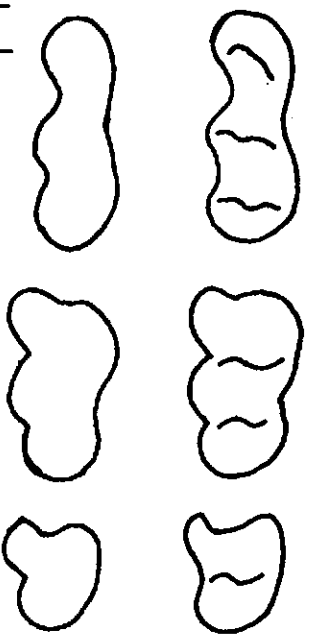
GP No. DIET

MANDIBULAR

SCORES		SCORES	
INCID.	SEVERITY	INCID	SEVERITY



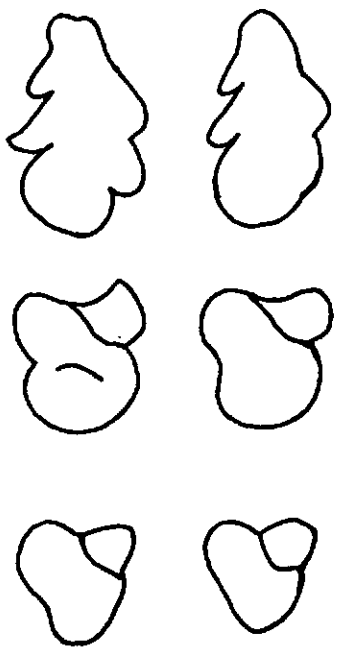
(SUBTOTAL)



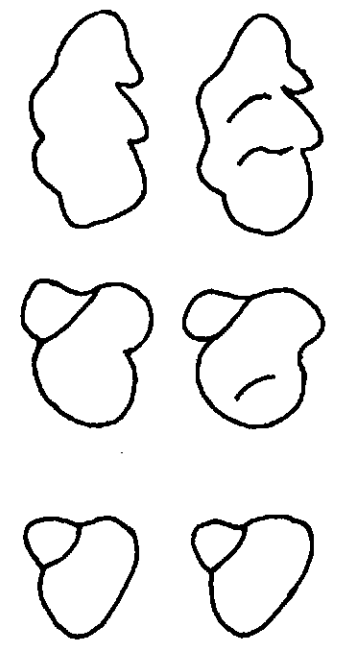
(SUBTOTAL)

MAXILLARY

SCORES		SCORES	
INCID.	SEVERITY	INCID.	SEVERITY



(SUBTOTAL)



(SUBTOTAL)

(TOTAL MANDIBULAR) + (TOTAL MAXILLARY) = TOTAL SCORE

_____ + _____ = _____

DISCUSSION

In this study we have been able to produce a carious lesion in-vivo with multibacterial innoculation. This is similar to the natural phenomenon. Up till now, no one has produced a carious lesion identical to the natural ones *in-vivo* either by acid gel or by monobacterial plaque (Zhou-Xue-dong et al, 1992).

Kleinberg and Jenkins (1967) showed that there are variations in the pH values at different intra-oral sites. These variations were found by these workers to be consistent with the relative proneness of these sites to dental caries or periodontal disease. That is, a low plaque resting pH is found in areas prone to periodontal disease.

In this experiment, the acids (Lactic, Formic, Acetic) produced by the mixed bacteria are most likely responsible for the demineralization of the surface enamel which led to the cavitation seen on the occlusal surfaces of the molar teeth of the experimental rat (Bibby and Mundroff, 1975). It is well known that bacterial plaque formed on surfaces of teeth plays an important role in the initiation and progression of dental caries (Yue Song-ling, Zhou Xue-dong and Li Jie, 1992).

Considering the multifactorial etiology which includes microbial, dietary and host factor, it is not sufficient that the three etiological factors be present in the right proportions, but also they must have enough virulence or degree of intensity to induce dental caries. This explains the reason why group (IV) animals show significant difference in incidence and severity of caries when compared with the control group (I).

We do not know whether increase in the number of animals in each group will show

significant difference in severity and incidence of dental caries amongst experimental groups II and III. With the creation of this model, cariogenic properties of various Nigerian foodstuffs can be determined. From further research on the association of specific foods and dental caries, rational advise from the dental profession to the public can be made. Thus it would be advisable that sugar-containing snack foods be replaced with savoury products for the preservation of dental health.

Fig. 2.2

Maxillary molars of Group I Animals (Control). Arrows show pin-point caries on first and the last molar.

HP, hard palate.

X 20

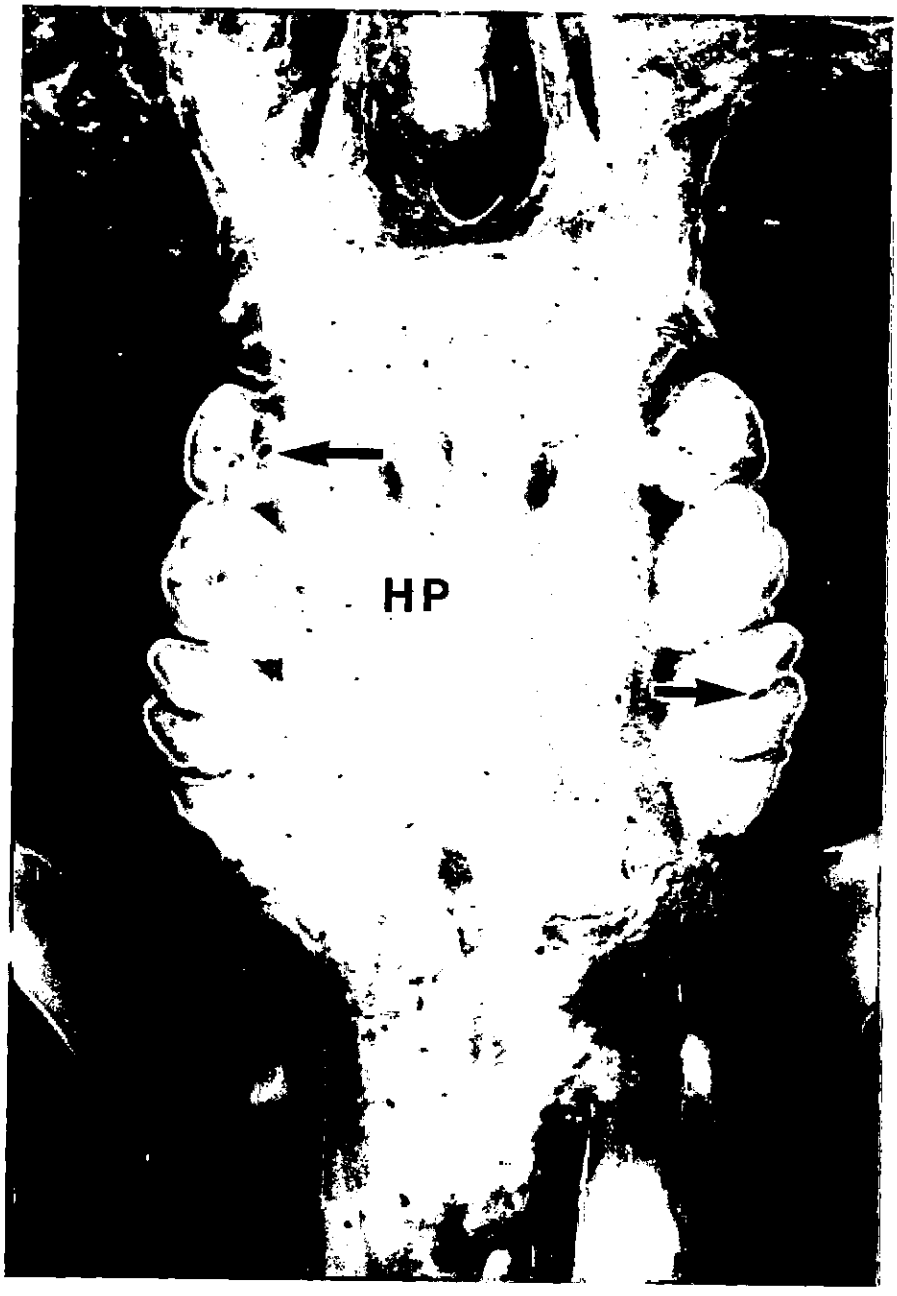

HP

Fig. 2.3

Mandibular molars of Group II animals (Experimental).

Arrow shows caries on the left mandibular teeth.

M, Mandible

X 20


M
M

Fig. 2.4

Maxillary molars of Group III animals (Experimental).

Arrow shows gross caries on the last left maxillary

tooth.

HP, hard palate

X 20

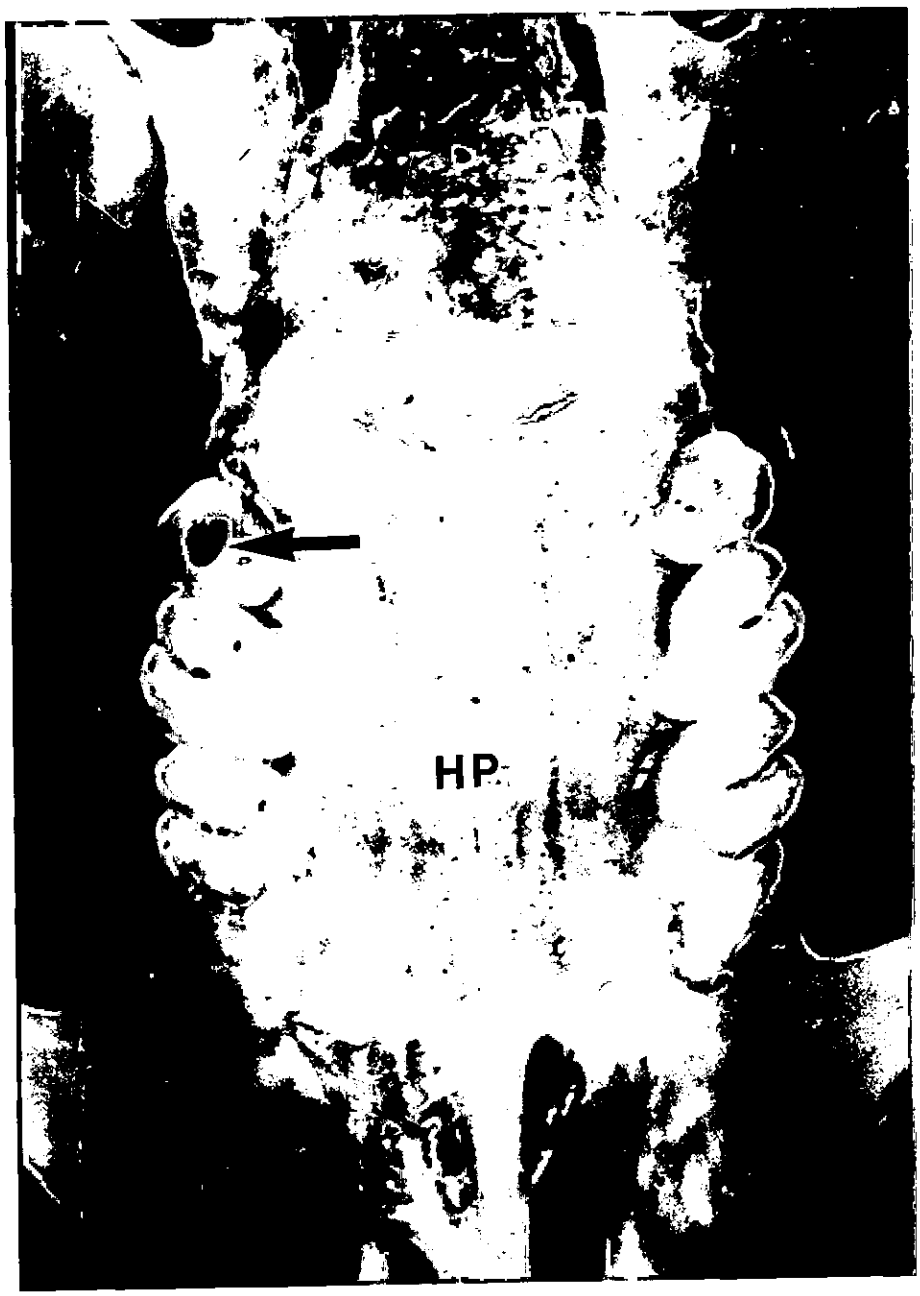

HP

Fig. 2.5

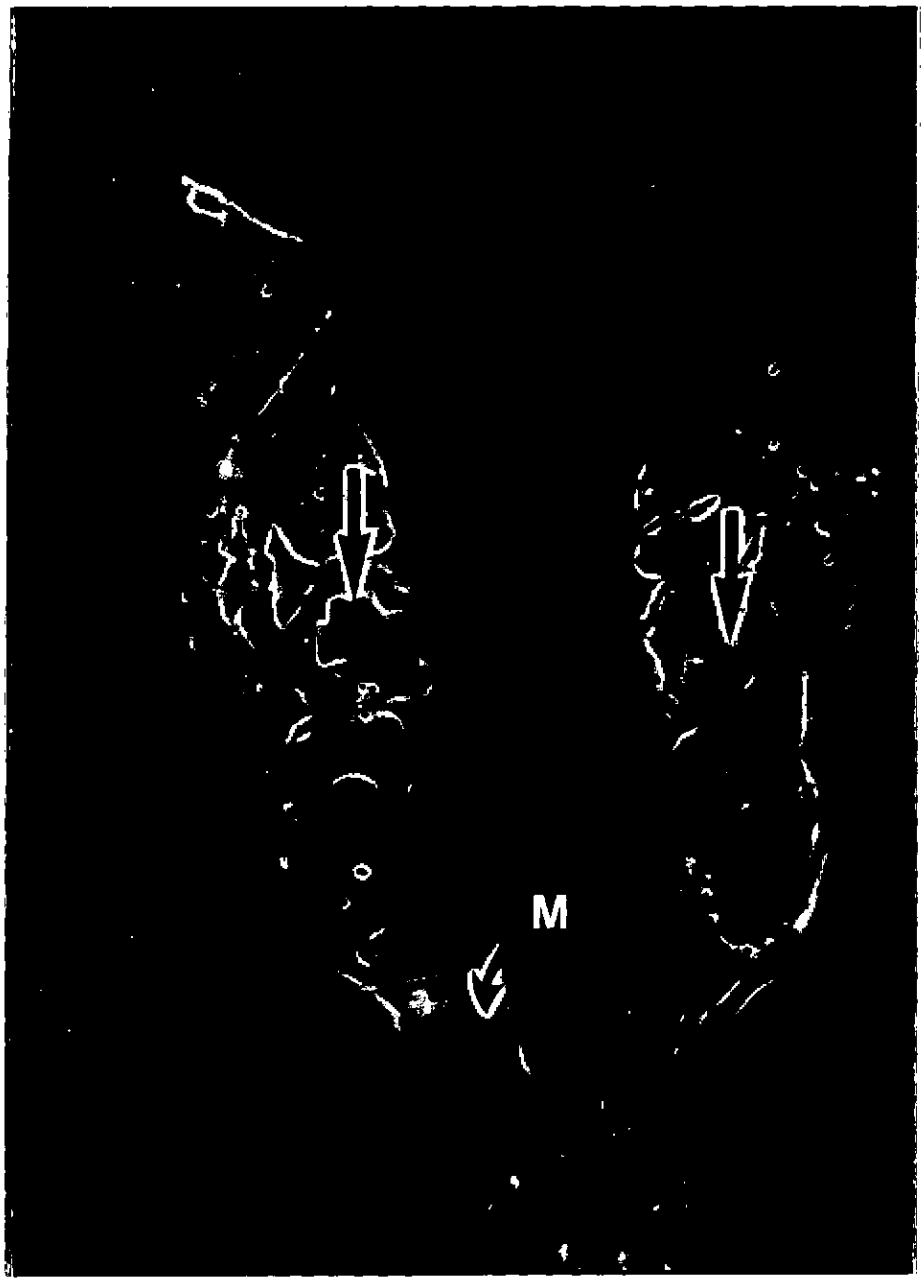
Mandibular molars of Group IV animals (Experimental).

Arrows show marked destruction of the cusps and fissure

of the 2_{nd} mandibular teeth bilaterally.

M, Mandible

X 20


M

CHAPTER 3

TO INVESTIGATE THE EFFECTS OF MATERNAL NUTRITION ON THE DEVELOPMENT OF DENTAL CARIES

CHAPTER 3

SUMMARY

The purpose of this study is to investigate the effects of maternal nutrition on the development of dental caries. Pregnant rats were divided into two groups at the begining of their pregnancy. Group I (GPI) placed on normal diet (ND) (lab. chow) and the other group (GP2) was placed on nutritionally defecient diet (DD) (cooked cassava) throughout the period of pregnancy. The offsprings of each group (GPI-ND and GPI-DD) were each divided into a further two groups making a total of four experimental groups.

I. GPI-ONDI were placed on cariogenic diet + bacteria innoculation.

II. GP2-OND2 were placed on normal diet + bacteria innoculation.

III. GP3-ODD3 were placed on cariogenic diet + bacteria innoculation.

IV. GP4-ODD4 were placed on cariogenic diet + bacteria innoculation.

The scores for the extent and penetration of lesions were 6.8 and 35.0 respectively, for the animals fed on cariogenic diet, (GPI) and (GP3), ($p < 0.001$). There was no statistically significant difference in the scores of the groups fed normal diet. Each rat in the group receiving cariogenic diet developed extensive caries. It was also found that the means of incidence and severity of dental caries between offsprings of adequately nourished and malnourished mothers were highly statistically significantly different ($p < 0.001$).

The resistance of malnourished rats is reduced to dental caries during their development (Speirs, 1967).

INTRODUCTION

The successful control of several diseases, especially rickets, by means of vitamin or mineral therapy stimulated a lot of research on nutrition and dental caries. It has been suggested that susceptibility to infection increases in children that are undernourished (Scrimshaw et al, 1960; Scrimshaw, 1975; Chandra, 1972; Allen, et al. 1990). Similar finding was observed in animals (Cooper et al, 1974). An explanation to this may be that the humoral and the cell mediated immune responses are affected (Deitchman et al, 1980). Malnutrition and susceptibility to infection can work synergistically (Scrimshaw, 1975).

Malnutrition in man causes a reduction in the number of T lymphocytes (Chandra, 1974; Neuman et al, 1975) as well as a reduced function of complement (Chandra, 1975). It has been shown by Chandra (1977) that the proportion of "null" lymphocytes increases in protein and energy malnutrition in man. A corresponding decrease for T lymphocytes is seen, whereas the B lymphocyte level stays unchanged. The serum immunoglobulin levels do not change during protein malnutrition unless T helper lymphocytes are needed (Chandra and Newberne, 1977).

Secretion of antibacterial components from some exocrine glands is reduced by malnutrition. The volume of breast milk and the total amount of sIgA secreted per minute are reduced in malnourished women (Carlsson et al, 1976). Protein deficiency causes lower sIgA concentration in vaginal secretions of the guinea pig (Chandra, 1977). However, Bell et al, (1976) could not demonstrate any reduction in the sIgA concentration in intestinal secretions of malnourished Indonesian children.

It has been reported that rats exposed to moderate protein deprivation from birth and in-utero (Navia et al, 1970; Menaker and Navia, 1973a) develop more caries than do controls. This caries susceptibility may depend partly on maternal diet. However, Spiers (1967) obtained negative results. It is therefore necessary to investigate if malnourished mothers give birth to caries susceptible or caries resistant offsprings using our local foodstuffs.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing between 80 - 100g were purchased from the animal laboratory centre, College of Medicine, University of Lagos, Idi-Araba. They were kept in temperature controlled room (25-28°C) with the light on from 0700-1900 hours daily. The rats were allowed to acclimatize to their enviroment, fed on normal rat feed for one week before commencement of the experiment.

The rats were mated. The pregnant rats were divided into two groups at the begining of their pregnancy. Group 1 (GP1) placed on Normal diet (ND) (Lab. Chow) and the other group (GP2) was placed on nutritionally deficient diet (DD) (Cooked Cassava) throughout the period of pregnancy. The offsprings of each group (GP1-ND and GP1-DD) were each further divided into two groups. Offsprings of GP1-ND were divided into OND1 and OND2. (OND = Offspring of mothers fed on normal diet). Offsprings of GP2-DD were divided into ODD1 and ODD2 (ODD = Offspring of mothers fed on dificient diet, making a total of four experimental groups.

I. GP1-OND1 were placed on cariogenic diet + Bacteria Innoculation.

II. GP2-OND2 were placed on Normal diet + Bacteria Innoculation.

III. GP3-ODD3 were placed on cariogenic diet + Bacteria Innoculation.

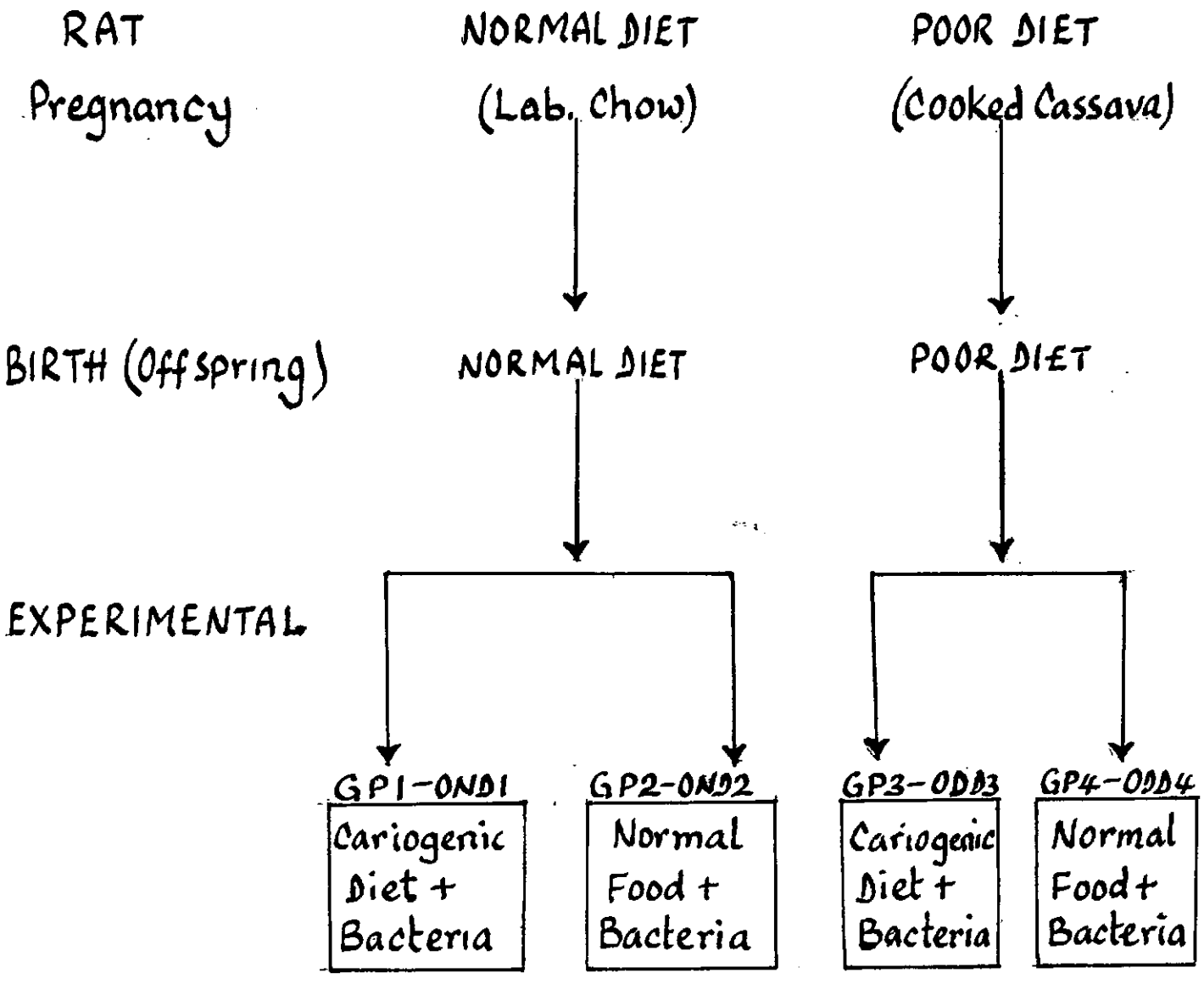
IV. GP4-ODD4 were placed on Normal food + Bacteria Innoculation.

The animals were sacrificed after ether anaesthesia at 24 weeks. The upper and lower jaws were defleshed and scored for caries using modified KEYES (1958) method. The salivary glands were fixed in 10% formol - saline. Sections for light microscopy were cut at 5um and stained with Haematoxylin and Eosin.

METHODOLOGY

Flow chart of the Maternal nutrition on the developement of Dental Caries in the offspring of rat.

METHODOLOGY



RESULTS

The daily weight gain of the rats in the four groups during the twenty four-week experimental period were low, approximately 4.0.g for the rats fed on Normal diet and 1.8g for the rats fed on cariogenic diet ($p < 0.001$). Since the normal daily weight gain on a standard laboratory pellet diet is about 5g, it is clear that the cariogenic diet did not provide complete nutrition.

The caries scores for the different groups are presented in Table 3.1. The scores for the extent and penetration of lesions (E +D) were 6.8 and 35.0, respectively, for the animals fed on cariogenic diet, (GP1) and (GP3), ($p < 0.001$). There was also a three-fold increase in total number of lesions (L) in the group (GP3) receiving cariogenic diet, and a relatively higher proportion of smooth surface caries (18.9) in group (GP3) than in group (GP1; 5.8) ($P < 0.001$). There was no statistically significant difference in the scores of the groups fed normal diet.

Each rat in the groups receiving cariogenic diet developed extensive caries. In the other groups, the distribution of caries between the animals was uneven, with a few animals developing a large number of lesions. The consistent difference in caries development between the two groups fed cariogenic diet is apparent in (Table 3.1)

TABLE 3.1

CARIES SCORE (M + SE) FOR THE FOUR DIFFERENT DIET GROUPS EXPRESSED AS NUMBER OF LESIONS PER RAT (L) AND THE SUM OF THE SCORES FOR THE EXTENSION (E) AND THE DEPTH OF PENETRATION (D) OF SULCAL(Su) AND SMOOTH (Sm) SURFACE CARIES

	GP_1 (Cariogenic Diet)				GP_3 (Cariogenic Diet)				GP_2 (Normal Diet)				GP_4 (Normal Diet)			
	OND_1				ODD_3				OND_2				ODD_4			
	L		E+D		L		E+D		L		E+D		L		E+D	
RAT	Sm	Su	Sm	Su	Sm	Su	Sm	Su	Sm	Su	Sm	Su	Sm	Su	Sm	Su
1	1	2	1	2	0	0	0	0	2	15	2	18	0	9	0	13
2	2	7	2	7	1	2	1	46	0	0	0	0	0	6	0	0
3	0	7	0	8	0	0	0	0	0	1	0	1	2	0	0	9
4	0	1	0	1	6	11	7	24	0	2	0	2	0	0	2	0
5	0	11	0	14	0	15	0	28	0	3	0	3	3	3	1	1
6	0	1	0	1	4	14	4	23	0	0	0	3	1	1	0	7
7	0	1	0	1	1	17	1	34	0	1	0	1	0	6	0	4
8	0	1	0	1	0	16	0	28	0	1	0	2	1	10	3	14
9	2	16	2	22	9	17	10	37	0	3	0	1	3	11	1	11
10	0	6	0	6	4	15	4	33	0	1	0	0	0	4	3	4
•	5	53	5	63	25	126	27	253	2	27	2	31	10	50	10	63
M	5.8		6.8		18.9		35.0		3.2		3.7		6.0		7.3	
SE	1.8		2.3		1.3		2.9		1.8		2.1		1.4		1.7	

TABLE 3.2

INITIAL AND FINAL WEIGHT DIFFERENCE IN THE RATS

NORMAL FEED (CONTROL GP)

INITIAL WT. (G)	FINAL WT (G)	WT. DIFF.	%
39.5	200.2	161	80.30
51.5	198.5	147	74.96
40.2	190.0	149.8	78.84
44.5	210.0	165.5	78.81
38.5	218.5	180	82.38
40.0	215.5	175.5	81.44
37.5	180.5	143	79.22
45.5	189.5	144	75.99
40.5	178.0	137.5	77.25
50.5	199	148.5	74.62

TABLE 3.3

INITIAL AND FINAL WEIGHT DIFFERENCE IN THE RATS

LAB. CHOW + GRANULATED SUGAR (56%)

INITIAL WT. (G)	FINAL WT. (G)	WT. DIFF.	%
42	120	78	65
50	140	90	64.29
52	123	71	57.72
53	140	87	62.14
32	125	93	74.4
46.5	136	89.5	65.81
38.5	140	101.5	72.5
48	150	102	68.00
44.5	145	100.5	69.31
46.5	140	93.5	66.79

TABLE 3.4

INITIAL AND FINAL WEIGHT DIFFERENCE IN THE RATS

CASSAVA DIET (BOILED)

INITIAL WT (G)	FINAL WT (G)	WT. DIFF.	%
37.5	56	18.5	33.04
50.3	60.5	10.2	16.86
40.5	68.5	28	40.88
42.0	70.5	28.5	40.43
45.0	75.0	30	40.00
44.5	60.5	16	26.45
38.0	65.0	27	41.54
39.0	75.5	36.5	48.34
46.6	80.0	33.5	41.88
42.5	78.5	36	45.86

DISCUSSION

The purpose of this study was to find out the effect of maternal nutrition on caries susceptibility of the rats born of adequately nourished and malnourished mothers. When rats are fed with carbohydrate at early age they are highly susceptible to caries which is indicated by the caries score. Some workers believe that the carbohydrate concentration of teeth is a factor which determines the susceptibility of the teeth to caries. Egyedi (1953) reported that the carbohydrate concentration of teeth from natives of Indonesia, who had a high resistance to caries, was lower than in teeth from inhabitants of Holland whose caries resistance was much lower.

Although the question is still unsettled, the most careful experiments suggest that an intake of excess carbohydrate during enamel formation does not affect caries susceptibility. Whether this is because the enamel carbohydrate is unchanged or because enamel carbohydrate does not affect caries has not been adequately investigated (Egyedi 1973). In this study, it was found that the means of incidence and severity of dental caries between offsprings of adequately nourished and malnourished mothers were highly statistically significantly different ($p < 0.001$). Low birth weight was also observed in offspring of malnourished mothers.

The resistance of malnourished rats to dental caries is reduced during their development (Speirs, 1967). The pathophysiology of this is not known for sure, but some researchers believe that the humoral and cell-mediated immune response are affected, (Chandra 1977). It is reported that malnutrition in man causes a reduction in the number of T lymphocytes. Neuman et al, (1975). It is also known that rats exposed to moderate

protein deprivation from birth and in utero (Navia et al., 1970; Menaker and Navia, 1973a) develop more caries than do controls. These rats have also been reported to attain lower levels of DNA, RNA, and protein in the submandibular gland (Menaker and Navia, 1974). A reduced rate of Saliva secretion has been found in protein-deficient animals in other studies as well (Deitchman et al, 1980). Total protein content was lower in saliva from protein-malnourished monkeys (Alvarez, 1979).

Saliva exerts considerable protection against dental caries (Johansson, 1985). It is observed in this study that the salivary gland activity in the malnourished rat is reduced due to the reduced number of secretory granules found in the histological section. Resistance to caries increases after rat's teeth have been in contact with oral fluids even for as little as 2 weeks. This increased resistance is called post-eruptive maturation and is believed to be the incorporation of additional mineral, and perhaps protein, from saliva into hypomineralized spaces still present when the tooth erupts (Speirs, 1967). It has also been reported recently that eruption of the primary teeth was significantly delayed in all malnourished children examined (Alvarez et al, 1979). Since salivary gland secretion is affected, resistance to micro-organism which acts on the carbohydrate to produce lactic acid is reduced therefore susceptibility to dental caries is very marked in offsprings of undernourished mothers (Scrimshaw, 1975). Since no work has ben reported in this part of the world to show correlation between children born of malnourished mothers and rampant caries, the results of this study has confirmed previous studies in animals and indirect human epidemiological evidence which had suggested a cause-effect relationship between early malnutriton and increased dental caries (Alvarez et al, 1979). Pregnant

women particularly the low socio-economic group, should be advised about the importance of balanced diet in relation to dental caries susceptibility, in their children.

CHAPTER 4

TO INVESTIGATE THE POTENTIAL OF NIGERIAN FOODSTUFFS AS CARIOGENIC AND PERIOPATHIC AGENTS

SUMMARY

The cariogenicity of the following Nigerian foods was tested in a rat model: cooked rice, peanuts (groundnuts), bread, cornstarch, sucrose and laboratory chow as control.

The means of incidence and severity of caries between the control group fed with laboratory chow and experimental groups fed with test foods are significantly different ($p < 0.001$).

Sucrose shows the highest mean caries scores (17.0) when compared with the other test foods such as bread (14.83), cornstarch (13.67) and cooked rice (13.17) repectively. The peanuts is least cariogenic among the test foods. The result suggests that some Nigerian foods contain intrinsic sugar and hydrolyzable starch which is easily converted into demineralizing acid.

INTRODUCTION

The caries process is multifactorial and involves the dynamic integration of several factors, including oral bacteria, saliva, tooth enamel, clearance, and dietary substrates. It is possible to learn a great deal about each of these factors by studying them individually or in limited combinations (Mundorff, et al, 1990). Many isolated aspects of the caries process have been evaluated, including sugar content of foods (Shannon and Westcott, 1975), acid production and enamel demineralization (Bibby and Mundroff, 1975), food retention in the mouth (Lundqvist, 1952), pH depressions produced in plaque (Edgar et al., 1975), demineralization of the enamel using in - vitro experiments (Featherstone et al, 1981).

Various sources of information can indicate whether some types of foods are more cariogenic or less cariogenic than others. These are:

- caries prevalence in relation to the use of a specific food or group of foods.
- tests on animal caries
- the production of experimental caries in human teeth;
- measurements of the caries-associated reactions of foods. This include acid production and enamel demineralization on fermentation of foods by mouth bacteria, acid production in plaques and retention of food in the mouth.

Hefferren (1986) has reviewed the numerous models that estimate the cariogenic potentials

of foods. The caries model incorporates many aspects of the caries process in vivo and is, therefore considered to be a valuable and appropriate model for the evaluation of the cariogenic potential of dietary substances (Bowen, 1986; Mundorff, 1988).

Since what has been described as a "caries explosion" is taking place in some of the developing countries of the world, where populations are abandoning their native diets and increasingly turning to western foods (Bibby, 1981), it is therefore necessary to determine the cariogenic properties of various Nigerian foodstuffs. The result of this study will help to give advice from the dental profession to the public as to which food to eat for the preservation of dental health.

MATERIALS AND METHODS

Sprague-Dawley albino rats, 21 days old weighing between 80 - 100g were purchased from the animal laboratory centre, College of Medicine, University of Lagos, Idi-Araba. They were kept in temperature controlled room (25 - 28°C) with the light on from 0700 to 1900 hours daily. The rats were allowed to acclimatize to this enviroment for one week before commencement of the experiment.

The oral cavity of the rats were innoculated with pure cultures of major oral bacteria obtained from grossly carious cavities from patients who attended Lagos University Teaching Hospital dental clinic. The plaque sample were grown in the Brain Heart Infusion (BHI) Broth. Equal volume (100ul) of each microorganism was innoculated and grown up for an additional 24 hours in BHI broth for 18 - 24 hours.

With the use of a swab and syringes, 0.2mls of oral bacteria (Streptococcus mutans and Lactobacillus casei) was innoculated into the oral cavity daily for 14 days. Various test foods were fed in their different groups. On the 21st. day, random plaque samples were collected with the aid of a dental scaler and dispensed in 1.0ml of sterile BHI broth. After serial dilution, appropriate dilution was plated on Blood Agar and incubated for 72 hours anaerobically and aerobically. Various colonies were identified to see the degeree of colonization of innoculated bacteria using Standard microbiological methods.

Animals were divided randomly into different experimental groups with 56% test food consisting of 6 animals per group.

Group I. - Water, Lab Chow, + bacteria innoculation.

Group II. - Water, Peanuts + bacteria innoculation.

Group III. - Water, Bread + Bacteria innoculation.

Group IV. - Water, Cornstarch/sucrose + Bacteria innoculation

Group V. - Water, sucrose + bacteria innoculation.

Group VI. - Water, cooked rice + bacteria innoculation.

The innoculation was for 5 consecutive days. Animals were sacrificed after ether anaesthesia at about 16 weeks. The upper and lower jaws were defleshed and scored for caries using modified KEYES (1958) methods.

RESULTS

The mean caries scores (incidence and severity) by food is shown in Table 4.1. Among the six experiments, sucrose gave the highest mean caries score.

The means of incidence and severity between Groups I and V are significantly different ($p < 0.001$). The means of incidence and severity between the control (Group I) and the experimental Group III to VI are significantly different, but the means of incidence and severity between Group I and Group II are not significantly different. Sucrose shows the highest mean caries scores when compared with the normal rat feed (Lab. Chow). Next to sucrose in caries severity is Bread, mean of (14.83); Cornstarch (13.67) and cooked rice (13.17) respectively. The peanuts showed the least caries incidence and severity out of all the test foods (6.7 and 6.67) (Fig. 4.1).

Figure 4.1

Bar chart showing the Incidence and Severity of

Dental caries in rats fed various Nigerian foodsfuffs

Fig. 4.1

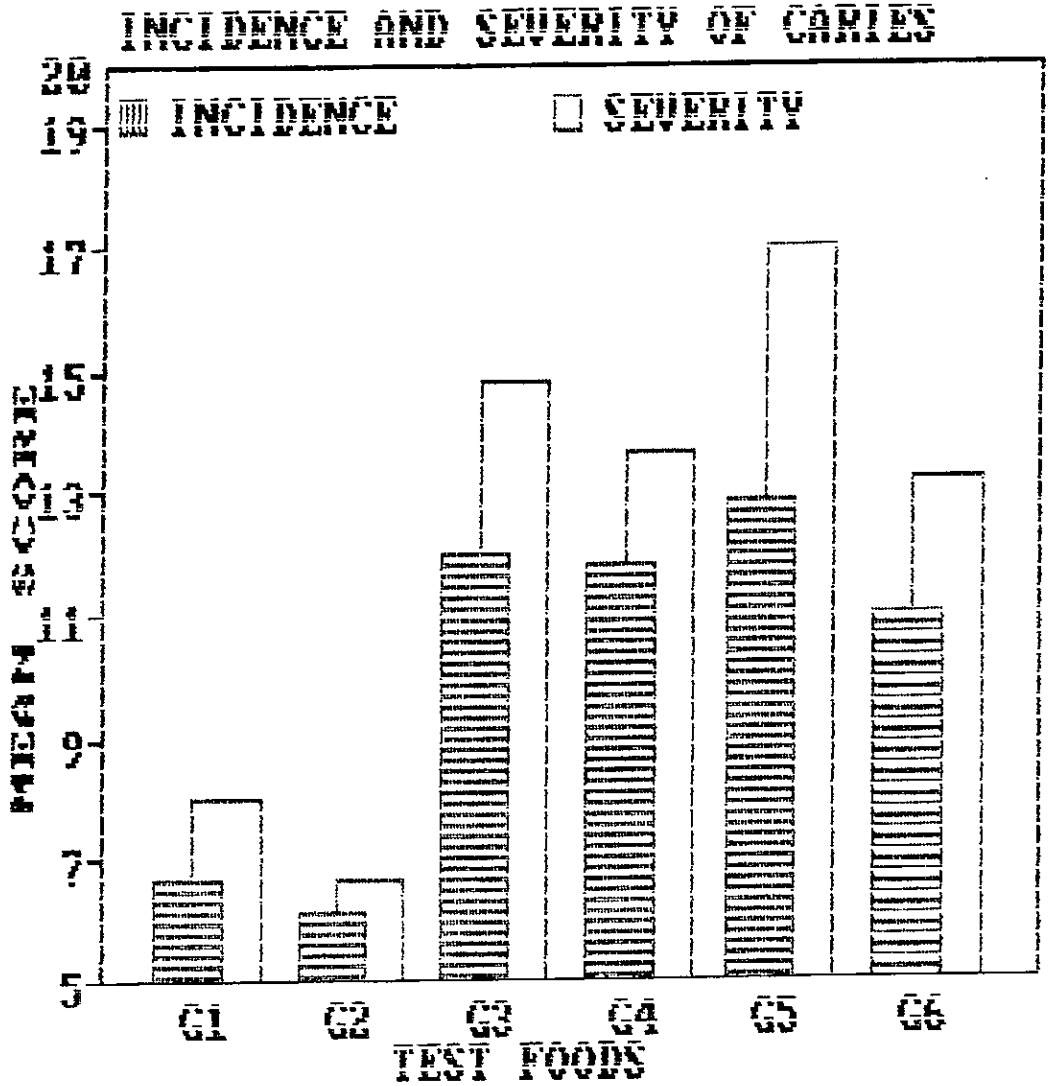

INCIDENCE AND SEVERITY OF CARIES
INCIDENCE
SEVERITY
MEAN DMFS SCORES
20
19
17
15
13
11
9
7
5
C1
C2
C3
C4
C5
C6
TEST FOODS

TABLE 41

INCIDENCE AND SEVERITY OF CARIES IN RATS TREATED WITH VARIOUS NIGERIAN FOODSTUFFS

	GROUP I LAB. CHOW CONTROL (56% T.F*)		GROUP II PEANUTS (56% T.F)		GROUP III BREAD (56% T.F)		GROUP IV CORN STARCH (56% T.F.)		GROUP V SUCROSE (56% T.F.)		GROUP VI COOKED RICE (56% T.F.)	
	INCD.	SEV.	INCD.	SEV.	INCD.	SEV.	INCD.	SEV.	INCD.	SEV.	INCD.	SEV.
	6	7	6	7	12	14	11	12	11	15	10	12
	7	8	6	6	13	14	12	14	12	16	11	13
	6	7	7	7	11	13	13	14	14	18	12	14
	7	9	6	7	13	17	12	16	15	19	10	13
	8	8	7	7	11	16	11	12	12	16	11	14
	6	9	5	6	12	15	12	14	13	18	12	13
X	6.67	8	6.17	6.67	12	14.83	11.83	13.67	12.83	17.0	11	13.17
S.D	0.816	0.894	0.752	0.516	0.894	1.472	0.753	1.505	1.472	1.549	0.894	0.753

* = Test Food

DISCUSSION

There is now no doubt that dietary sugars are the most cariogenic item in the human diet and in their absence, very little caries develop. Laboratory studies on the cariogenic potential of foods by determination of plaque pH (Edgar et al., 1975; Rugg-Gunnet; 1978) or by rat caries tests (Bowen et al; 1980; Mundorff et al., 1990) have shown that there is a relationship between cariogenic potential and the presence of sugars in a food. The amount or concentration of sugars is not quantitatively related to the cariogenic potential. For example, the fall in plaque pH after eating a bar of chocolate containing perhaps 20g of sucrose is smaller than that following consumption of a boiled sweet weighing 3g. Similarly, in the study of Bowen et al (1980), no significant difference was shown in levels of caries induced in rats fed breakfast cereals containing between 8 and 60% sucrose. Mundorff et al (1990) suggested that it was the

interaction between sugars and hydrolyzable starch which was important for cariogenicity.

The result of this study, suggests that some of our test food contain **intrinsic** sugars (those naturally integrated into the cellular structure of a food), **extrinsic** sugars (those 'which are free in the food or added to it') and hydrolyzable starch. Sucrose has the highest mean caries score (17.0) amongst the test food, while the peanuts has the lowest mean caries score (6.67).

CHAPTER 5

THE EFFECT OF LOCAL CHEWING STICK EXTRACTS ON CARIES PROCESS.

SUMMARY

The effect of chewing stick extracts (Serindea warneckei)on caries process was determined by dividing Sprague Dawley rats into 3 groups. Group 1 was placed on high sucrose diet + chewing stick extract; Group II was placed on similar high sucrose diet and distilled water; Group III was placed on normal Laboratory chow and water (control group). The significant reduction in the means of incidence and severity of dental caries in the group treated with the extract may be due to reduction in bacterial growth and significant decrease in adherence of bacteria on the surfaces of the teeth.

The results of this study suggest that aqueous extracts of S. warneckei inhibited the caries process in Sprague Dawley rat. The ready availability of chewing stick at low cost and its wide acceptability amongst African people also make its use as a preventive agent for oral diseases a very attractive idea.

INTRODUCTION

A large number of Nigerian population still use chewing sticks as an alternative to the conventional toothbrush when cleaning their teeth. It has recently been observed and documented that there is a decreased incidence of caries and periodontal diseases among persons who chew these sticks (Enwonwu, 1974). Although part of their beneficial effect may be due to mechanical cleansing and removal of plaque, it seemed worthwhile to consider inhibitory or bactericidal effects which may be exerted by substances released from the chewing sticks. It has been suggested that sticks might contain chemotherapeutic agents which specifically inhibit plaque formation (Newbrun, 1978). Some Nigerian researchers have been able to show that buffered extracts of several chewing sticks inhibited the growth of oral bacteria on agar plates (El-said et al., 1971).

The adherence of Streptococcus mutans to the surfaces of teeth and their subsequent colonization is thought to play an important role in the development of plaque and dental caries (Gibbons and Van Houte, 1975). The mechanisms by which this adherence occurs is still unclear and controversial, however, it has been hypothesized (Gibbons, 1977) that the adherence of this organism to the tooth surface and subsequent plaque formation occurs in two stages.

The first is the reversible initial attachment of the bacterial cell to the pellicle-coated enamel surface. The second stage involves the accumulation of S. mutans and is dependent upon the production of extracellular glucans produced by bacterial glucosyl-transferase enzymes from sucrose. Distruption of either of these stages results in reduced bacterial colonization, and hence reduced plaque formation (Wolinsky and Sote, 1984). It has been

reported that 1% (w/v) aqueous extracts of these commonly used chewing sticks (e.g. Serindea warnecki) significantly reduced adherence of S. mutans to glass surface (Wolinsky and Sote, 1983), and inhibited acid production in a test tube (Salako, 1988). No study, however, has dealt with the effect of this extract on caries process.

The purpose of this study was to determine whether in the created model aqueous extracts of the Nigerian chewing sticks can inhibit the caries process.

MATERIAL AND METHODS

EXTRACTION OF CHEWING STICK EXTRACT

Nigerian chewing sticks (Serindea warnecki) were shaved using a sharp knife. The shavings were then powdered with commercially available blender. One hundred grams of this powder was extracted with distilled water at 4°C for 48 hours. The extract was decanted and centrifuged at 2000g for 15 minutes. The supernatant, that is the extract, was decanted and stored at 4°C before use.

EXPERIMENTAL PROCEDURE

Sprague-Dawley rats were divided into 3 groups. Group I was placed on high sucrose diet (containing 56% granulated sugar + lab. chow) and the chewing stick extract was applied with a camel brush or syringe twice a day for 5 days a week throughout the experimental period. Group II was placed on similar high sucrose diet and distilled water. Group III was palced on normal lab. chow and water (control group). Experimental groups (I and II) were innoculated with multibacterial culture from human carious lesion. The experiment was terminated at 24 weeks. Animals were sacrificed after ether anaesthesia. The upper and lower jaws were defleshed and scored for caries using modified KEYES (1958) Method.

RESULTS

The means of incidence and severity between the groups (GP1) treated with chewing stick extract and the group placed on similarly high sucrose diet and distilled water are significantly different ($p < 0.001$). Whereas the means of incidence and severity between group 1 and the control group (GP III) are not significantly different ($p = 0.1950$). The means of incidence and severity between group II and groups III are significantly different ($p < 0.001$). The similarity in the means of incidence and severity between the group treated with extracts and the control group shows the inhibiting action of the extract on the caries process (Fig. 5.1 and Table 5.1).

TABLE 5.1

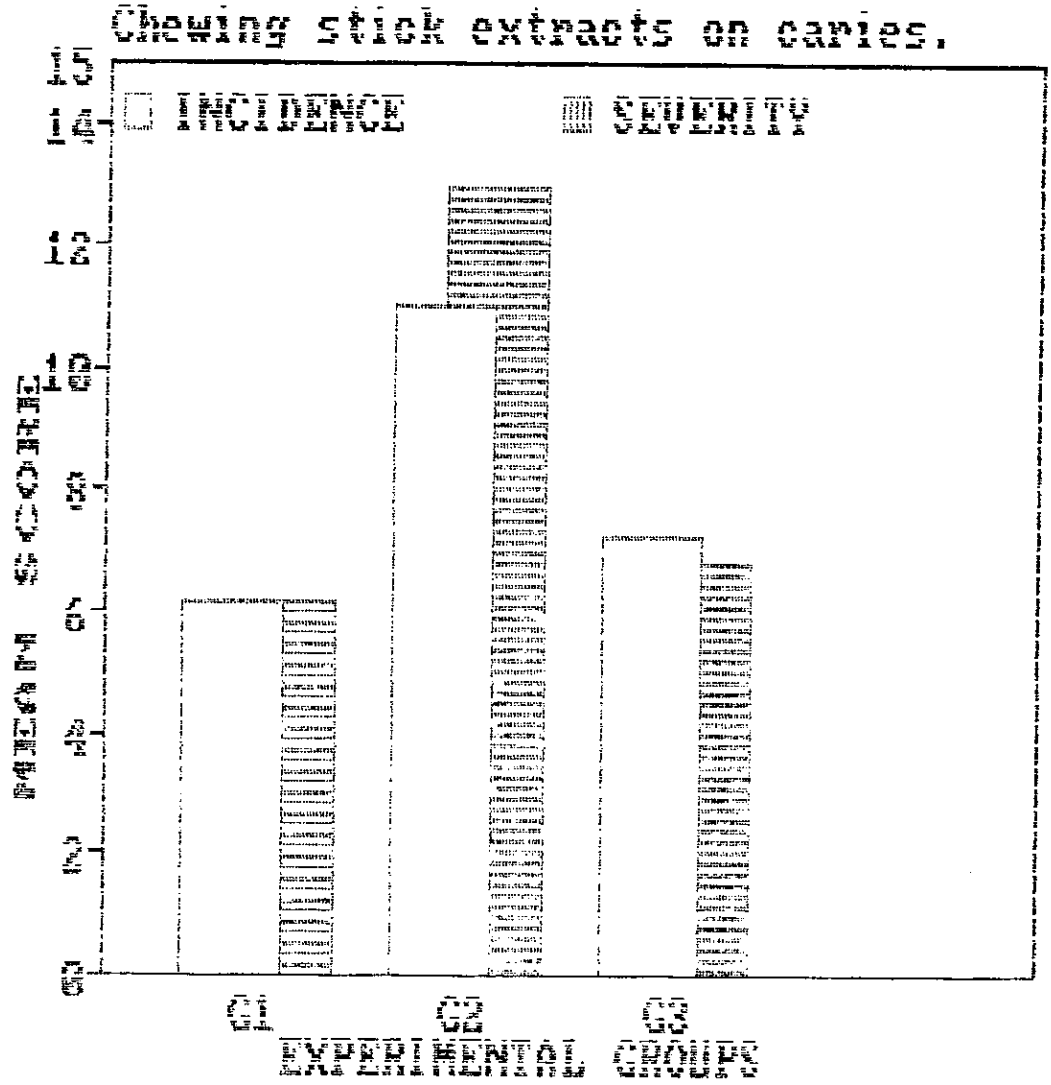
INCIDENCE AND SEVERITY OF CARIES IN RATS TREATED WITH CHEWING STICK EXTRACTS

	GROUP I		GROUP II		GROUP III	
	INCD.	SEV.	INCD.	SEV.	INCD.	SEV.
	6	7	11	12	8	7
	7	6	12	13	8	8
	7	6	11	13	7	7
	6	6	10	13	6	6
	5	6	11	14	7	6
$\bar{X}$	6.20	6.20	11.00	13.00	7.20	6.80
S.D	0.836	0.447	0.707	0.707	0.836	0.836

Figure 5.1

Bar chart showing the incidence and severity of dental caries in rats treated with chewing stick extracts.

FIG. 5.1


Chewing stick extracts on caries.
INCIDENCE
SEVERITY
MEAN SCORES
EXPERIMENTAL GROUPS

DISCUSSION

The mechanical advantage derived by the fibrous nature of the Nigerian chewing stick has been used to explain the observed lower caries incidence among the population using them. However, the hypothesis that these sticks contained some antiplaque constituents could not be overlooked (Wolinsky, and Sote 1984). It has been reported that Nigerian chewing stick (Serindeia warnecki) contain some water-soluble compounds such as tannins or flavins (Wolinsky and Sote, 1984). Tannic acid or hydrolysable tannins are complex phenol-rich polymers found in many foods and plant materials (Price and Butler, 1980). They are described as water soluble metabolites of plants with a molecular weight of 500 or greater, capable of precipitating gelatin and other proteins in aqueous solution (Gupta and Haslam, 1980). The main active antibacterial agent in the chewing stick has been suggested to be a tannin-like material.

The mechanism whereby tannic acid exhibits its antibacterial (Wolinsky and Sote, 1983; 1984; Rotimi and Mosadomi, 1987), antifungal, antiglycolytic and antiureolytic activities (Salako, 1988) have not been fully ellucidated. However, tannins are known to bind proteins especially those with open-box structure, with a high proportion of hydrophobic amino acids and a high proline content (Hagerman and Butler, 1980). The binding of such proline-rich content (PRPs) which are abundant in saliva (Mandell, Thompson and Ellison, 1965; Levine, Well and Ellison, 1969) by tannin may prevent metabolism of such protein by oral bacteria, therefore reducing production of ammonia. Similar binding of carbohydrates have also been reported (Mehansho, Butler and Carlson, 1987). This binding may therefore reduce the availability of these substrates for bacterial

degradation. The significant reduction in the means of incidence and severity of dental caries in the group treated with the extract may be due to reduction in bacterial growth and significant decrease in adherence of bacteria on the surfaces of the teeth. Gibbons and Dankers (1981) showed that naturally occuring plant products could have a drastic effect upon in-vitro bacterial adherence. In some earlier work, Stralfors (1967) reported on the caries-inhibiting tannins from Cocoa in hamster feeding experiments. The mode of action of tannins is by interaction of hydrogen bond of their phenolic groups with proteins thereby resulting in an inhibition of bacterial adherence. Alternatively, the tannins may interact with surface bound lipoteichoic acids, as in the case of salivary acidic glycoproteins resulting in bacterial aggregation (Hogg and Embry, 1982).

The results of this study showed that aqueous extracts of S. warneckei inhibited the caries process in Sprague-Dawley rat. The ready availabitlity of chewing stick at low cost and its wide acceptability amongst African people also make its use as a preventive agent for oral diseases a very attractive idea. There is, however, the need for a controlled clinical trial on its efficacy and cost-effectiveness.

CHAPTER 6

TO QUANTITATE THE CHANGES IN DENTAL CARIES AND ORAL TISSUE STRUCTURES USING *STEREOLOGICAL METHODS*

REQUIREMENTS FOR STEREOLOGICAL ANALYSIS

Uniform object sampling in Stereology.

Quite a number of existing stereological procedures require some kind of isotropy (Gundersen, et al, 1988). Plane sections of the structure in question must be isotropic, uniform random (IUR) planes, or the structure itself must be statistically isotropic. These basic requirement poses a problem due to the fact that biological structures are most of the time anisotropic. A pratical solution to the problem of anisotropy is VERTICAL SECTION (Gundersen, 1986; Gundersen et al, 1988; Cruz-Orive and Weibel, 1990).

Stereological measurements of all the parotid acini were carried out on vertical sections. It has been pointed out that unbiased stereological estimates relying on point counting can freely be obtained from vertical sections. However, estimates obtained by the use of test lines must take into consideration the "*vertical setting*" of the sampling (Baddeley et al, 1986; Gundersen et al, 1988). This lead to the requirement that on the vertical section, a test line is given a weight proportional to the sine of the angle between the test line and the vertical direction. This is not so much a requirement as a description of how to construct suitable test systems. There are many ways to create correct test lines (Baddeley et al, 1986) but for the practical purpose of surface estimation on vertical section, CYCLOID ARCS are the most suitable, since only the total intersection number is then counted and no numerical weighing is required (Gundersen et al, 1988).

Definition of Vertical Sections

A vertical section of a tissue is a planè section perpendicular to a given "horizontal" plane. The meaning of a horizontal plane is only a plane of reference, which defines the orientation of the section. This is a rather handy definition, since the horizontal plane can be given by the tissue itself or generated artificially by the observer. Examples of tissues naturally possessing a horizontal plane are for instance tissues covered with squamous (flat) epithelium like skin. The possibility to produce a horizontal, reference plane of any organ of arbitrary shape makes the principle of vertical sections applicable to every experimental setting.

Sampling Procedure For Vertical Sections.

In the practical handling of tissue for use in vertical section stereology, four requirements must be fulfilled (Baddeley, et al, 1986). The first three deal with the cutting of tissue specimens whereas the fourth requirement concerns the stereological test system employed for the measurement. The number of steps involved in the handling of specimen may vary considerably in different applications.

REQUIREMENT 1

Either the tissue must intrinsically possess an identifiable directional (vertical) axis or the investigator must generate such a direction.

REQUIREMENT 2

All the vertical sections must be parallel to the vertical, i.e. normal to the horizontal, and the vertical direction must be identified in each section.

REQUIREMENT 3

Relative to the common horizontal plane, the vertical sections must have random positions and random (i.e. isotropic) orientation.

REQUIREMENT 4

On the vertical section, a test line is given a weight proportional to the sine of the angle between the test line and the vertical direction.

The test line system is unsuitable for the present study because of the arbitrary shape of the parotid acini. A suitable test system (the cycloid arc) has been designed for situations such as this (Rene et al., 1988).

Estimation of the number of Parotid acinar profiles per area

The number of profiles of an irregularly shaped structure per area (i.e. its *numerical density*) is given by:

Q_A (prof/sect) = Q (prof)/A (sect) = # of profile/Area of frame.

Where|

Q_A is the numerical density

Prof is the profile (structure)

sect is the section

A is the area of reference space (i.e. space containing Q profiles).

In this study the total number of acini in reference space (area covered by counting frame is estimated.

Estimation of the Areal Fraction of Acini

The areal fraction (A_A) is a stereological method of estimating "*how much there is* " of the structure in the profiles (Gundersen et al, 1988)

A_A (struc/sect) = Ep(struct/Ep(sect) = total area of struct/ total area of sect.

Ep (struct) can be estimated by counting points which hit the structure (see point counting test system used; Fig.6.1.

Ep (sect) can be estimated directly from the area of the "window" on the point counting test system used.

Estimating the mean profile area ($\bar{a}$) profile of acini

The mean profile area is derived directly from the relative area of profile (A_A) and the relative number of profiles (Q_A).

$\bar{a}$(prof) = A_A/Q_A = relative area of profile/ relative number of profile

UNBIASED STEREOLOGICAL ESTIMATION OF PAROTID ACINAR VOLUME USING SYSTEMATIC SECTIONS AND CAVALIER'S PRINCIPLE.

An unbiased and highly efficient estimate of the volume 'V' of a solid object of arbitrary shape is easily obtained by the following two steps (Gundersen and Jensen, 1987):

Step 1

A convenient direction is chosen and the object is cut by a series of paralled sectioning planes a distance 'T' apart. To preserve unbiasedness it is essential that the position of the plane is uniformly random in location within a range of length 'T', independently of the object to be cut. The direction of cutting does not affect the unbiasedness of the volume estimator, but affects its error variance in general.

The sectioning planes will determine in the object a random number 'm' of systematic sections of areas A1, A2, ...Am at distance "T" apart. Cavalier's principle implies that:

$$est_1V = T.\ (A1 + A2 + \ldots\ldots + Am)$$

is an unbiased estimator of V.

Step 2

Planar areas may be unbiasedly and efficiently estimated by point counting. To do this, a test system with P/a test points per unit area is superimposed uniformly at random on a section image of area A. If the section image is hit by P test point, then,

$estA = (a/P).P$

is an unbiased estimator of A.

Combining this result with Eq. (above) we get the final unbiased estimator of V, namely

$$est_2V = T.\ (a/P).M^{-2}.\ (P1,\ +\ P2\ +\ .....\ +\ Pm).$$

Where

M is the final linear magnification of the section images.

Effect of Section thickness

Under and overprojection effects may induce a negative and a positive bias, respectively, in the Cavalieri estimators $estV_1$, $estV_2$. However, simple corrections exist which will remove such bias almost completely, (Gundersen, 1986; Cruz-orive, 1987; Gundersen and Jensen, 1987).

Error of Variance of est_2V.

The standard error of est_2V cannot be predicted in the usual way (i.e. via SD(P)//m, where SD denotes standard deviation) because A1, A2, ... Am are not independent. A useful approximation of the standard error, fairly robust with respect to object shape, may still be calculated (Gundersen and Jensen, 1987) using Matheron's theory of regionalized variables (Matheron, 1965; 1971). The relevant formula is as follows. First compute the sums:

$$g_o = E \cdot Pi^2$$

$$g_1 = E \cdot PiPi+1$$

$$g_2 = E \cdot PiPi+2$$

The estimator of the coefficient of error of est_2V (namely of its standard error divided by V) is:

est CE (est_2 V/V) = $\{[3g_o+g_2-4g_1]/12\}^{1/2}/E.Pi.$

It should be noted that the preceding coefficient of error depends in general on the cutting direction.

The Cavalieri estimator above is usually more precise than one would anticipate. As demonstrated empirically by Gundersen and Jensen (1987), with 5-7 systeamtic sections the CE (est_2V/V) already drops to less than 0.05 for a wide variety of object shapes. For an arbitrary triaxial ellipsoid cut by a fixed number m of systematic sections the exact result is CE (est_1V)=$(1/5)m^{-2}$, (Cruz-Orive, 1985), already about 0.05 for 'm' = 3 sections.

MATERIALS AND METHOD

Salivary glands were removed from control rats, those fed normal diet (laboratroy chow), those fed sugar diet and those fed on cassava. The tissues were placed in a petri-dish and dissected free from periglandular fat microscopically using a dissecting

stereomicroscope at X 2 magnification. All dissections were carried out in fixative. Small pieces of tissue were fixed in Formol-Saline for 24 hours and processed for routine histology - briefly, were dehydrated in graded series of water/alcohol (50%, 70%, 90%) and three changes of absolute alcohol, two quick changes of xylene and were subsequently embedded in parafin. Sections for histological observations were cut at 5um, mounted on microscopic slides and stained with Haematoxylin/Eosin.

For stereological analysis, histological sections were used to determine the number of acinar profiles per unit area of glandular tissue (i.e. the numerical density) in all three groups of rats, also, the Areal Fraction of acini (A_A) were determined. The mean profile area ($\bar{a}$) of acinar in glandular tissue were then estimated. The theoretical basis of these estimates have been discussed earlier.

Estimation of Q_A.

A point counting test system with a (12.5/p/a) was used (see Fig. 6.1. A series of between 5 and 10 frames were counted per section. The means of total number of profiles were then calculated. From values obtained the A_A (areal fraction), $\bar{a}$ (mean profile area) were derived.

Q_A = # of profiles/area of frame

Estimation of A_A

The areal fraction of the profiles of acini per area of test system used for Q_A was estimated as follows:

A_A = E P (struc)/E P (sect)

= total area of structure/total area of section

Estimation of $\bar{a}$

The mean profile area is the average area of "a typical profile". It is computed as follows:

$\bar{a}$ (prof) = A_A (prof/sect /Q_A (prof/sect)

= relative area of profiles/ relative # of profiles.

[1]

[1]NOTE: E is the statistical summative symbol

RESULTS

(HISTOLOGY)

Histological sections of the three groups already show distinct differences in the architecture of acini at low magnification (Figs. 6.2, 6.3, 6.4). In the control group the glands are characterized by well defined acini and ductal system in panoramic view. At higher magnifications the differences become even obvious. Cells of the normal end pieces of the gland consist of cells varying from polygonal to pyramidal in shape. The central lumina are in most acini discernible but not patent. The nuclei are located in the basal part of the cells. The supranuclear cytoplasm consists of a uniformly lighter staining material (mucous). In some sections the seromucous arrangement of the demilunes are apparent. Myoepithelial cells were not identifiable even at higher magnifications. Acini from the control group show a more discrete circular arrangement than those of acini in sugar and cassava fed rats. In all the three groups, the ductal system remained patent. Terminal ducts are identifiable in low magnification sections. Intercalated ducts are also identifiable but only at higher magnifications in all three groups. There were no discernible differences between the ductal system in control and experimental groups.

Acini are generally supported by connective tissue which occupy the interglandular (interacinous) spaces. Cells of the connective tissue cannot be clearly distinguished as fibroblasts, macrophages e.t.c. Acini of cassava fed rats show some degree of distortions in the typical circular to spheroidal shaped one in laboratory chow fed rats (Figs. 6.2a and 6.4a).

RESULTS (STEREOLOGY)

TABLE 6.1

COMPARISON OF THE AREAL FRACTIONS

OF ACINAR END PIECES IN CONTROL,

SUGAR AND CASSAVA FED RATS.

CONTROL	$\frac{60 \times 12.5\ mm^2}{70 \times 12.5\ mm^2}$	= 0.85
SUGAR	$\frac{55 \times 12.5\ mm^2}{70 \times 12.5\ mm^2}$	= 0.79
CASSAVA	$\frac{35 \times 12.5\ mm^2}{70 \times 12.5\ mm^2}$	= 0.50

23

[2]Point to point distance on point counting test system used is 12.5mm: see Fig. 6.1

[3]E P (struct) : no. of points falling on profile (acini);
E P (sect) : no. of points on reference space; Reference space is area covered by frame window.

TABLE 6.2

COMPARISON OF THE Q_A (NUMERICAL DENSITY) OF

THE THREE GROUPS OF RATS (CONTROL, SUGAR AND CASSAVA FED)

Q_A = # of prof/area of frame

CONTROL $\dfrac{9}{4.2\text{mm}^{-2}} = 2.1\text{mm}^{-2}$

Q_A = # of prof/area of frame

SUGAR $\dfrac{10}{4.2\text{mm}^{-2}} = 2.4\text{mm}^{-2}$

Q_A = # of prof/area of frame

CASSAVA $\dfrac{16}{4.2\text{mm}^{-2}} = 3.8\text{mm}^{-2}$

TABLE 6.3

COMPARISON OF THE MEAN PROFILE AREA ($\bar{a}$)

OF THE THREE GROUPS OF RAT (CONTROL,

SUGAR AND CASSAVA FED)

CONTROL

A_A (prof/sect) relative area of profile 0.85

$\bar{a} = \frac{\quad}{\quad} = \frac{\quad}{\quad} = \frac{\quad}{\quad} = 0.4mm^2$

Q_A (prof/sect) relative # of profiles 2.10

SUGAR

A_A (prof/sect) relative area of profile 0.79

$\bar{a} = \frac{\quad}{\quad} = \frac{\quad}{\quad} = \frac{\quad}{\quad} = 0.33mm^2$

Q_A (prof/sect) relative # of profiles 2.40

CASSAVA

A_A (prof/sect) relative area of profile 0.50

$\bar{a} = \frac{\quad}{\quad} = \frac{\quad}{\quad} = \frac{\quad}{\quad} = 0.13mm^2$

Q_A (prof/sect) relative # of profiles 3.80

FIGURE 6.1

Stereological Test System used to determine

Q_A, A_A, $\bar{a}$.

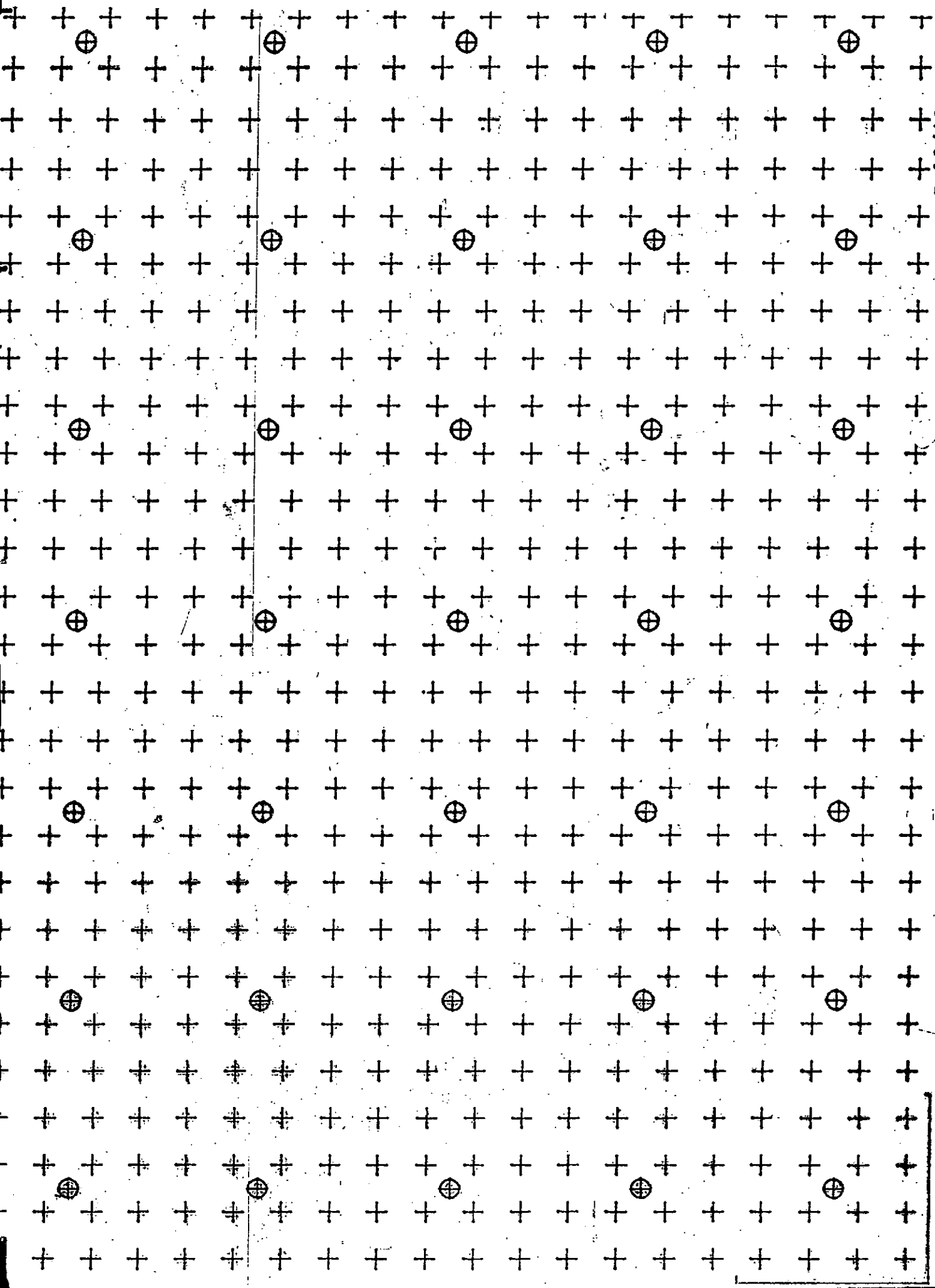


Fig. 6.2

Low-power photomicrograph of a salivary gland from rats fed on normal diet (lab. chow). The parenchyma is supported by connective tissue septa.

Arrows show acini. dt, ductal system; ct, connective tissue septa.

X10

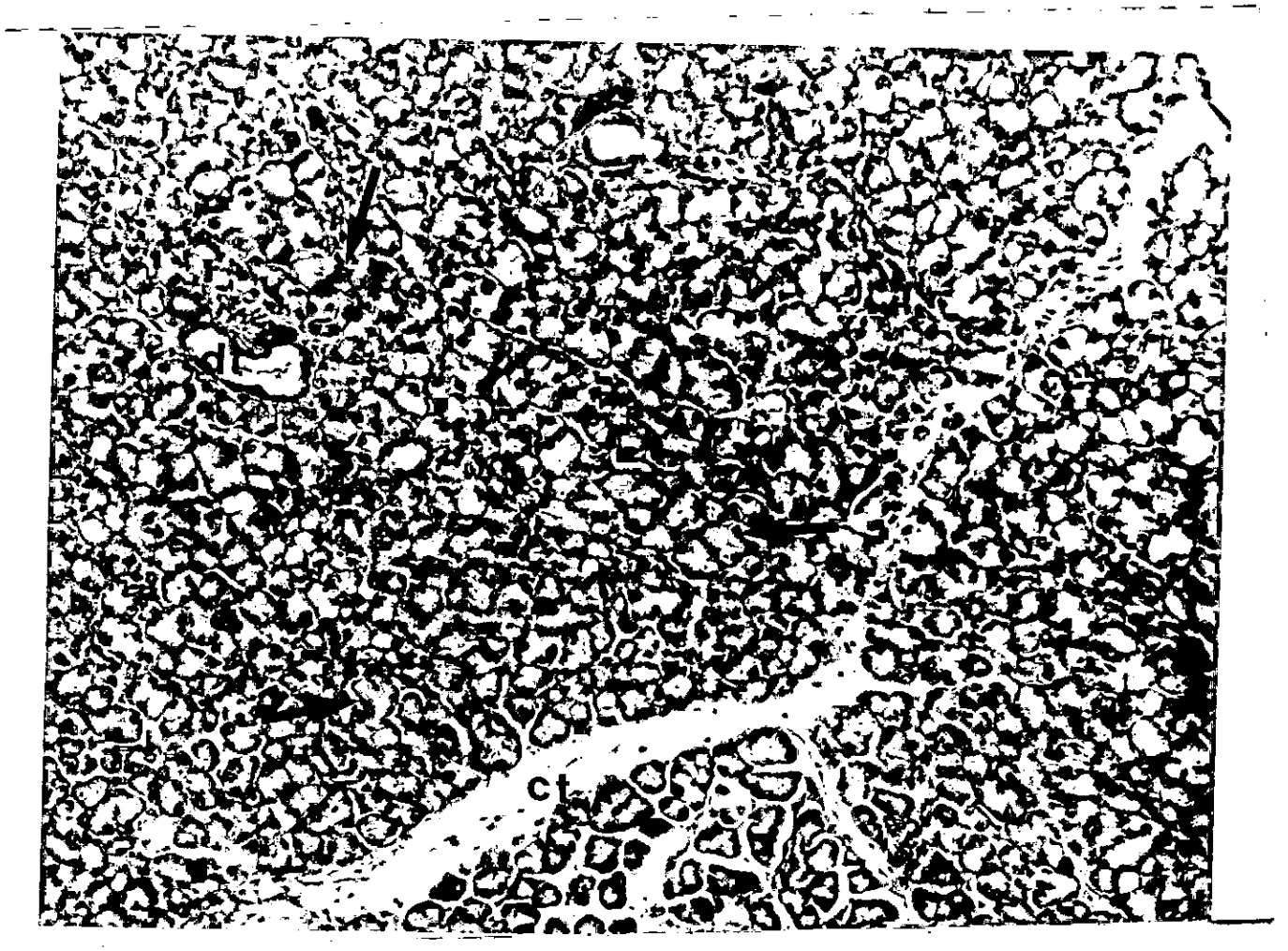

ct

Fig. 6.3

Low-power photomicrograph of a salivary gland from rats fed on cariogenic diet (56% sugar diet).

The acini are not well demarcated as compared to the normal diet fed rats. dt, ductal system; ct, connective tissue septa.

X10

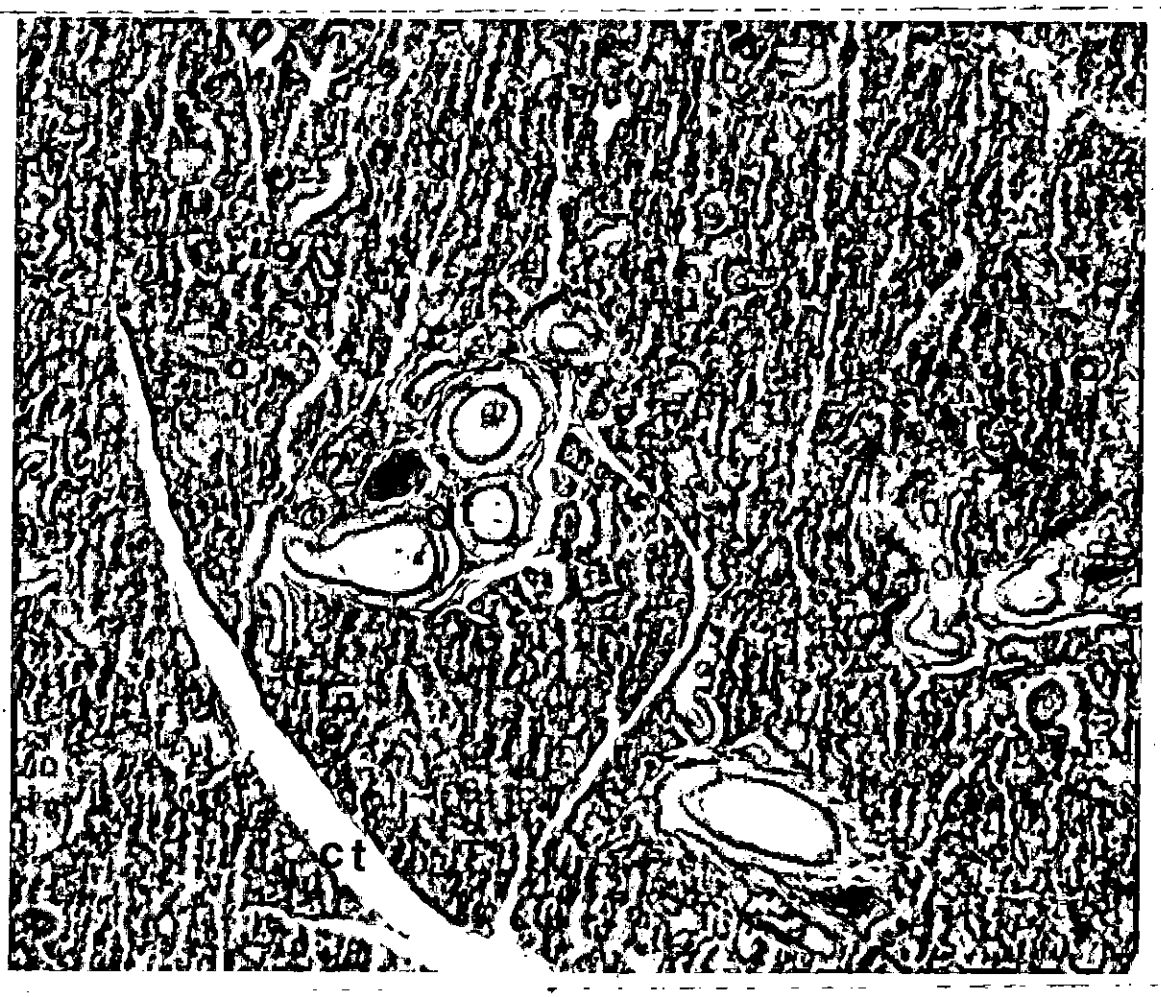


Fig. 6.4

Low-power photomicrograph of a salivary gland from rats fed on cassava diet .The architecture is not as defined as the normal diet fed rats.

Arrow points at the lamina of the connective tissue

septa. ct, connective tissue

X 10

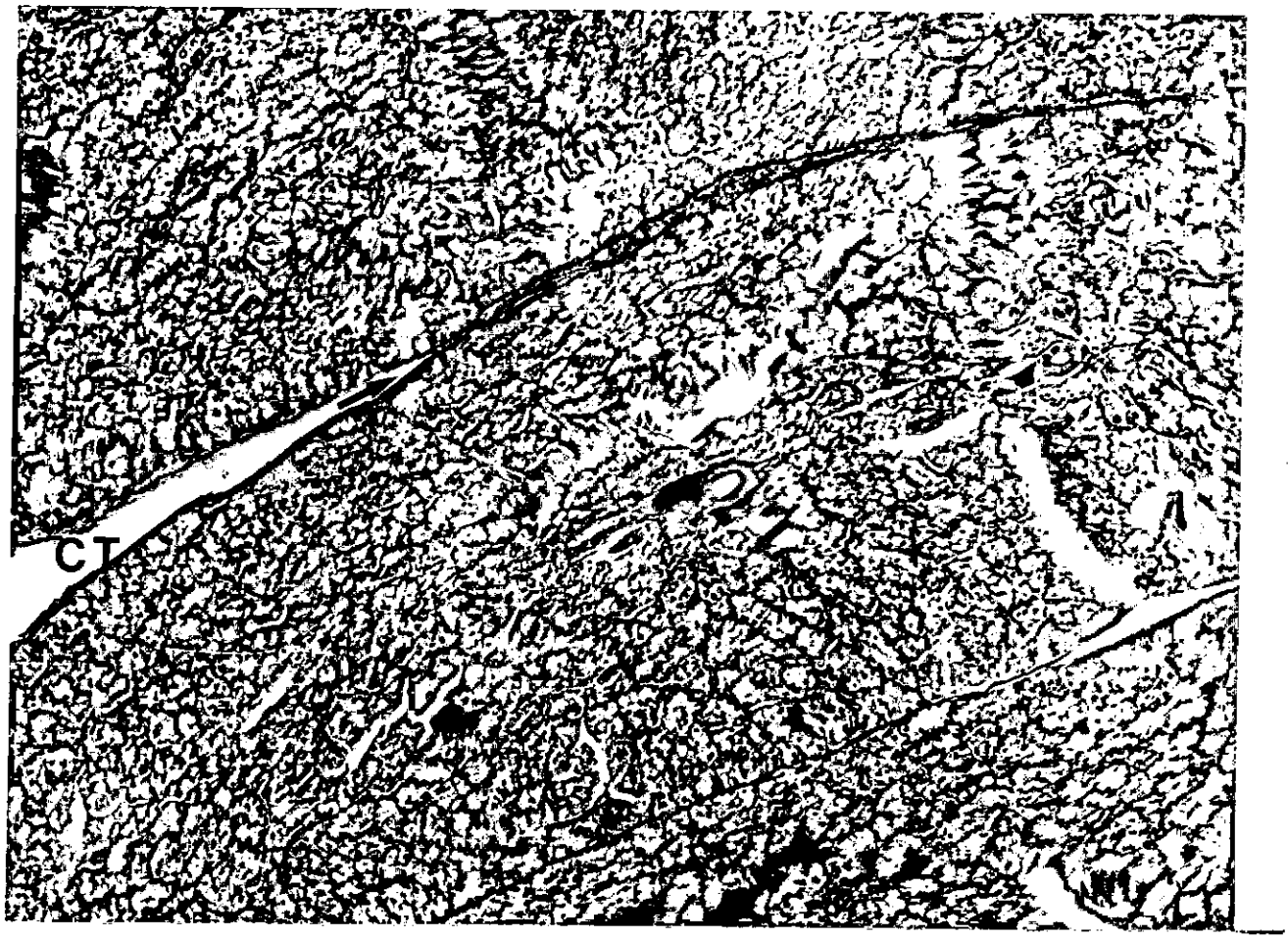

CT
A

Fig. 6.2a

Tubular secretory end piece of mucous cells from rats fed on normal diet (lab. chow).

Arrow shows lumen. ac, acini

X 40

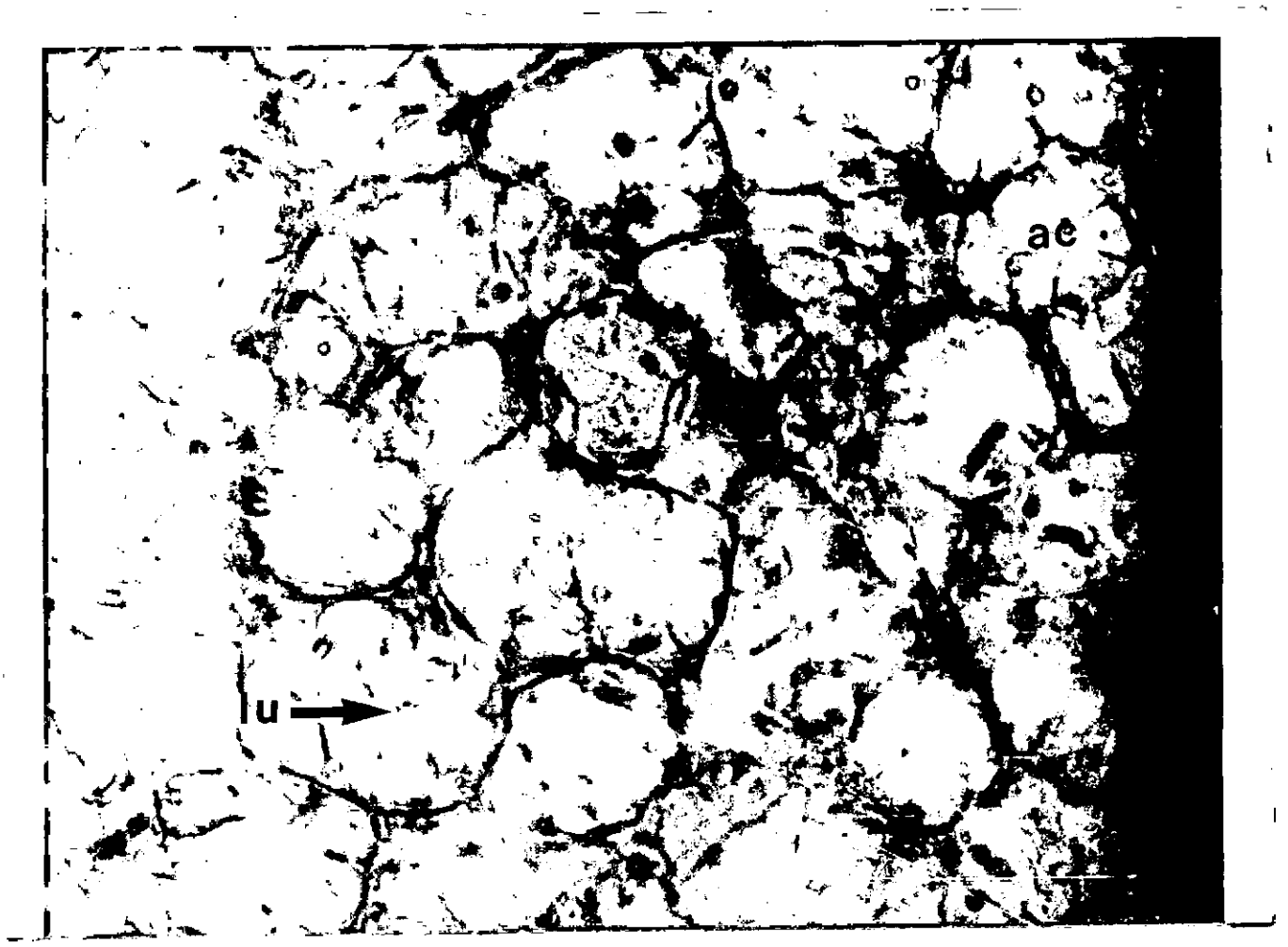

ae
lu

Fig. 6.3a

Photomicrograph of salivary gland from rats fed with cariogenic diet (56%) sugar. Spherical and tubular acini are numerous.

Arrow point to connective tissue septa; dt, ductal system.

X 40

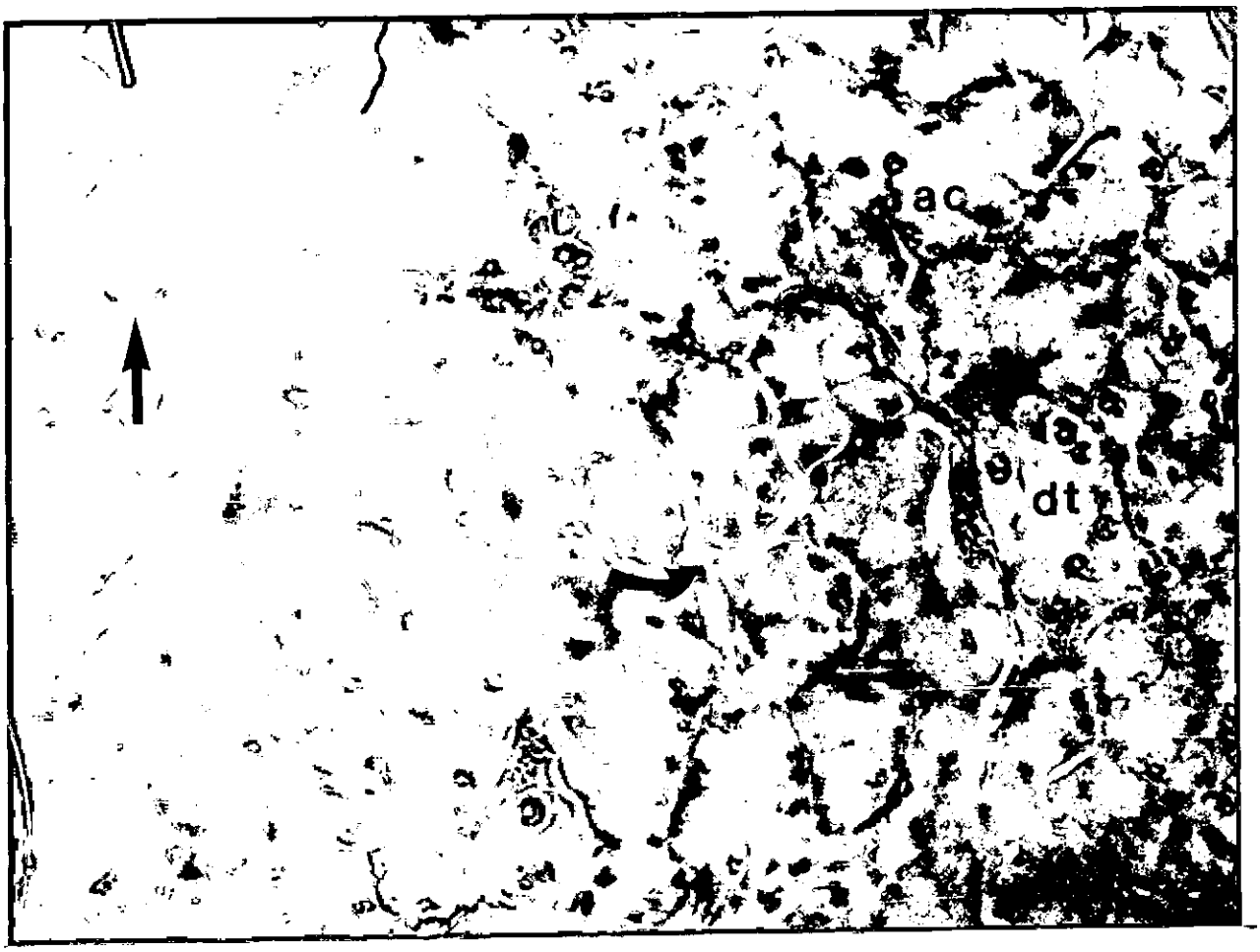

ac
dt

Fig. 6.4a

Photomicrograph of a salivary gland from rats fed with cassava diet. The architecture of the gland is distorted.

dt, ductal system; ct, connective tissue septa

X 40

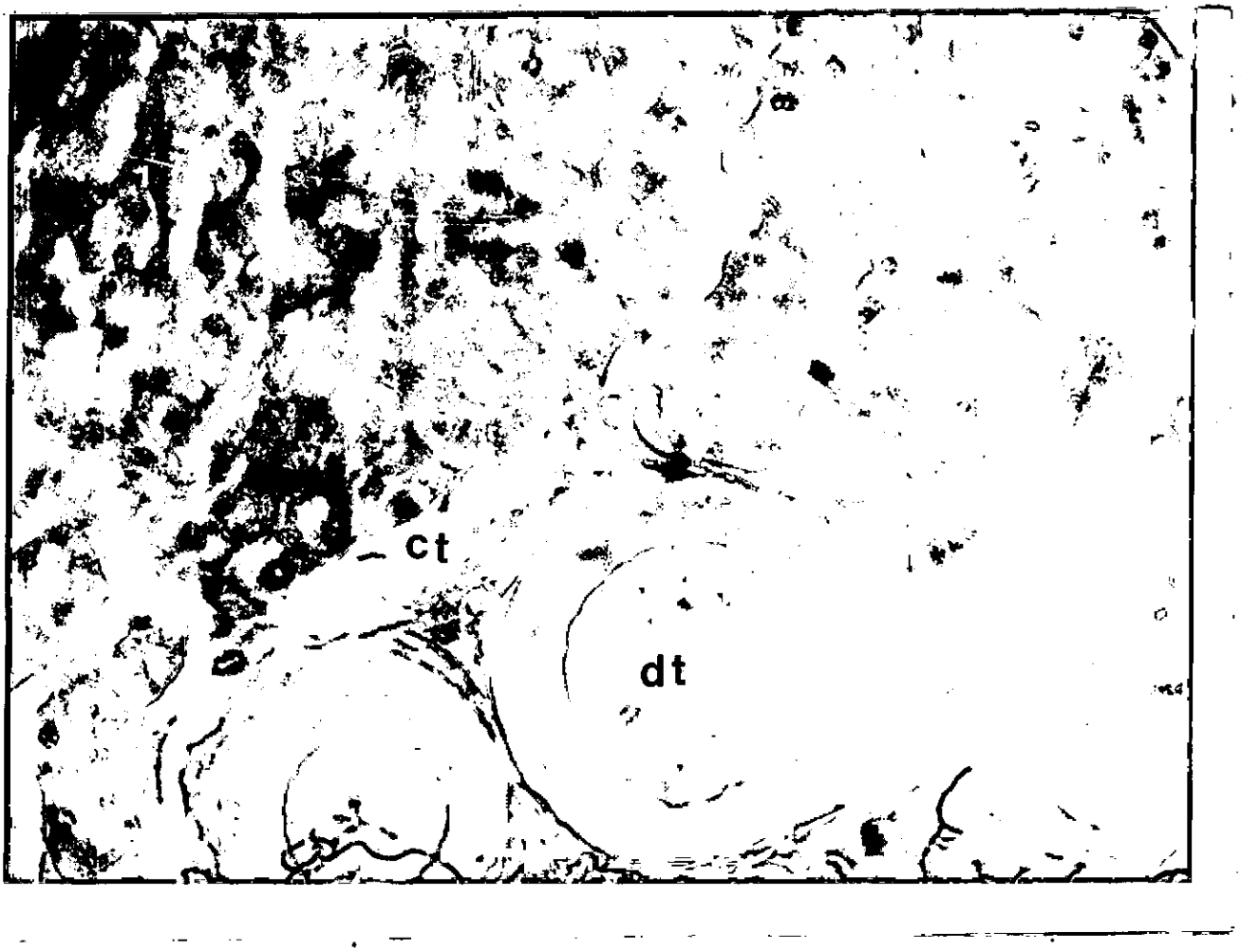

ct
dt

Fig. 6.2b

Mucous cells from laboratory chow fed rats.

Note the pyramidal cells with basally situated nuclei

and the patent lumen. Their cytoplasm appears empty in

Hematoxylin and Eosin stained sections.

Arrow indicate canaliculi. ct, connective tissue.

X 100


n
pc
Can
lu
ct
n

Fig. 6.3b

Parotid gland acini from sugar fed rats.

Note the basal position of the nuclei and the columnar acinar epithelium.

Arrows show nuclie. ac, acini; ct, connective tissue septa.

X 100

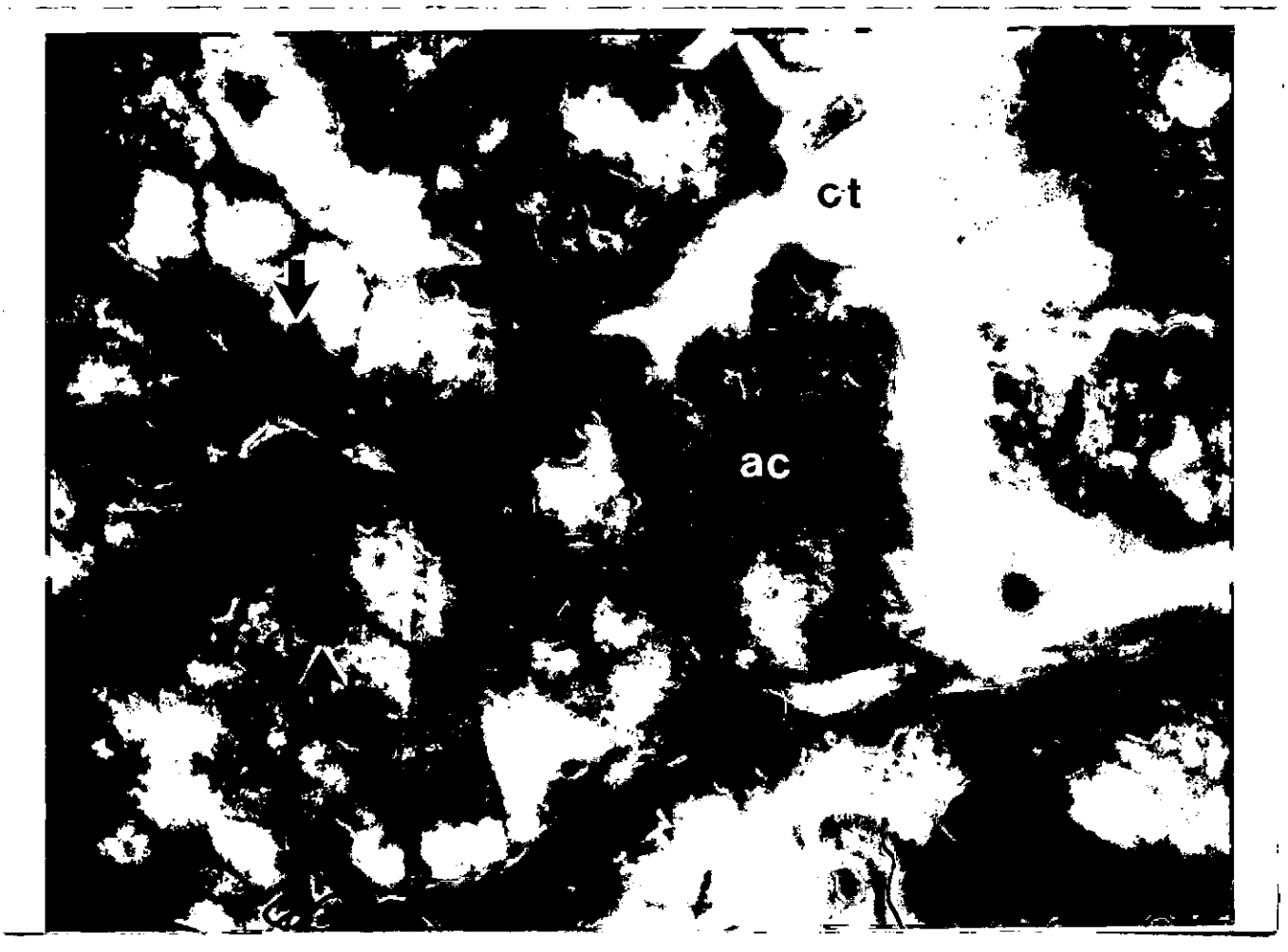

ct
ac

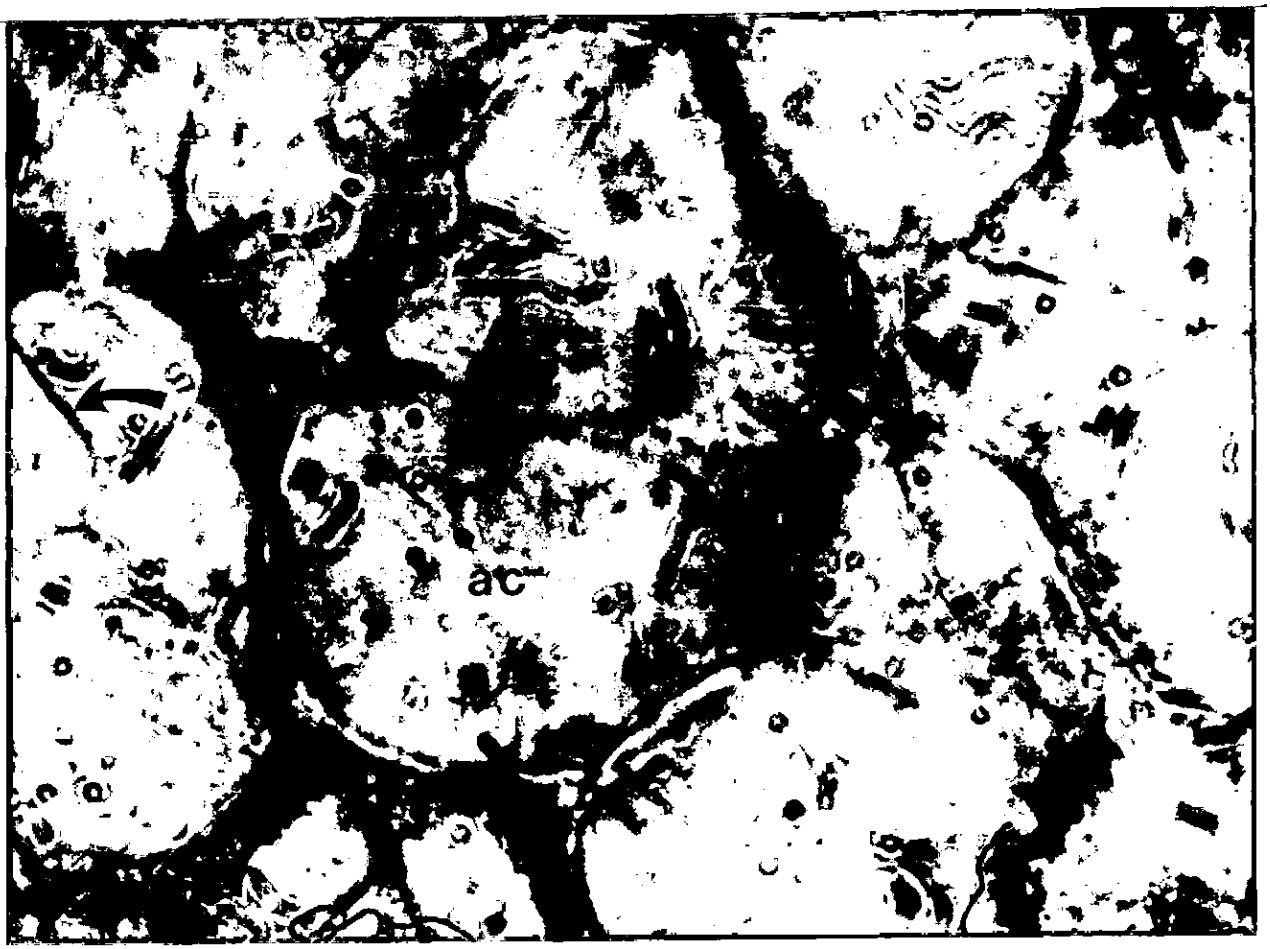
Fig. 6.4b

Photomicrograph of salivary gland acini from rats fed cassava diet.

Note the occluded lumen and undefined boundaries from end piece to end piece.

Arrow shows canaliculi. ac, acinus.

X 100


ac

CHAPTER 7

GENERAL DISCUSSION

GENERAL DISCUSSION

In this study, we have been able to look at the various factors which enhance and inhibit the disease called dental caries. Dental caries is a localized, progressive destructive disease of the teeth that starts at the external surface (usually the enamel) with the apparent dissolution of the inorganic components by organic acids and leading to cavitation.

A lot is known about the aetiology of dental caries but there are still aspects of the disease which need further elucidation. The caries process is multifactorial and involves the integration of several factors, including oral bacteria, saliva, tooth enamel, clearance, and dietary substrates. It is possible to learn about each of these factors by studying them individually or as a group. Many isolated aspects of the caries process have been evaluated, including sugar content of foods (Shannon and Westcott, 1975), acid production and enamel demineralization (Bibby and Mundorff, 1952), pH depressions produced in plaque (Edgar et al, 1975), demineralization/remineralization of the enamel using in vitro experiments (Featherstone et al, 1981).

Although a vast amount has been written about the effects of various foods and drinks on the teeth, little is known specifically of the cariogenicity of Nigerian local foodstuffs.

It has generally been accepted that the common dietary sugars are cariogenic because their small molecules are easily fermented to acids close to the tooth surface by a selection of oral microorganism. Although sugars and starches both belong to the carbohydrate family and can supply the body with energy, and although the starch molecule is in fact built of large numbers of linked glucose units. It has been held that these two classes of

carbohydrates differ in their biochemical properties because the complex, long-chain nature of the starch molecules prevents them being metabolised readily in the mouth, so that relatively little acid can be formed from them. However, this does not imply that the starch is degraded as far as to liberate free glucose, but when it is processed, it either distorts its spatial molecular sturcturte or partially breaks down the long chains of glucose unit to release oligosaccharide fragments (short chains facilitating attack by microbial enzymes (Grenby, 1990).

In this study we have been able to produce a carious lesion in-vivo with multibacteria innoculation. This is similar to the natural phenomenon occuring in the mouth. Up till now, no one has produced a carious lesion identical to natural ones in-vivo, either by acid gel or by monobacterial plaque (Zhou-Xue-dong et al, 1992).

The results of this present study show that the incidence and severity of dental caries were highly statistically significantly different ($p < 0.001$) between offsprings of adequately nourished and malnourished mothers. Low birth weight was also observed in offspring of malnourished mothers. The resistance of malnourished rats to dental caries is reduced during their development (Speirs, 1967). Some researchers believe that the humoral and cell-mediated immune response are affected, Chandra (1977). It is reported that malnutrition in man causes a reduction in the number of T lymphocytes, Neuman et al, (1975). It is also known that rats exposed to moderate protein deprivation from birth and in utero (Navia et al, 1970; Menaker and Navia, 1973a) develop more caries than do controls.

Since no work has been reported in this part of the world to show correlation

between children born of malnourished mothers and rampant caries, the results of this study has confirmed previous studies in animals and indirect human epidemiological evidence which had suggested a cause-effect relationship between early malnutrition and increased dental caries (Alvarez et al, 1979). From this study, pregnant women particularly the low socio-economic group, should be advised about the importance of balanced diet in relation to dental caries susceptibility.

There is now no doubt that dietary sugars are the most cariogenic item in the human diet and in their absence, very little caries develop. From the result of this study we can say categorically that some Nigerian foodstuffs contain intrinsic sugars (those naturally intergrated into the cellular structure of a food), in other words, some Nigerian foodstuffs are more cariogenic than others and it is high time the public is educated about this fact.

We have also been able to show from this study that the caries process can be inhibited by a commonly used chewing stick extract. No such experiment has been carried out in our enviroment, where caries has been inhibited in animals. This study corroborates and is a logical extension of the work done by Salako (1988), Wolinsky and Sote (1983, 1984) who showed that acid production in a test tube and microorganism in the dental plaque can be inhibited using chewing stick extracts.

The histological studies included in this study show that there is significant morphological differences betweeen cariogenic diet and cassava diet. This is evidenced by distortions in the parotid gland end pieces of cassava fed animals when compared to controls. The lighter staining of the end pieces of the glands is due to the fact that Haematoxylin and Eosin stains do not stain mucus distinctly. Mucoid tissue can be

demonstrated more readily by PAS (Periodic Acid-Schiff reagent) technique. The salivary gland's ductal system remain patent in all groups of rats, it can therefore be concluded that the diet does not affect the ductal system. Occlusion of the central lumina in cassava fed rats may be due to salivary gland response to changes occassioned by diets administered to the experimental group. This observation is in agreement with those of earlier workers who showed that the nature of food eaten affects the quality (pH, viscosity, e.t.c.) and flow of saliva (Runn-Gunn et al., 1975; Geddes et al., 1977).

A personal observation during the course of this study is the preponderance of mucoid acini in the salivary glands of rats in general (control and experimental groups). It is already known that the esophagus in rodents is lined by keratinizing epithelium, perhaps this explains why the extra mucous is needed to reduce friction and expedite passage of food along the esophageal tract (Dale, 1985).

That myoepithelial cells could not be demonstrated in histological sections is due to their inability to pick up Hematoxylin and Eosin stain. They could be better demonstrated by histochemical ATPase technique (Cutler and Chaudhry, 1973). Functionally, myoepithelial cells are known to aid in the discharge of acini contents by contraction of the myofibril they contain and the ramification of their podocytic extensions to several acinar cells. In addition, it has been suggested that they act as support for the secretory cells, preventing an over distention of secretory products which accumulate within the cytoplasm (Emmelin and Gjostrup, 1973).

The quantitative aspect of this study (Stereology) is a novel approach to quantitate morphological changes in salivary gland structure in animal models fed cariogenic diets and

controls. Our quantitative methods indicate that rats fed cariogenic diet (sugar) do show slight but discernible differences in several stereological parameters (numerical density, areal fraction and mean acinar profile area).

From these results there are many factors responsible for the caries process. Both the genetic background and the enviroment play a big role. For these reasons different cultures and different genetic make up will necessarily affect caries process in different parts of the world.

This study is a pioneering work in trying to elucidate how Nigerian local environmental factors such as diet and oral hygiene technique may determine the prevalence of caries in our society.

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