A COMPARISON OF CERTAIN SALIVARY PROPERTIES FROM SPECIFIC MAJOR SALIVARY GLANDS OF CARIES RESISTANT AND CARIES SUSCEPTIBLE RATS

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Arnold S. Jaffe
Major professor

ABSTRACT

A COMPARISON OF CERTAIN SALIVARY PROPERTIES FROM SPECIFIC MAJOR SALIVARY GLANDS OF CARIES RESISTANT AND CARIES SUSCEPTIBLE RATS

By Charles J. Sylvester

Certain physical, biochemical and biological properties of parotid, submaxillari-sublingual, and whole rat saliva were studied in order to understand further the factors that contribute to resistance and susceptibility to dental caries.

Specific salivary glands were removed to provide parotid and submaxillari-sublingual salivas. Littermate unoperated rats were used to supply whole saliva. The secretions were collected from anesthetized animals by pilocarpine stimulation.

To study the effect of whole and submaxillari-sublingual saliva from resistant and susceptible rats on microorganisms, the growth rates of rat oral lactobacilli and rat oral streptococci were determined photometrically. Five out of six strains of lactobacilli were not stimulated by any of the salivas tested when compared with a saline control. On the other hand, the four strains of streptococci tested were stimulated by all salivas tested. This stimulation was evident in the maximum limits of growth, but not in the rate of growth as compared with a saline control. Whole saliva, but not submaxillari-sublingual saliva, from susceptible rats supported a greater average maximum amount of growth of the streptococci, than did whole resistant saliva.

Amylase activity of rat saliva was expressed as milligrams of reducing sugar as glucose formed per milliliter of saliva (using an excess of soluble starch as substrate) at a constant time and temperature of reaction. More than 99 percent of the amylase activity of saliva originated in the parotid glands of these animals. Parotid and whole salivas from susceptible rats showed greater amylase activity than these salivas from resistant rats. However, no significant correlation existed between amylase activity and caries activity.

Relative viscosity was determined by timing the fall of saliva between two marks on a narrow bore glass tubing, and dividing by the time required for distilled water to drop the same distance. No difference in relative viscosity was found in the parotid secretions between the two lines of rats. Whole and submaxillari-sublingual salivas from resistant rats were more viscous than these salivas from susceptible rats, when collected at room temperature. However, when whole saliva was collected in tubes submerged in ice, the difference between the resistants and susceptibles no longer existed. Comparison of salivas within the lines of rats showed parotid saliva to be less viscous than submaxillari-sublingual saliva; whereas, whole saliva gave intermediate values. A correlation analysis of caries experience and relative viscosity revealed no significant relation between these two traits.

The rate of flow of saliva from pilocarpine-stimulated glands was recorded on a milliliter per minute basis. There was no essential difference in the rates of flow in the three types of saliva from resistant and susceptible rats. However, within each line of rat, the rate of flow between salivas differed materially. Whole saliva from unoperated rats showed the greatest flow rate, parotid saliva was the slowest, and submaxillari-sublingual saliva was intermediate.

The pH of saliva was measured by a Beckman Glass Electrode pH Meter. The mean pH values of the various salivas tested fell within a narrow range, indicating that there was no significant difference between the salivas.

Buffering capacity was determined as titratable alkalinity; that is, the number of millilieters required to adjust one ml of saliva, diluted one to five, to pH 4.5 $\pm$ 0.2. Whole and submaxillari-sublingual saliva from susceptible rats had a significantly greater buffering capacity than these salivas from resistant rats, but this property was not correlated with caries activity.

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By

Charles J. Sylvester

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INTRODUCTION

Inasmuch as this problem is directly related to dental caries, it would be pertinent to define at the onset, certain basic terms relative to this disease. According to Dorland's American Illustrated Medical Dictionary (20th edition), caries is defined as "the molecular decay or death of a bone, in which it becomes softened, discolored and porous" and the term dental caries is the "discoloration and disintegration of the enamel and dentin by the action of acid-producing bacteria and their products." The latter term has been defined in accordance with the predominant theory for the development of the carious lesion as W. D. Miller proposed in 1895 (Pickerill, 1923). He presented evidence that implicated acidogenic oral microorganisms and carbohydrate food material. He believed that these organisms, while attached to the tooth surface, metabolized the carbohydrate to organic acids, which in turn dissolved the inorganic constituents of the enamel. Accordingly, dental research has since attempted to elucidate the carbohydrate-bacterial relationship and to determine the etiologic agent of the lesion.

Lactobacilli and streptococci have been associated with dental caries to a far greater degree than any other groups of microorganisms studied. However, it has not been definitely established yet as to whether either or both of these groups of bacteria constitute the etiologic agent. That

dental caries is indeed a bacterial disease has been unequivocally established by the experimental evidence reported by Orland, Blayney, Harrison, Trexler, Wagner, Gordon, and Luckey (1954). Their findings indicate that rats reared under germfree conditions remained entirely free of even microscopically demonstrable dental caries. Virtually all of the conventional control rats, possessing the usual mixed oral flora, developed caries when maintained on the same kind of dietary regimen as the germfree animals. It was concluded, therefore, that dental caries in the rat is not possible in the absence of microorganisms.

Later, Orland, Blayney, Harrison, Reyniers, Trexler, Erwin, Gordon and Wagner (1955) inoculated otherwise germfree rats with known bacterial cells in which an enterococcus resembling Streptococcus fecalis was the predominating organism. All these rats developed carious lesions in the molar teeth. These animals were fed the same standard diet that was fed to all conventional control rats having an unknown complex bacterial flora. These control rats regularly developed caries during the 150 day test period. The above studies by Orland, et al., were confirmed by Fitzgerald, Jordan, Stanley, Poole, and Bowler (1960) inoculating the test animals with an alpha-hemolytic streptococcus isolated from the oral cavity of the rats receiving the cariogenic diet.

Kite, Shaw and Sognnaes (1950) eliminated food from intact and desalivated rats by feeding them by stomach tube.

These animals did not develop caries, but the intact and desalivated control animals consuming orally the same diet, did develop caries. These differences were highly significant and showed that with all other factors being controlled and equal, tooth decay is prevented in rats when the direct effects of food in the oral cavity are eliminated.

That the carbohydrate must be present locally was confirmed by Kamrin (1954) when he fed dextrose to the right parabiont of united, genetically similar, pairs of rats. A high incidence of dental decay was observed in the right parabiont, but little or no caries in the left parabiont.

One of the major factors contributing to the development of dental caries is heredity, as demonstrated in rats by Hunt and Hoppert (1939,1941, 1944, 1948b), Hunt Hoppert and Braunschneider (1947) and Hunt, Hoppert and Erwin (1944). As a result of their work, caries-resistant and caries-susceptible lines of rats (Rattus norvegicus) have been produced by progeny testing, close inbreeding and selection.

Recently, a report by Keyes (1960) challenged heredity as a factor to explain caries resistance. He presented data interpreted as suggesting that caries is an infectious and transmissible disease. He found caries activity markedly reduced in both hamsters and rats after penicillin-sensitive oral flora had been depressed prior to feeding a high cargohydrate diet. Furthermore, hamsters whose penicillin-sensitive oral flora had been depressed, in some cases, produced several generations of progeny with negligible activity.

This suggested that the Hunt-Hoppert rats were caries-resistant because of the absence of a cariogenic flora. To test this hypothesis Rosen, Hunt and Hoppert (1961a,b) had caries-resistant litters, within a day after birth, fostered by caries-susceptible mothers, and conversely, newly born caries-susceptible litters fostered by caries-resistant mothers. In another study, rats from both lines were maintained on penicillin until weaned. Littermates from each line were then orally inoculated with feces from caries-resistant and caries-susceptible rats. In both of the above experiments, the rats behaved the same as their natural parents with respect to dental caries.

Like most phenotypic expressions, the carious lesion is the end result of many prior and interrelated reactions and processes. Many lines of investigation have been undertaken to determine any factor that might be associated with resistance or susceptibility to caries in these rats, and thereby aid in the better understanding of this disease. For example, sex was found to be of no significance in the carious process of the animals (Hunt and Hoppert, 1948a). Slight differences were found between the two strains with respect to weight of the teeth, percent ash, and phosphorous content (Hoppert and Shirley, 1950). Fissures of the molar teeth were found to be significantly wider in susceptibles than in resistants, on the average, but the fissure width of some resistants was wider than those of some susceptibles (Kifer, Hunt, Hoppert and Witkop, 1956).

Study of the oral flora revealed striking differences between the two stocks of animals. Using selective culture media, lactobacilli and Streptococcus salivarius were recovered more frequently and in greater numbers from caries-susceptible than from caries-resistant rats (Rosen, Benarde, Hunt and Hoppert, 1955 and Rosen, Hunt, and Hoppert, 1956). No differences in the types of lactobacilli, however, could be detected in the two strains of rats by Rosen, Ragheb, Hunt and Hoppert (1956). They found penicillin very effective in retarding the development of caries in susceptible rats, and inhibiting the acidogenic bacteria (lactobacilli and streptococci). However, terramycin also inhibited the acidogenic bacteria, but did not appreciably inhibit caries (Rosen, Ragheb, Hoppert, Hunt, 1956).

Since saliva provides an intimate environment for the teeth, it was assumed that some of the factors contributing to resistance or susceptibility to caries may be present in this secretion. In their study on rat saliva, Rosen, Benarde, Fabian, Hunt and Hoppert (1957) could find no real difference in the saliva of resistants and susceptibles with regard to antibody titers against lactobacilli, pH, surface tension, refractive index and specific gravity. However, relative viscosity was about ten percent higher in saliva from resistant animals. The significance of this difference was minimized by the variability of the values obtained from one experiment to another.

Willett (1955), extending this study of rat saliva, assayed for various enzymes. He found protease activity 2.5 to 3 times greater in susceptible saliva than in resistant saliva, but could demonstrate no important differences in the activity of acid and alkaline phosphatase, lysozyme, or sulfatase. Hyaluronidase and urease could not be detected. Since the large difference in protease activity in the saliva of mature rats was not observed in young rats of 49-55 days of age, the age when caries development has been initiated in susceptible rats, it was concluded that the protease activity and the degree of susceptibility are independent traits fixed by the process of inbreeding.

Rosen, Sreebny, Hoppert, Hunt and Bachem (1958, 1959a) showed that sialoadenectomy reduces resistance. However, caries developed later in the sialoadenectomized resistants than in control susceptibles, which indicates that extrasalivary factors also contribute to caries-susceptibility.

It is generally accepted by others that extirpation of all of the major salivary glands of certain experimental animals results in an increase in dental caries. This has been amply shown by Kondo, Ichikawa and Arai (1938), Cheyne (1939), Weisberger, Nelson and Boyle (1940), Gilda and Keyes (1947), Dale (1948), Shaw and Weisberger (1949), Kite, et al., (1950), Klapper (1953), Klapper and Volker (1953), Schwartz and Shaw (1953, 1955), Fanning, Shaw and Sognnaes (1954), Bixler, Muhler and Shafer (1954, 1955), Muhler and Shafer (1954) and Blechman, Gupta and Bartels (1960).

When specific glands were removed, however, there was some disagreement as to the effect upon the development of caries. Schwartz and Shaw (1955) using a high sucrose cariogenic diet found that removal of the parotid glands from rats caused an increase in caries, as was the case when the submaxillary and sublingual glands were removed. But Keller, Hunt and Hoppert (1954) using a coarse particle cariogenic diet reported that interruption of parotid secretions did not affect the caries incidence.

Since the main difference between the two studies was the difference in diet, Schwartz, Resnick and Shaw (1958) repeated the experiment studying the effect of diet on selective sialoadenectomy. They found that caries increased in rats in the absence of parotid saliva when fed a high sucrose diet, but not when fed the coarse particle diet, thus explaining the difference in the above-mentioned results.

Rosen, et al., (1959a) found that extirpation of the submaxillary and sublingual glands from rats on the coarse particle cariogenic diet (also called the Hoppert-Webber-Canniff diet) caused a decrease in caries-resistance, whereas removal of the parotid ducts resulted in an increase in caries resistance.

It was of interest, therefore, to study specific salivary secretions from physical, biochemical and microbiological points of view to provide more information regarding the factors that contribute to resistance and susceptibility to dental caries.

GENERAL METHODS AND MATERIALS

Caries-resistant and caries-susceptible rats obtained from the colony of Hunt and Hoppert were used in this study. The rats were maintained on a modified Hoppert-Webber-Canniff coarse particle cariogenic diet (1931, 1932), which consists of the following ingredients:

Coarsely ground rice so that 1 to 2 percent is retained on a 20-mesh screen	66 percent
Whole powdered milk	30 percent
Alfalfa	3 percent
Sodium chloride	1 percent

Thirteen-week old rats of each litter had either a portion of their parotid ducts excised and the severed ends ligated, or their submaxillary and major sublingual glands removed, and the other rats of the same litter served as unoperated controls. Approximately four weeks following the operations, saliva was collected from all rats according to the method of Benarde, Fabian, Rosen, Hoppert and Hunt (1956). This involves lightly anesthetizing the animals by injecting sodium pentabarbital (Nembutal-Abbott) intraperitoneally followed by a subcutaneous injection of the sialogogue, pilocarpine hydrochloride (Merck). This procedure does not elicit a detectable amount of secretion from the minor salivary glands, or from the mucus glands of the oral cavity. No saliva was obtained from six rats which had their parotid

ducts, as well as their submaxillary and sublingual glands removed. Accumulated food and other debris in the mouth were removed by scrubbing the molars with a number 0 stencil brush and their mouths flushed with water using a 5 cc syringe without an attached needle. The residual water was blotted with cotton swabs.

The saliva from each animal was allowed to flow into a graduated conical centrifuge tube facilitated by a short stem funnel (see figure 1). The saliva was collected at room temperature for most of the experiments. For the antibacterial study and for the second set of determinations of amylase activity and viscosity, saliva was collected in tubes immersed in ice. Care was exercised to prevent nasal and lacrimal secretions from mingling with the saliva.

Throughout the experimental period, some of the rats were observed biweekly for macroscopic caries in the mandibular molars.

Figure 1. Device used for the collection of rat saliva.

EFFECT OF SALIVA ON THE GROWTH OF MICROORGANISMS

Literature Survey

A variety of investigations have been made on the effect of human saliva on oral microorganisms.

Hugenschmidt (1896) attributed the removal of microorganisms to the "washing action" of saliva. This was later demonstrated by Bloomfield (1920a, b, c).

Sanarelli in 1891 first reported a salivary bactericidal property against certain pathogens using Chamberland filtrates of saliva. However, Triolo (1897) found that unfiltered saliva showed bactericidal activity, whereas filtered saliva had no effect. No difference was observed in parotid and submaxillari-sublingual secretions. Dold and Weigmann (1934) found that the diphtheria bacillus, among other microorganisms, died in a few hours when inoculated into fresh saliva, but grew in the same saliva after it had been heated to 56° C for 30 minutes. They concluded this was due to an inherent property of saliva and named this factor "inhibine." Dold and Weigmann's conclusion was corroborated by a number of researchers: Clough (1933), Dold, Lächele and Du Dscheng Hsing (1936), Weigmann and Koehn (1936), Shaefer (1936), Brawley and Sedwick (1936), Pesch and Damm (1936), Bibby and Ball (1937), Weigmann and Noeske (1937), Polezhaeva (1938), Dold (1938), Rolleston (1938), Berg (1938), Mühlenbach (1939), Skrotskii, Makhlinovskii and Slutskaia (1939), Lande (1939), Dold (1942),

Dawson and Blagg (1948, 1950), and Bönicke, Reif and Arndt (1953). Many of these workers found the inhibitory principle to vary greatly in different individuals. They included a wide range of test microorganisms, and employed *in vitro* methods to demonstrate this inhibitory activity of saliva. Appleton (1936, 1937) was successful in showing pneumococci inhibition *in vivo* by injecting mice with saliva suspensions and broth suspensions of the pneumococci. On the other hand, Bezi (1932) could not demonstrate conclusively *in vivo* anti-diphtheria properties in saliva.

Unfortunately the workers cited above did not cannulate the salivary glands in order to obtain sterile saliva. The antibacterial activity could have been due to the activity of other microorganisms as shown by Bartels (1933), Besta and Kuhn (1934), Clauberg (1935), Appleton and Dietz (1937), Prica (1937), Mühlenbach (1939), Lande (1939), Weigmann and Hölzl (1940), Thompson and Shebuya (1946), Bethege, Soehring, and Tschesche (1947), Thompson and Johnson (1947, 1951), White and Hill (1949), Hegeman (1950), Scrivener, Myers, Moore and Warner (1950a, b, 1951), Scrivener, Warner, Myers, and Moore (1951), Lammers (1952), Scrivener (1952), Berger (1952), and Armstrong and Jenkins (1953). Some of these workers have suggested that H_2O_2 produced by streptococci is the agent causing inhibition.

Many investigators have concentrated their studies on the effects of saliva on microorganisms which have been suggested as etiologic agents of dental caries.

Miller (1930a) was not successful in demonstrating the inhibition by human saliva of a "caries bacterium." McIntosh, James and Lazarus-Barlow (1925) could find no appreciable bactericidal action against L. acidophilus in human saliva. However, Clough (1934, 1935) and Clough, Bibby and Berry (1938) found virtually all salivas tested to be inhibitory in varying degrees against L. acidophilus, whereas filtered saliva was not inhibitory. This salivary property was not correlated with caries. Taylor and Bibby (1935), Thompson (1941) and Van Kesteren, Bibby and Berry (1942) confirmed the fact that there was an anti-lactobacillus factor in whole human saliva. Hine (1936) found the inhibitory agent active against L. acidophilus was present in 91 percent of saliva samples tested and varied from person to person and from day to day. Armstrong and Jenkens (1953) demonstrated an inhibitory substance against "bacteria described as causing caries" in saliva from virtually all dogs tested. Williams and Oshtry (1957) presented evidence showing that sterile, human parotid saliva lacked metabolites for the multiplication of a homofermentative lactobacillus, among other organisms.

Hill (1939), Curotto Devoto (1940) and Hill and Kniesner (1941) indicated that saliva from a caries-susceptible person supported the growth of L. acidophilus, whereas, caries-resistant saliva did not. However, Rosebury (1930), failed to detect in saliva from caries-free humans and dogs growth inhibitory properties against aciduric bacilli isolated from carious teeth.

Grove and Grove (1942), Kesel, O'Donnell, and Kirch (1945), Kesel, O'Donnell, Kirch and Wach (1946, 1947) and Kesel (1948) attributed the anti-lactobacillus property of caries-immune saliva to ammonia produced by the oral flora. Clark and Carter (1927) had previously reported that most of the ammonia occurring in human saliva was the result of enzymatic activity other than that elaborated by bacteria, but Kirchheimer and Douglas (1950) could not implicate ammonium ions. Hill, White, Matt and Pearlman (1949) obtained a fraction of saliva that was inhibitory to certain bacteria and was recovered in larger amounts from the saliva of persons resistant to caries than from persons susceptible to caries. Green and Dodd (1956) showed an association of an anti-lactobacillus factor with dental caries in humans. Green and Dodd (1957), and Green (1958, 1959) showed this anti-lactobacillus factor to be associated with the globulin fraction of immune saliva, but not susceptible saliva. Grisamore and Toto (1958) found in the globulin fraction II of human sera antibodies which inhibit the growth of L. acidophilus.

Kerr and Widderburn (1958a, b) demonstrated inhibitory properties in sterile, cannulated, human, parotid and submaxillari-sublingual secretions. Zeldow (1955) found as much bactericidal activity against L. acidophilus in parotid saliva as in whole saliva, and concluded that, since the bacterial count of parotid saliva was only one percent that of whole saliva, this activity could not have a bacterial origin. In 1959 Zeldow quantitated this bactericidin,

finding levels in the parotid salivas equal to or greater than in submaxillary salivas. He also showed that this salivary factor required a dialyzable heat-stable cofactor for its activity. He not only established the difference between this agent and lysozyme, but also precluded its similarity to salivary amylase.

After Fleming (1922) demonstrated lysozyme in saliva and other biological fluids, Bartels (1934), Skrotskii, et al., (1939), and Chauncey, Lionetti, Winer and Lisanti (1954) confirmed the presence of lysozyme in saliva, but failed to find it correlated with dental caries. Rudinu (1954) found a correlation of salivary lysozyme with caries in men but not in women.

By incorporating saliva from a caries-resistant and a caries-susceptible person into the drinking water of hamsters, Granados, Glavind and Dam (1950) demonstrated that saliva from a caries-resistant person contained a factor (or factors) which has the ability to decrease dental caries activity in hamsters. Blechman, et al., (1960) found that pooled saliva from caries-immune humans added to the drinking water of sialoadenectomized Sprague-Dawley rats, resulted in a significant decrease in the average extent of carious lesions than in sialoadenectomized rats given pooled saliva from caries-susceptible individuals.

In the only study where saliva from rats was used, Rosen, et al., (1957) was not able to demonstrate an antagonistic effect against rat oral lactobacilli using whole, stimulated saliva from Hunt-Hoppert caries-resistant and caries-susceptible rats.

Methods and Materials

1. Collection and Pretreatment of Saliva

For this phase of the study, 25 litters of rats were used: 13 resistant and 12 susceptible. The size of the litters ranged from 4 to 16 in number with an average of approximately 8 rats per litter. Since parotid saliva was not used for this experiment, only parotidectomies were performed. Unoperated littermates were retained to obtain whole saliva. Thus, only four types of saliva were used. These were submaxillari-sublingual and whole saliva from resistant and susceptible lines of rats. The saliva of littermates receiving the same treatment was pooled, refrigerated overnight to allow precipitation of mucin, and centrifuged. The supernatant liquid was then sterilized using a Morton Filter Apparatus (Corning). All operations described thus far were carried out either in an ice bath or in the refrigerator. One ml of the filtered saliva was tested for sterility in Bacto Brain Heart Infusion Broth (Difco), which was incubated three days at 37° C, and the remainder was stored in the frozen state until needed.

2. Test

To determine the effect of rat saliva upon the various test organisms the procedure, in brief, was to follow the growth rates of the organisms photometrically.

Six strains of rat oral lactobacilli and seven strains of rat oral streptococci as well as Staphylococcus aureus, Escherichia coli, and Bacillus subtilis served as the test

organisms. Cultures of the lactobacilli and streptococci were kindly supplied by Dr. Samuel Rosen, and also the information contained in tables 24 and 25 of the Appendix, which indicates some of their biochemical and cultural characteristics. Stock cultures were maintained in Bacto Micro Assay Culture Agar (Difco). The inoculum for the test was prepared by subculturing from the stock culture into Bacto Micro Inoculum Broth (Difco) supplemented with one percent Bacto Dextrose (Difco) and incubated for 24 hours at 37° C. From this suspension, another subculture was made into the same type of broth and incubated under the same conditions. This resulted in actively growing cultures.

The test medium consisted of 2 ml of double strength Bacto Micro Inoculum Broth supplemented with one percent Bacto Dextrose and 2 ml sterile saliva. Each day this experiment was performed, a saline control was used which consisted of 2 ml of the broth and 2 ml of 0.085 percent NaCl (the approximate concentration of chloride ion in rat saliva). This medium was contained in optically matched 100 mm x 13 mm test tubes. Each tube was inoculated with 0.2 ml of the broth suspension of a test organism. Uninoculated tubes served as blanks. All tubes were incubated at 37° C and growth observed in terms of optical density at zero time and at suitable intervals thereafter, using a Bausch and Lomb Spectronic 20 at wavelength 620 mu after setting the instrument at zero optical density with the uninoculated blank. Growth cruves were plotted on semilogarithmic graph paper.

Results

The results of the effect of rat saliva (i.e., whole and submaxillari-sublingual from resistant and susceptible animals) on rat oral lactobacilli and rat oral streptococci, as well as on Staphylococcus aureus, Escherichia coli, and Bacillus subtilis can be seen in table 1 of the Appendix. A summary of these data appears in table 1 on page 19.

All the rat oral lactobacilli (Nos. 1, 4, 9, 10 and 14), with one exception (No. 11), were not materially affected on the average by the different saliva samples. That is, the mean rates and mean maximum limits of growth of these organisms in the presence of the salivas were virtually the same as when cultured in the presence of the saline control. This is graphically illustrated in figure 2, which shows a typical growth curve of a representative organism from this group.

The one strain of labtobacillus (No. 11) that responded differently from the others, was stimulated by all types of saliva tested. This stimulation was reflected in both the rate and maximum limits of growth when compared to the saline control. Mean values of four tests indicate that essentially no difference existed among the four types of salivas as to the extent of their stimulatory property for lactobacillus No. 11. Figure 3 shows this stimulation in a typical growth curve. B. subtilis was stimulated in a way similar to that of lactobacillus No. 11.

The rat oral streptococci, when cultured in the presence of the four types of saliva, showed an increase in the

TABLE 1. Effect of caries-resistant and caries-susceptible rat salivas on the mean slopes and mean maximum limits of growth of several bacteria

ORGANISM	RESISTANT SALIVA				SUSCEPTIBLE SALIVA				SALINE CONTROL	
	WHOLE		S-S[a]		WHOLE		S-S			
	SLOPE	MAX.[d]	SLOPE	MAX.	SLOPE	MAX.	SLOPE	MAX.	SLOPE	MAX.
Lactobacilli 1, 4, 9, 10, 14	1.06	0.35[b]	1.07	0.33[b]	0.99	0.32[b]	1.02	0.28[b]	1.04	0.32[b]
Lactobacillus 11	3.56	1.52[b]	3.67	1.06[b]	3.51	1.55[b]	3.32	0.92[b]	2.65	0.42[b]
Streptococci	3.08	1.07[b]	3.06	0.89[b]	3.34	1.53[b]	2.91	0.98[b]	3.16	0.58[b]
S. aureus	3.07	1.23[c]	-	-	3.38	1.13[c]	-	-	2.85	0.79[c]
E. coli	4.50	0.74[c]	-	-	4.80	0.70[c]	-	-	4.18	0.75[c]
B. subtilis	3.80	0.80[c]	-	-	3.32	0.77[c]	-	-	2.69	0.59[c]

a= S-S=sublingual and submaxillary salivas.

b= Maximum limits of growth in optical density after 25± 4 hours incubation at 37° C.

c= Maximum limits of growth in optical density after 13 ± 1 hour incubation at 37° c.

d= Maximum limits of growth.

Δ——Δ SALINE CONTROL
o——o RESISTANT SUBMAXILLARI-SUBLINGUAL SALIVA
o--o RESISTANT WHOLE SALIVA
X——X SUSCEPTIBLE SUBMAXILLARI-SUBLINGUAL SALIVA
X--X SUSCEPTIBLE WHOLE SALIVA

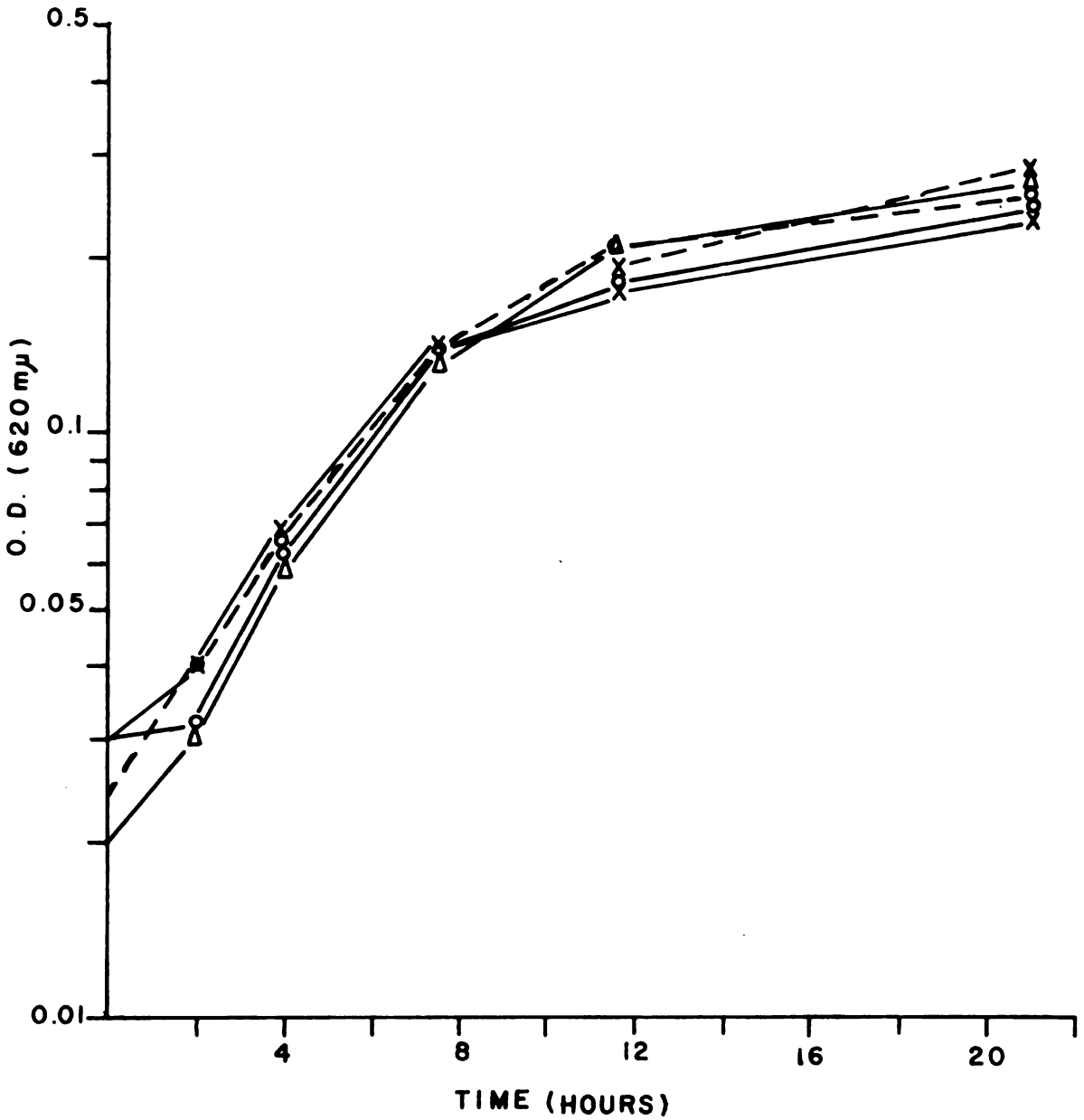


Figure 2. Typical growth curves of a rat oral lactobacillus showing small effect of rat saliva.

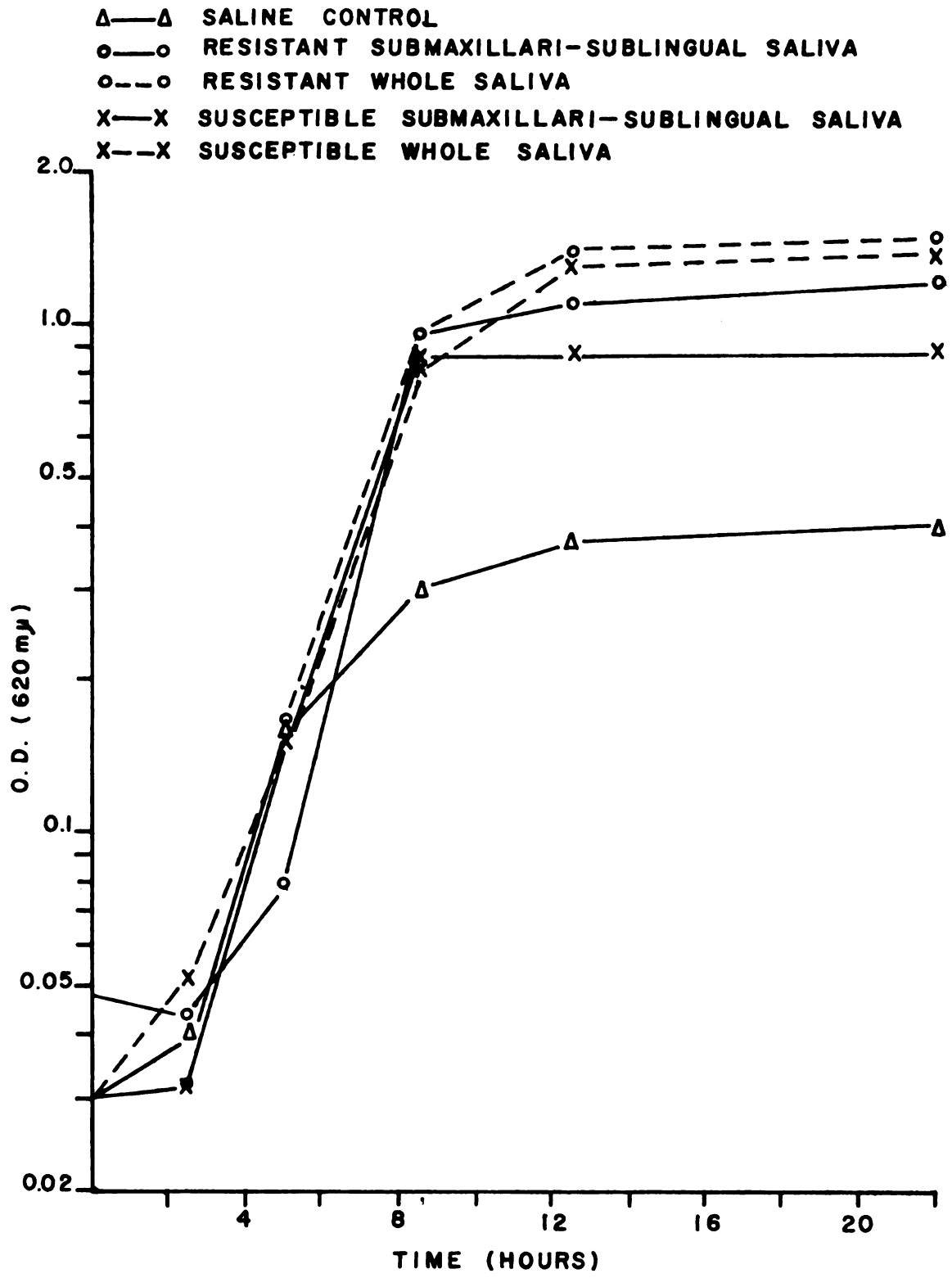


Figure 3. Typical growth curves of rat oral lactobacillus No. 11 showing the stimulatory effect of various rat salivas.

maximum amount of growth (See table 1). This difference is statistically significant ($P < 0.01$) as determined by using the Paired Analysis "t" test (table 2). However, the salivas did not influence the rate of growth (tables 1 and 2).

Whole susceptible saliva supported greater average maximum growth of the streptococci than did whole resistant saliva. This difference is also shown to be statistically significant ($P < 0.01$). On the other hand, submaxillari-sublingual susceptible saliva did not stimulate growth to a significantly greater degree than this type of saliva from resistant animals as indicated in table 2. Tables 2 to 13 of the Appendix show details of these analyses. Figure 4 is a typical growth curve of a rat oral streptococcus and the stimulatory effect of the salivas.

The maximum limits of growth of S. aureus increased when cultured in the presence of resistant and susceptible whole salivas, with no material difference between the two.

E. coli was stimulated only slightly, if at all, by the resistant and susceptible whole salivas.

Discussion

It has been established by Clough (1935), Dold, et al., (1936), Dold (1938), Skrotskii, et al., (1939), Thompson (1941), and Van Kesteren, et al., (1942) that human saliva contains an antibacterial factor other than lysozyme, that is inhibitory to certain bacteria including those types of organisms used in this study. Human parotid and submaxillary secretions have been shown to be active against L.

TABLE 2. Statistical significance of results of the effect of rat saliva and saline on the growth response of rat oral streptococci

COMPARISON	MAXIMUM GROWTH		GROWTH RATE	
	t VALUE	P	t VALUE	P
Resistant Whole Saliva with Susceptible Whole Saliva	6.061	<0.01	0.731	>0.5
Resistant S-S* Saliva with Susceptible S-S Saliva	1.518	>0.2	0.811	>0.4
Resistant Whole Saliva with Saline Control	8.088	<0.01	0.257	>0.5
Resistant S-S Saliva with Saline Control	10.040	<0.01	0.302	>0.5
Susceptible Whole Saliva with Saline Control	10.312	<0.01	0.034	>0.5
Susceptible S-S Saliva with Saline Control	21.654	<0.01	0.855	>0.5

*S-S=Submaxillari-Sublingual Saliva

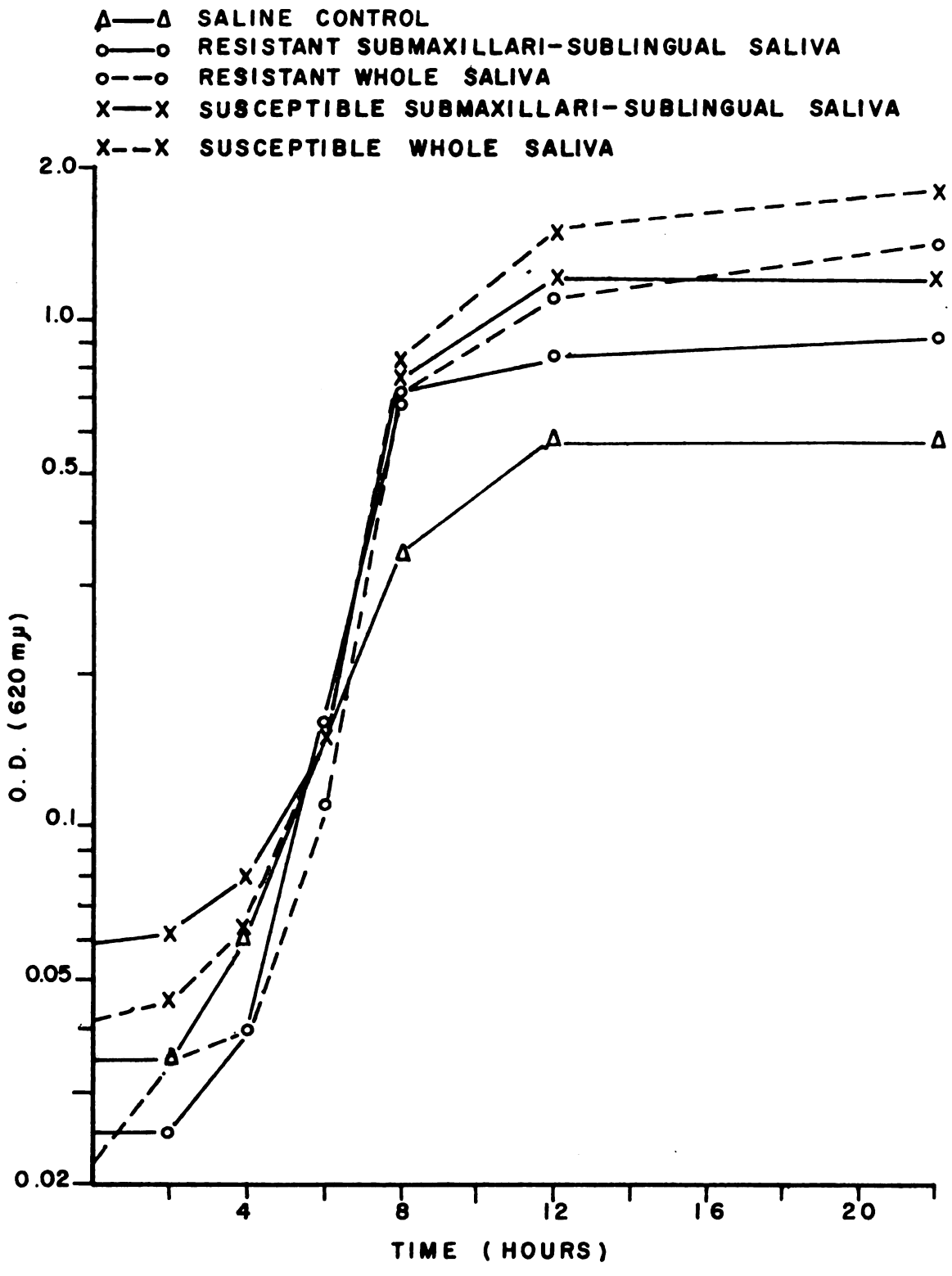


Figure 4. Typical growth curves of a rat oral streptococcus showing the stimulatory effect of various rat salivas.

acidophilus by Kerr and Widderburn (1958a, b) and Zeldow (1955, 1959). Others (Granados, et al., 1950 and Blechman, et al., 1960) presented evidence suggesting that only caries-resistant saliva had this property.

Rosen, et al., (1957) detected no anti-lactobacillus factor in whole saliva from caries-resistant and caries-susceptible rats using the deep well-agar plate method. However, the possibility that such a factor might be present in submaxillari-sublingual saliva from these rats was suggested when Rosen, et al., (1959b) found that caries-resistance decreased when the submaxillari-sublingual glands were removed, but that caries-resistance increased when the parotid duct was removed. Apparently, something in submaxillari-sublingual saliva, not present in parotid saliva, contributes to caries-resistance.

When the submaxillari-sublingual saliva and whole saliva from the resistant and susceptible rats was tested in the experiments described in this report for inhibitory activity agains lactobacilli, streptococci and other organisms, none was detected with the method employed.

However, the total growth of rat oral streptococci was decidedly stimulated by salivas from rats. What may be particularly significant is that whole saliva from the susceptible rats enhanced the growth of these organisms to a greater degree than did the whole saliva from resistant rats. This is especially interesting since streptococci, which are common inhabitants of the mouth and occur in

large numbers, have been implicated recently as etiologic agents of dental caries in experimental animals, rather than the lactobacilli.

The initiation of caries by streptococci was clearly shown by Orland, et al., (1955) in their work with gnotobiotic rats. They succeeded in producing caries in germfree animals ingesting a "cariogenic" diet and inoculated with an enterococcus or an enterococcus plus a proteolytic bacillus. No lactobacilli were present. This enterococcus had been isolated from a carious rat tooth and closely resembled Streptococcus fecalis. Uninoculated animals remained free of even microscopic caries. The authors believed at the time, that these results did not preclude the possibility that certain lactobacilli could also produce dental caries under similar conditions. However, Orland (1957) decided to confine the germfree-caries studies to the enterococci, since the lactobacilli studied under similar conditions failed to produce typical lesions. The above work of Orland, et al., (1955) was confirmed by Fitzgerald, et al., (1960) who also used germfree rats in their study.

Fitzgerald and Keyes (1960) succeeded in inducing dental caries in a strain of "caries-inactive" hamsters. They introduced single or pooled cultures of streptococci isolated from a carious lesion in a hamster. However, inoculation with strains of lactobacilli and diphtheroid organisms from "caries-active" hamsters and strains of strep-

tococci isolated from the oral cavity and feces of "caries-inactive" hamsters was without effect.

That the streptococci used in this study were strongly stimulated by rat saliva, supports the hypothesis that streptococci rather than the lactobacilli, constitute the main etiologic agents of dental caries. Relative to the findings of Rosen, et al., (1958, 1959a, b), however, this hypothesis raises a question. When resistant rats were sialoadenectomized, the incidence of caries and the numbers of lactobacilli increased, but the numbers of streptococci did not change materially. If this hypothesis is correct, why did the streptococci not increase, either along with, or instead of the lactobacilli? One explanation for this phenomenon is that a record of the total numbers of streptococci does not give information about the possible change in the relative types of streptococci occurring in the mouths of these rats as a result of sialoadenectomy.

It has already been shown that the types of streptococci occurring in experimental animals vary with regard to dental caries. Rosen, et al., (1955) recovered Streptococcus salivarius twice as frequently in caries-susceptible rats than in caries-resistant rats. Further, an unidentified oral streptococcus producing a rough, crateriform colony was found in every resistant rat, but only in 18 percent of the susceptible rats. Fitzgerald and Keyes (1960) found two types of streptococci in hamsters' oral cavities, some of

which could produce dental caries when inoculated orally into caries-inactive hamsters and some that could not.

It would be of interest, then, to compare the cariogenic potential of the various strains of rat oral streptococci in a future study. Grouped according to whether they were stimulated by rat saliva or whether they were not (if such can be found), these strains would be inoculated orally into caries-susceptible rats after the rats had been maintained for a period on a diet containing penicillin. Such a diet has already been shown to inhibit dental caries completely in caries-susceptible rats (Rosen, Ragheb, Hoppert and Hunt, 1956).

Since an antibacterial factor was not detected in this study, it is possible that the conditions of the test were such as to mask any inhibitory influence exerted by the saliva. That is, the inhibitory effects of the saliva against the lactobacilli could have been overcome, because the enriched culture medium used supported abundant growth of these organisms. This situation could also be investigated in a future study. If it can be demonstrated that lactobacilli under minimal growth conditions are in fact inhibited by rat saliva, and that the streptococci are stimulated under the same conditions, this would be further evidence that streptococci rather than lactobacilli are the etiologic agents for dental caries in experimental animals.

The abundant growth supported by the enriched culture medium could have masked another type of response of the

test organisms to rat saliva. Since a greater total growth of the streptococci was stimulated by susceptible whole saliva than by resistant whole saliva, one would expect that the rate of growth of the streptococci would also be stimulated to a greater degree by these salivas. However, no difference in growth rates was detectable.

Inasmuch as the stimulatory property of only the whole saliva was significantly greater in the susceptibles than the resistants, it would be of interest to investigate the parotid secretion in this regard. One would expect that the parotid saliva is contributing a stimulatory principle to whole saliva, at least in part, either additively or complimentarily.

AMYLASE

Literature Survey

There has been some disagreement as to whether amylase activity of whole human saliva is related directly or inversely with caries incidence, or whether any relation exists at all.

Those investigators who found a direct relation between amylase activity and caries susceptibility include Michel (1915), Gore (1935), Florestano, Faber and James (1941), Turner and Crane (1944a, b), Turner and Crowell (1947) and Turner, Anders and Becker (1957).

Myers and Adams (1932) and Schneyer (1951) recognized that the chloride content of saliva has an important influence upon the amylolytic index of a given individual. Anders (1956) and Carter, Englander, Mau and Hoerman (1957) demonstrated a significantly greater salivary chloride content in caries-active than in cares-free persons.

On the other hand, Pickerill (1924a, b) and Day (1934) found a direct relation between amylase activity and caries resistance; whereas, Hubbell (1933), Bergeim and Barnfield (1945), Bárány (1947), Hess and Smith (1948) and Ericsson, Hellström, Jared and Stjernström (1954) found no correlation between salivary amylase and dental caries in whole human saliva, and Rosen, et al., (1957) found none in rat saliva.

Studies were conducted to determine the amylolytic activity of saliva secreted from specific major glands, or to

determine the amylolytic activity of the gland tissue itself. Gore (1938b), Schneyer (1956b) and Köstlin and Rauch (1957) found greater amylase activity in parotid saliva than in submaxillari-sublingual saliva of humans. The preponderance of amylase activity in the parotid glands was shown to be the case also in experimental animals. Schneyer and Schneyer (1956, 1960) demonstrated it in the salivary glands of rats, while Gorden and Utrias (1957) confirmed it in the salivary glands of rats and mice. Ryan (1909) found that rabbit parotid saliva showed amylase activity to about the same degree as human saliva, but that the submaxillary saliva showed no activity. However, Raynaud and Rebeyrotte (1950) demonstrated that the amylase activity of the submaxillary gland of mice exceeded that of the parotid gland.

The data of McGeachen and Gleason (1956) showed that although amylase activity varied widely in the saliva of individual rats, the average is still several times that of human saliva. Latimer and Warren (1897) found high levels of amylase in both parotid and submaxillary glands of rats and mice.

Chittenden and Ely (1883) reported that the variations in the titratable alkalinity are within too narrow limits to exercise any appreciable influence on the amylolytic action of human saliva. However, Sullivan and Storvick (1950a) showed a significant positive correlation between buffer capacity and starch hydrolyzing time.

Carlson and Crittenden (1910) found parotid amylase to vary directly with the rate of flow as influenced by various stimuli. Deakins, Cheyne, Bibby and Van Kesteren (1941) working with whole human saliva found these two properties were not correlated as determined by a statistical analysis of the data.

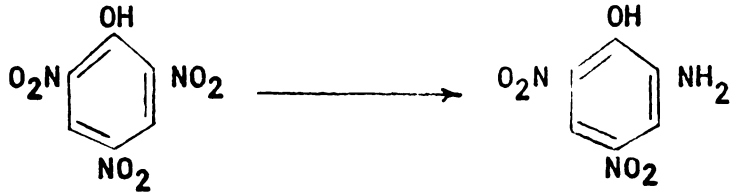
Methods and Materials

As soon as possible after collection, usually within an hour, the assay for amylase activity was carried out. The six types of saliva under study, i.e., whole, parotid and submaxillari-sublingual from caries-resistant and caries-susceptible rats, were collected at room temperature. This constituted the first experiment. A number of determinations were made in a second experiment with whole saliva that had been collected in tubes submerged in ice.

The method used to determine amylase activity was an adaptation of one by Myers, Free and Rosinski (1944). The modifications were the substitution of saliva for serum, and a reduction in the incubation time of the enzyme-substrate reaction mixture from 15 to 10 minutes. Each saliva sample from individual rats was tested in duplicate, using the glucose standard in triplicate.

The test involves the incubation of enzyme (contained in saliva) with a soluble starch solution as the substrate. After a specified period of time, the enzymatic reaction was stopped by the addition of picric acid (2, 4, 6-trinitrophenol) in a quantity to yield a saturated solution of the

acid. Upon boiling in a solution made basic by sodium carbonate, picric acid is reduced to picramic acid by the aldehydic groups produced during the enzymatic hydrolysis of the starch.



Picric Acid Picramic Acid

The concentration of picramic acid, a colored compound, was determined photometrically, using a Bausch and Lomb Spectronic 20 colorimeter. The amount of picramic acid formed is directly proportional to the number of reducing groups. By comparing the amount of reduction occuring in the system containing saliva and starch with the reduction produced by a known concentration of glucose, the amylase activity could thus be expressed as milligrams of reducing sugar as glucose formed per milliliter of saliva, after 10 minutes incubation at 40° C. This was calculated according to the formula

$$\frac{R}{S} \times D \times 0.6 \times \frac{9.1}{3} = \text{mg glucose/ml saliva}$$

where R = Optical density of the saliva assay tube

S = Optical density of the standard (glucose) tube

D = Dilution factor of saliva

0.6 = mg Glucose in the standard tube

$\frac{9.1}{3}$ = Factor to correct for the fraction of saliva taken from the enzyme-substrate mixture.

Results

The data for amylase activity are given in table 3. The parotid gland was found to be virtually the sole source of amylase in rat saliva, since the activity produced by the submaxillari-sublingual secretion was so slight that it might be considered negligible. There was greater activity in the saliva from susceptibles than from resistants ($P < 0.01$). This difference was evident in the parotid saliva as well as in whole saliva.

Correlation analysis (see table 4) between amylase activity in whole susceptible saliva and caries age yielded correlation coefficients (r) of +0.082 and +0.105 for the first and second experiments, respectively. A similar analysis of combined data from resistant and susceptible whole salivas and caries age indicated $r = +0.270$. Caries age is defined as the age in days of a rat when a macroscopic carious lesion is first detectable. The above r values are not significant at the 5 percent level. However, significant values (at the 2 percent level) were obtained in a correlation between amylase activity and caries age with whole resistant saliva ($r = +0.651$).

The data and formulae upon which the values in table 4 are based are presented in tables 14 through 16 and page 127 in the Appendix.

TABLE 3. Amylase activity of whole, parotid, and submaxillari-sublingual saliva from caries-resistant and caries-susceptible rats

Type of Saliva	Strain of Rat	No. of Rats	Mean Amylase Activity[a] and Standard Error	Difference	t	P
			First Experiment[b]			
Whole	Resistant	24	1,013±99	540±161	3.4	< 0.01
	Susceptible	25	1,552±127			
Parotid	Resistant	12	1,944±370	1,954±690	2.8	< 0.01
	Susceptible	18	3,890±581			
Submaxillari-Sublingual	Resistant	18	1.87±0.62	0.49±0.696	0.7	> 0.4
	Susceptible	22	1.37±0.32			
			Second Experiment[c]			
Whole	Resistant	18	1,145±96	915±218	4.2	< 0.01
	Susceptible	19	2,060±196			

a Milligrams glucose per milliliter of saliva.
b Saliva collected at room temperature.
c Saliva collected in tubes immersed in an ice bath.

TABLE 4. Correlation analyses of rat saliva properties and caries age

	r	P
Amylase Activity[a] and Caries Age[b]		
Susceptible Whole Saliva (first experiment)	+0.082	>0.05
Susceptible Whole Saliva (second experiment)	+0.105	>0.05
Resistant Whole Saliva (second experiment)	+0.651	<0.02
Resistant and Susceptible Whole Saliva (second experiment)	+0.270	>0.05

a Amylase Activity = mg glucose per ml saliva.

b Caries Age = days of age when a carious lesion first appeared.

Discussion

The test for amylase activity employed in this study does not distinguish between alpha or beta amylase. McGeachen and Gleason (1956) found that rat salivary amylase was probably the alpha type, as is also the case in human saliva. Confirmation of the type of amylase in saliva from the rats used in this investigation was not considered necessary.

The elaboration by the parotid glands of virtually all of the salivary amylase in rats, as shown in table 3, supports the findings of Schneyer and Schneyer (1956, 1960), and Gordon and Utrias (1957). Even though parotid saliva from susceptible rats showed a significantly greater level of amylase activity than did parotid saliva from resistant rats, this difference is not considered to be of great importance, since it does little to explain the caries behavior of the two lines. This can be explained by a consideration of the following facts.

When parotid ducts alone were removed from resistant animals, thus blocking essentially all salivary amylase, the effect on caries incidence depended upon whether the rats were maintained on a diet which included either a simple carbohydrate (sucrose) or a complex carbohydrate (coarse particle rice). If the carbohydrate was sucrose, caries susceptibility increased, but when rice was used, the susceptibility of the rats to caries either did not change or decreased (Keller, et al., 1954; Schwartz and Shaw, 1955; Schwartz, et al., 1958; and Rosen, et al., 1959a). This is

what one would expect if greater amylase activity in susceptible rats was interpreted as accounting for susceptibility. However, the incidence of caries increased when the saliva from all the major salivary glands was interrupted, thus blocking the source of salivary amylase in resistant animals maintained on the rice diet (Rosen, et al., 1958, 1959a). Caries should have decreased if salivary amylase contributed materially to susceptibility.

Furthermore, the level of amylase activity, although lower in resistant whole saliva than in susceptible whole saliva, is still high, and probably sufficient to catalyze the hydrolysis of large quantities of starch into simple carbohydrates needed by cariogenic bacteria.

Another aspect of amylase activity which minimizes its importance in accounting for a difference between the two lines of rats is revealed in the results of correlation analysis between amylase activity and caries age (table 4). The correlation coefficients of + 0.082 and + 0.105 for susceptible rats in two separate experiments, and + 0.270 for combined data of resistant plus susceptible rats are not significant at the 5 percent level. Although the caries age in the susceptible and resistant lines are widely different, the type of analysis carried out with the combined data corrected for this fact, since the correlation analysis of two variates assumes a normal distribution.

When the data obtained from the resistant rats alone was analyzed, a significant positive correlation ($r = + 0.651$)

was obtained. This might appear somewhat puzzling, since these animals have a higher mean caries age, but lower mean amylase activity than the susceptibles. From this, one might expect to obtain a negative rather than a positive value for r. It seems apparent that when the resistant rats were selectively inbred for their low caries activity (high caries age), the trait corresponding to a high salivary amylase activity was also selected, even though this trait probably has no causal relation to caries-resistance.

VISCOSITY

Literature Survey

Lohmann (1904), Rathje and Fröhlich (1949) and Rathje (1951) reported a direct relation between the degree of viscosity of whole human saliva and susceptibility to caries. Willsmore (1937) reported no relation existed between viscosity of "resting" saliva (saliva secreted in the absence of overt stimulation) and general caries susceptibility, but that a "resting" saliva with a viscous tendency is a predisposing factor in the susceptibility to gingival caries. Rosen, et al., (1957) in studying rat saliva found that the relative viscosity was slightly, but significantly higher in the saliva of caries-resistant than in caries-susceptible rats. Shafer, Clark and Muhler (1957) found that higher levels of thyroxine administered to rats for two months resulted in a lower incidence of dental caries and a less viscous saliva. These findings were substantiated by Shafer, Clark, Bixler and Muhler (1958b) who demonstrated that a dysfunction of the rat thyroid gland caused by propylthiouracil and radiothyroidectomy resulted in an increase of salivary viscosity. Thyroxine reversed this effect and restored the function of the gland.

Lothrop and Gies (1910), Rae and Clegg (1949) and Dewar and Parfitt (1954b) could find no relation between viscosity and caries activity.

Salivary mucin, a glycoprotein, has been investigated as to its possible role in viscosity of saliva and dental caries. Willsmore (1937) concluded that mucin is probably the major factor contributing to the viscosity of saliva.

Gore (1938b) and Simmons (1941) showed that enzymes in bacteria-free saliva catalyzed the breakdown of mucin in a way that resulted in its depolymerization, as reflected in a decrease in viscosity, and a concomitant release of the prosthetic carbohydrate group, as evidenced by the liberation of reducing sugars. Knox (1953b) decreased the viscosity of salivary mucoid using trypsin, thus depolymerizing the mucoid, and then liberated a reducing sugar from this trypsinized mucoid using hyaluronidase. Gore (1938b) further reported that human submaxillari-sublingual saliva contained a greater concentration of carbohydrate than parotid saliva.

Dewar and Parfitt (1954a) demonstrated a highly significant positive correlation between viscosity of saliva and polymerized mucin. When Shafer, Clark, Bixler and Muhler (1958a) bilaterally ligated the ducts of the submaxillari-sublingual glands of rats, thus blocking the flow of mucous saliva from the sublingual gland, a significant reduction occurred in salivary viscosity. Tests for mucoprotein in parotid secretion were shown by Bramkamp (1936) to be negative, but positive for mixed saliva. However, Bagnell and Young (1930) indicated that the viscosity of saliva bears no direct relationship to its mucin content.

Lohmann (1904) and Miller (1904, 1905) concluded that saliva containing much mucin was conducive to caries. Rogers (1948) presented evidence to support this view when he showed that some groups of organisms in raw saliva are able to break down and make available the salivary mucins as a fermentable carbohydrate source for streptococci, staphylococci and lactobacilli. On the other hand, Lothrop and Gies (1910) and Dewar and Parfitt (1954b) could find no relation between the concentration of mucin in a given fraction of saliva and the state of the teeth.

Methods and Materials

Two experiments were conducted for the determination of viscosity. In the first experiment, whole, parotid and submaxillari-sublingual salivas from the two lines of rats were collected at room temperature. In the second experiment, only whole saliva from the two lines of rats was collected in tubes immersed in an ice bath, then placed in the refrigerator until tested. These samples were brought to room temperature quickly and their viscosities measured. In all cases, saliva from individual rats was used rather than pooled samples.

Since the purpose of the study was to detect any difference in viscosity between the various salivas, relative values instead of absolute values were determined. This was accomplished by timing the fall of saliva between two arbitrarily spaced marks on a narrow bore glass tubing, and dividing by the time required for distilled water to drop the same distance.

Usually within one-half hour after collection, the salivas were centrifuged to remove any particles, and the relative viscosity was determined at room temperature (27° ± 3° C). The viscometer was cleaned for each saliva sample passing an acid-dichromate solution through it, followed by a sequence of rinsing solutions. These were distilled water, 70 percent ethanol and acetone. Finally, the viscometer was air-dried before evaluating the next sample of saliva.

Results

The data for relative viscosity are given in table 5. In the first experiment (saliva collected at room temperature) the relative viscosity of whole saliva from resistant rats was 1.92 ± 0.037 seconds, whereas that from susceptible rats was 1.66 ± 0.066 seconds. The difference of 0.26 ± 0.066 seconds is highly significant (at the 1 percent level). However, in the second experiment (saliva collected in tubes immersed in ice), the relative viscosity of whole resistant saliva was 1.99 ± 0.043 seconds, while that from susceptible rats was 1.89 ± 0.033 seconds. This difference (0.10 ± 0.054 seconds) is not significant at the 5 percent level. It can be seen that the viscosity of resistant whole saliva remained very much the same in both experiments, but the susceptible whole saliva was more viscous when collected and kept at a colder temperature.

The relative viscosity of parotid saliva from resistant rats was 1.29 ± 0.035 seconds, and that from the susceptible

animals was not significantly different with a relative viscosity of 1.35 ± 0.034 seconds. Resistant rats produced a more viscous submaxillari-sublingual saliva than did the susceptible rats. The relative viscosity of submaxillari-sublingual saliva from resistant rats was 2.14 and that from susceptibles was 1.94. The difference of 0.20 ± 0.084 seconds is statistically significant. The saliva from specific salivary glands used in the viscosity determinations were collected at room temperature.

Comparison of the relative viscosity of parotid, submaxillari-sublingual and whole salivas within each line of rat (tables 5 and 6) yielded marked differences, which are significant at the 1 percent level. Parotid saliva was less viscous than submaxillari-sublingual saliva, whereas whole saliva, a natural mixture of the other two types, gave intermediate values.

Correlation analysis between the relative viscosity of whole saliva and caries age (table 7) did not reveal any significant relation at the 5 percent level, when these two traits were considered in resistant animals *per se* or in susceptible animals *per se*. The data and formula used in these analyses are presented in tables 17, 18 and 19 of the Appendix.

The correlation coefficient between these two traits was not significant when the data from the two lines of rats were combined. The caries age in the susceptible and resistant lines are widely different, but the type of analysis

TABLE 5. Relative viscosity of whole, parotid, and submaxillari-sublingual saliva from caries-resistant and caries-susceptible rats

Type of Saliva	Line of Rat	No. of Rats	Relative Viscosity* (Seconds)	Difference	t	P
Whole (First Experiment)	Resistant	25	1.92 ± 0.037	0.26 ± 0.066	3.97	< 0.01
	Susceptible	26	1.66 ± 0.054			
Whole** (Second Experiment)	Resistant	23	1.99 ± 0.043	0.10 ± 0.054	1.84	> 0.05
	Susceptible	20	1.89 ± 0.033			
Parotid	Resistant	12	1.29 ± 0.035	0.06 ± 0.049	1.23	> 0.05
	Susceptible	18	1.35 ± 0.034			
Submaxillari-Sublingual	Resistant	18	2.14 ± 0.044	0.20 ± 0.095	2.10	< 0.05
	Susceptible	22	1.94 ± 0.084			

* Relative viscosity is the time in seconds taken by saliva to fall between two marks in a glass tube, divided by the time taken for distilled water to fall the same distance.

** Saliva was collected in tubes immersed in an ice bath, for the second experiment. All other salivas listed above were collected at room temperature for the first experiment.

TABLE 6. Comparison of differences in relative viscosity of salivas within the resistant and susceptible strains of rats

Line of Rats	Salivas Compared	Difference (Seconds)	t	P
Resistant	Whole with Parotid	0.63 ± 0.050	12.49	<0.01
Resistant	Whole with S-S*	0.22 ± 0.058	3.82	<0.01
Resistant	Parotid with S-S	0.85 ± 0.056	15.08	<0.01
Susceptible	Whole with Parotid	0.31 ± 0.064	4.82	<0.01
Susceptible	Whole with S-S	0.28 ± 0.100	2.79	< 0.01
Susceptible	Parotid with S-S	0.59 ± 0.091	6.49	<0.01

* S-S = Submaxillari-Sublingual Saliva.

TABLE 7. Correlation analysis of relative viscosity of rat whole saliva and caries age

	r	P
Susceptible Whole Saliva (first experiment)	+0.251	>0.05
Susceptible Whole Saliva (second experiment)	+0.452	>0.05
Resistant Whole Saliva (second experiment)	-0.004	>0.05
Resistant and Susceptible Whole Saliva (second experiment)	+0.038	>0.05

carried out with the combined data corrected for this fact, since the correlation analysis of two variates assumes a normal distribution. See page 131 of the Appendix for details of this analysis.

Discussion

That saliva from submaxillari-sublingual glands is decidedly more viscous than that from the parotid glands, confirms similar findings by Shafer, et al., (1958a). They reported a significant reduction in viscosity of rat saliva when the submaxillari-sublingual ducts were ligated, which blocked the flow of mucous saliva from the sublingual gland. Related to these findings also is the work of Gore (1938b), who reported that the "thick" human mandibular (submaxillari-sublingual) saliva has much more mucin than parotid saliva.

The greater degree of relative viscosity of whole resistant saliva than of susceptible whole saliva confirms the findings of Rosen, et al., (1957). Little, if any, importance can be attached to this difference, however. When it was noticed that the relative viscosity of saliva decreased on standing at room temperature, an experiment was conducted to determine the rate of decrease in whole saliva from a resistant rat and from a susceptible rat when these salivas were stored for a number of hours at refrigerator and at room temperature. As can be seen in figure 5, the decrease was considerably greater at room temperature with both salivas, especially with susceptible saliva.

Other workers reported similar findings. Gore (1935, 1938b) observed the spontaneous autolysis of human salivary mucin at 37° C, 40° C - 45° C, and 75° C, which had the effect of decreasing the viscosity of saliva and simultaneously increasing reducing sugars. He attributed this autolysis to the hydrolytic activity of salivary amylase on the carbohydrate component of mucin. Although, Ericsson and Stjernström (1951) also noticed that the viscosity of human whole saliva decreased upon standing at room temperature, they showed that neither alpha amylase nor salivary bacteria had any effect. Instead, ascorbic acid in the presence of hydrogen peroxide, which bacteria are known to elaborate, or in the presence of traces of cupric ion, reduced the viscosity rapidly. Mandibular saliva was stable or changed slowly. Dewar and Parfitt (1954a) noted that a 30 percent destruction (depolymerization) of mucin present in saliva occurred within 30 minutes, and 50 percent within two hours at room temperature, but that only slight destruction occurred if the sample was placed on ice immediately after collection. Even in the cold, however, they observed a 30 percent destruction within two hours.

Knox (1953a, b) attributed the decrease in viscosity in saliva upon standing to mucinase, an enzyme having the ability to depolymerize mucin, and occurring normally in the mouth. He found that several microorganisms with mucolytic activity were included among the human oral flora. Not only did Knox demonstrate the ability of mucinase to

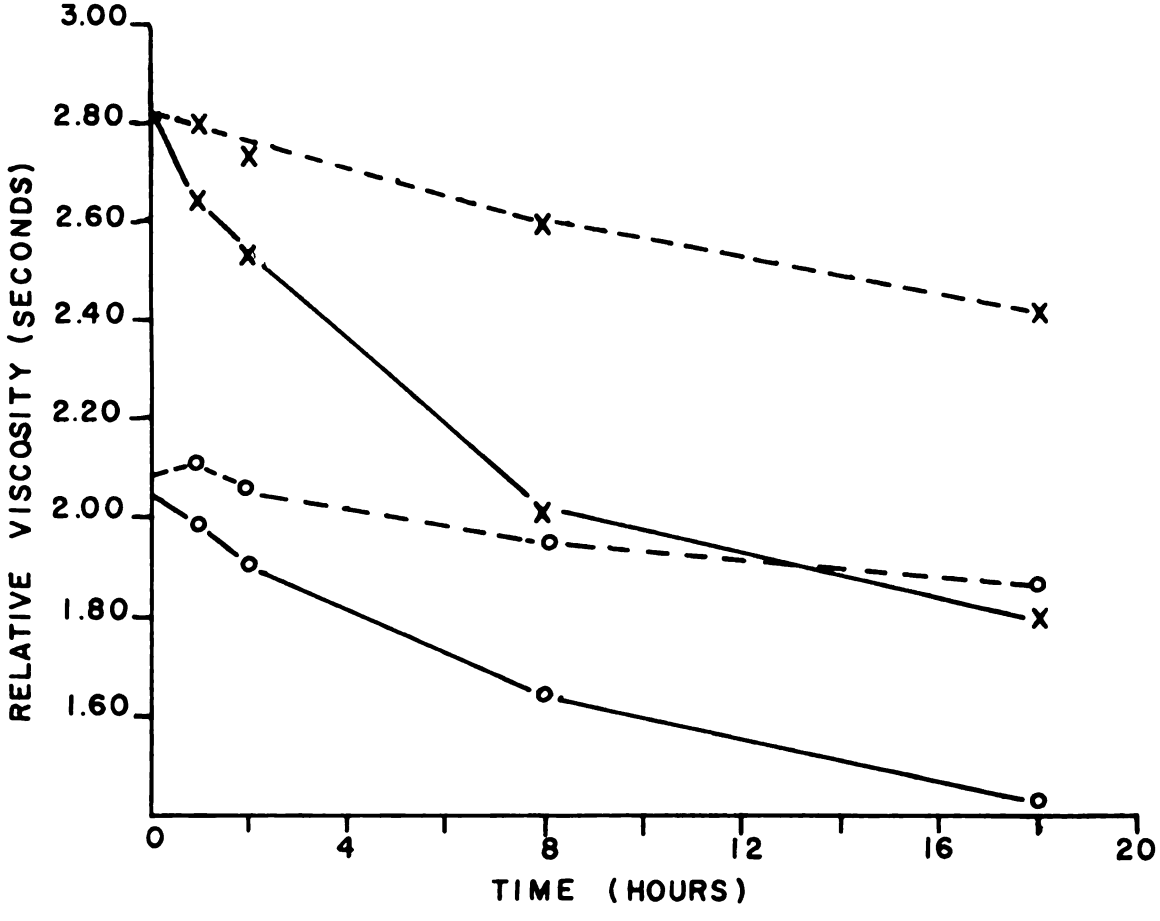


Figure 5. Change in relative viscosity in caries-resistant and caries-susceptible rat salivas when stored at room temperature and refrigerator temperature.

affect a reduction in salivary mucoid viscosity, but that trypsin (a protease) was similarly active. It is perhaps a protease that causes the observed change in relative viscosity at room temperature in saliva from the two lines of rats used in this study, particularly from susceptible rat saliva. Willett, (1955) reported a $2\frac{1}{2}$ to 3 times greater protease activity in whole susceptible saliva than in whole resistant saliva.

RATE OF FLOW

Literature Survey

There has not been complete agreement as to whether there exists a correlation between the rate of salivary flow and the incidence of dental caries. Rigolet (1901), Pickerill (1924b), Trimble, Etherington and Losch (1938), Gurley (1939), Losch and Weisberger (1940), Cushman, Etherington and Thompson (1940, 1941), Rathje and Fröhlich (1949), Rathje (1951), Rovelstad (1957) and Rovelstad, Geller and Cohen (1958) concluded that there is a direct relationship between rate of flow and resistance to caries.

In their study with rats, Muhler, Bixler and Shafer (1957) reported that the salivary flow-dental caries relationship is not a simple one. Although daily administrations of 2 mg of pilocarpine, a sialogogue, significantly reduced caries, 6 and 12 mg did not.

Shafer, Clark and Muhler (1957) found that higher levels of thyroxine injected into rats for two months resulted in a greater rate of flow of pilocarpine-stimulated saliva and a lower incidence of dental caries. Shafer, Clark, Bixler and Muhler (1958b) demonstrated that a dysfunction of the rat thyroid gland caused by propylthiouracil and radiothroidectomy resulted in a reduced salivary flow and a greater incidence of dental caries. Thyroxine reversed this effect.

Miller (1903b, 1904) indicated that the rate of salivary flow was directly related to dental caries only in extreme cases; that is, when the flow was very strong or very scant. Even in cases where the flow was strong, he did not feel this would affect the incidence of caries that originate in deep fissures "where saliva scarcely penetrates." Burrill and Fosdick (1944) and Bárány (1947) concluded that there was only a tendency to a larger quantity of saliva in persons with relatively little caries.

On the other hand, Becks, Wainwright and Young (1941, 1943) and Karshan (1942) found no significant difference between the rates of flow of whole human saliva in caries-active and caries-free groups. This was confirmed by Englander, Mau, Hoerman and Chauncey (1958) who studied the flow rates of human parotid saliva.

Becks and Wainwright (1939) thought that resting (unstimulated) saliva is better for routine analytical purposes, because they found that subjects with originally low or high rates of flow of resting saliva arrive at approximately the same rate when the salivary glands are stimulated. This would disguise the true secretory capacity of the resting gland, which differs greatly among individuals.

Little agreement can be found in the literature regarding the rates of flow of the individual salivary glands. Gore (1938a) found no difference in the rates of flow of the parotid and submaxillari-sublingual "resting" glands. When these glands were stimulated, however, the rate of flow of

the parotid secretion was more than three times greater. Zipkin and Soban (1957) likewise found that stimulated parotid glands contributed more saliva (60 percent of whole saliva) than did the submaxillari-sublingual glands. Later, Gore (1956) pointed out that this rate difference was less in persons on a high carbohydrate diet, especially during the nocturnal hours.

This was not confirmed by Henriques and Chauncey (1958) who found no difference in rates of flow between stimulated parotid and submaxillary glands. Chauncey, Weiss and Lisanti (1956) detected no difference in rates of flow in the left and right parotid glands either before or after eating.

Schneyer and Levin (1955a) in their study with essentially resting glands of humans, calculated that the parotid glands secreted 26 percent, submaxillary glands 69 percent, and sublingual glands 5 percent. The volume of whole saliva collected from the "resting" glands was 42 percent greater than the combined volume of the individual secretions. When the glands from the same subjects were stimulated (1955b), the submaxillary secretion still accounted for the largest fraction, the sublingual secretion the smallest fraction, and the parotid secretion for an intermediate portion of the combined volume. However, with increased stimulation, the relative proportion contributed by the parotid glands increased. Here again, whole saliva collected from these stimulated glands was 47 percent greater than the combined volume of the individual secretions.

Schneyer (1956a) attributed the discrepancy of the greater volume of whole saliva secreted to the method used for collection of whole saliva, and not to the secretion of mucosal glands.

In the attempt to explain the wide variation that occurs in the rates of flow of specific salivary glands, Schneyer, Pigman, Hanahan and Gilmore (1956) concluded, as a result of their work, that saliva collected routinely in the laboratory as "resting" saliva is, in fact, stimulated or activated secretion. Gross variations in the rate of secretion, then, are due to fluctuations in the intensity and frequency of internal stimulation.

Methods and Materials

Selectively desalivated and intact caries-resistant and caries-susceptible rats were lightly anesthetized with Nembutal, then injected with pilocarpine to stimulate the flow of saliva. Each animal was allowed to salivate into a graduated 15 ml conical centrifuge tube. The collection was continued for 20 minutes, unless the rat died before this time, or the effects of anesthesia diminished. The number of minutes corresponding to the period of collection was noted. Accordingly, the rate of flow was calculated on a milliliter per minute basis.

The rats that had their submaxillari-sublingual glands removed yielded essentially only parotid saliva. Those that had a section of their parotid ducts removed and the cut ends ligated yielded essentially only submaxillari-

sublingual saliva, and the unoperated (intact) animals secreted whole saliva. The method employed for collecting saliva does not elicit a detectable amount of secretion from the minor salivary glands, or from the mucous glands of the oral cavity. No saliva could be collected from six rats which had their parotid ducts and submaxillari-sublingual glands removed.

In order to ascertain what effect the removal of the parotid ducts might have on the weight of the submaxillari-sublingual glands, both previously desalivated rats and littermate controls were sacrificed when they were an average of 31 weeks of age (an average of 18 weeks following desalivation). Their submaxillari-sublingual glands were removed and weighed on an analytical balance. Prior to removing the glands for weighing, their total body weights were noted.

Results

The data pertaining to the salivary rates of flow are presented in table 8. The mean rate of flow of the various salivas was not essentially different in resistant and susceptible rats, but the rate of flow of the various salivas within the lines of rats differed materially. Whole saliva from unoperated (intact) rats showed the greatest flow rates (0.102 and 0.103 ml/min.); those of submaxillari-sublingual secretion were intermediate (0.082 and 0.084 ml/min.); and parotid secretion showed the slowest rate (0.040 and 0.034 ml/min.).

TABLE 8. Mean rates of flow of whole, parotid, and submaxillari-sublingual saliva from caries-resistant and caries-susceptible rats

Type of Saliva	Strain of Rat	Number of Rats	Rate of Flow (ml/minute)
Whole	Resistant	27	0.102
	Susceptible	27	0.103
Parotid	Resistant	14	0.040
	Susceptible	14	0.034
Submaxillari-Sublingual	Resistant	18	0.082
	Susceptible	21	0.084

Whole saliva is made up of a natural mixture of parotid, submaxillary and sublingual salivas, and their combined rates of flow contribute to the observed rate of whole saliva. However, when the separate rates of flow of parotid and submaxillari-sublingual saliva were added together, their sum was 15 percent greater than the rate for whole saliva. Therefore, it was of interest to see if removal of one set of glands affected the size of the remaining set of glands.

The body weights, and the absolute and relative submaxillari-sublingual gland weights were analyzed statistically using the "t" test, comparing rats that had their parotid ducts intact with those that did not. The results of the analyses are presented in table 9. The data and formulae used in the analyses are found in tables 20 through 22, and page 135 of the Appendix, respectively.

The total body weight of male rats with parotid ducts intact was 405 ± 49.2 g, whereas those with parotid ducts removed was 401 ± 45.7 g. In these two groups of male rats, the absolute weight of the submaxillari-sublingual glands was 682 ± 65.5 mg and 676 ± 96.4 mg, respectively. The relative weights (mg of gland weight per 100 gm of body weight) of the submaxillari-sublingual glands in male animals with parotid ducts intact weighed 170 ± 17.0 mg, and those with parotid ducts removed had glands that weighed 169 ± 20.4 mg. The differences observed above, whether for total body weight, absolute gland weight or for relative gland weight, are not significant at the 5 percent level.

TABLE 9. The effect of removing the parotid duct from male and female rats upon the body weight, and absolute and relative weights of submaxillari-sublingual glands

Sex	Treatment	No. of Rats	Total Body Wt. (Grams) Mean	t	P	Absolute S-S* Gland Wt. (Milligrams) Mean	t	P	Relative S-S** Gland Wt. (Milligrams) Mean	t	P
M	Par. Duct Intact	36	405±49.2	0.357	>0.05	682±65.5	0.309	>0.05	170±17.0	0.182	>0.05
	Par. Duct Removed	36	401±45.7			676±96.4			169±20.4		
F	Par. Duct Intact	36	244±35.4	0.976	>0.05	535±60.7	0.106	>0.05	220±19.6	2.265	<0.05
	Par. Duct Removed	36	237±24.3			549±52.4			233±28.3		

* S-S=Submaxillari-Sublingual

** Relative Gland Weight=Milligrams absolute gland weight per 100 grams of body weight

In female rats, the total body weight with parotid ducts intact was 244 ± 35.4 g, and 237 ± 24.3 g with the parotid ducts removed. The difference of 7 grams is not significant. Similarly, no significant difference occurred in the absolute weight of the submaxillari-sublingual glands obtained from these two groups of female rats (535 ± 60.7 mg and 549 ± 52.4 mg, respectively). However, when considering the relative weights of the submaxillari-sublingual glands in these females, those with parotid ducts intact had significantly lighter glands (220 ± 19.6 mg) than those with parotid ducts removed (233 ± 28.3 mg). This difference is significant at a level of 5 percent.

Discussion

Since the values for the rate of flow of stimulated saliva was essentially the same in resistants and susceptibles, indicating that probably no direct relation exists between rate of flow and incidence of caries, no additional information would be expected by studying the relation of these two characteristics in rats by correlation analysis.

There may still be a difference in the rate of flow of unstimulated saliva between the resistant and susceptible rats, even though there does not exist a difference in the rate of flow of stimulated saliva. As Becks and Wainwright (1939) pointed out, the wide variation in the secretory capacity of the resting salivary gland among human individuals

was masked by stimulating the glands.[1] That is, the subjects with originally low or high rates of flow of resting (unstimulated) saliva arrived at about the same rate when the salivary glands were stimulated. Perhaps, this phenomenon is also operative in the salivary glands of rats. However, the determination of the rate of flow of resting saliva in the rat would be very difficult, if possible at all. In the first place, the conscious animal has swallowing reflexes that would interfere with collections. Secondly, tongue and cheek muscles are active in the conscious animal, which stimulates the salivary glands in varying degrees. To overcome the swallowing and other mouth movements by anesthesia, would introduce a factor that would very possibly affect the secretory activity of their salivary glands.

The results obtained in this study pertaining to the moderately significant increase in the relative weight of the submaxillary and sublingual glands when the parotid ducts were tied and severed in the female rats (see table 9) are in agreement with those of Schwartz and Weisberger (1955). They found that a significant hypertrophy of the remaining salivary glands took place in partially sialoadenectomized rats. They, too, measured the increase in terms of the ratio of gland weight to body weight.

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[1] The saliva of rats was stimulated by pilocarpine. This is a different kind of stimulation than that caused by the senses of smell or taste and by chewing. Human subjects are usually stimulated by chewing.

Schwartz and Shaw (1955) found a highly significant increase in the weight of submaxillary glands of rats when the parotid glands were removed. This was confirmed by Schwartz, Resnick and Shaw (1958) who found a moderately significant increase in the weight of the submaxillary glands of rats when either the parotid ducts were tied and severed, or when the parotid glands were extirpated.

pH

Literature Survey

Conflicting reports have appeared which have attempted to discern a relation between salivary pH and incidence of dental caries.

Those who concluded that a direct relationship exists between a low pH in whole human saliva and caries-susceptibility, and conversely, a high pH and caries-resistance have been Röse (1905), McIntosh, et al., (1925), Entin (1927), Entin and Stark (1928), Skosovsky (1935), Staz (1938), Krasnow (1938), Hanke (1939), Belding and Belding (1939) and Sullivan and Storvick (1950b). In addition to these, Karshan, Krasnow and Krejci (1931a,b) and Krasnow (1932, 1936) noticed a tendency for such a correlation. Focusing their attention on children, who seem to develop caries at a more rapid rate than adults, Turner, Scribner and Bell (1953, 1954) also found a high salivary pH tended to be associated with caries-immunity, and a low salivary pH with a high caries incidence.

However, many more investigators believe that no consistent relation exists between salivary pH and dental caries activity. These investigators include: Miller (1904), Lothrop and Gies (1910), Marshall (1916a), D'Alise (1921), Gans (1926), Kallhart (1928), White and Bunting (1935), Grove and Grove (1935), Ziskin and Hotelling (1937), Swerdlove (1942),

Whyte (1943), Mackenzie (1945), Rovelstad (1957), Rovelstad, et al., (1958), Muracciole and Castro (1959), and Englander, Mau, Hoerman and Chauncey (1958).

Furthermore, the following researchers concluded that there was no consistent pH difference in saliva of caries-resistant and caries-susceptible children: McKeag (1928), Roskin (1928), Magee, Drain and Boyd (1929), Stern (1931), Brodsky (1933), Hubbell (1933), White and Bunting (1935, 1936), Stones, Lawton, Bransby and Hartley (1950) and Muracciole and Castro (1959).

Rosen, et al., (1957) found no significant difference in salivary pH between caries-resistant and caries-susceptible rats.

In a study of parotid saliva from caries-active and caries-resistant persons, Englander, Mau, Hoerman and Chauncey (1958) could not correlate pH with caries activity, although they did find that pH varied directly with the rate of flow of stimulated parotid saliva. This correlation was confirmed by Chauncey, Lisanti and Winer (1958). Not only was there no significant difference between the secretions of the left and right parotid glands of humans, as shown by Chauncey, et al., (1956), but also Shafer, et al., (1958a) and Henriques and Chauncey (1958) found no marked difference in pH between the parotid and submaxillari-sublingual rat salivas.

The pH of the isoelectric zone of saliva protein was demonstrated by Arnold and McClure (1941) to be a relatively

constant characteristic of each person's saliva, but varies among individuals. This variation was not, however, correlated with caries activity.

Krasnow and Oblatt (1933) found a greater interdependence of pH and titratable alkalinity in caries-susceptible cases than in caries-resistant cases. Similarly, Sullivan and Storvick (1950a) showed a significantly positive correlation between pH and buffer capacity, although they made no attempt to relate this to caries activity.

Methods and Materials

As soon as possible (usually within an hour) after collecting parotid, submaxillari-sublingual, and whole saliva from caries-resistant and caries-susceptible rats, the pH of the samples was determined using a Beckman Glass Electrode pH Meter, model H2. Each saliva sample was from an individual rat, and kept at room temperature until the pH determination was made.

Results

The pH of the various salivas listed in table 10 ranged from 8.61 to 8.77. The constant pH observed in these salivas indicates that probably there is no significant difference between the pH of salivas from resistant and susceptible rats, or between the salivas within each line of rat.

The pH of whole resistant and whole susceptible salivas were 8.65 and 8.68, respectively. Parotid saliva showed a similar pH (8.77), on the average, in both lines of rats.

Submaxillari-sublingual saliva from the resistants showed a pH of 8.75, and that from the susceptibles was 8.61. Thus, the various salivas were within 0.2 pH units of each other.

Discussion

The data of table 10 do not show any relation between the pH of the various pilocarpine-stimulated salivas (i.e., parotid, submaxillari-sublingual and whole) and caries activity, as this activity relates to resistance or susceptibility to caries in the rats used in this study. This confirms the findings of Rosen, et al., (1957) who studied whole saliva from these two lines of rats. Furthermore, Shafer, et al., (1958a) found that the pH of parotid and submaxillari-sublingual rat salivas ranged from 8.1 to 8.2. Henriques and Chauncey (1958) found no significant difference in the pH of parotid and submaxillari-sublingual human salivas.

The average pH of all salivas in table 10 is 8.70. This is close to the pH of 8.3 of pilocarpine-stimulated whole rat saliva reported by Shafer, et al., (1958a). There is some indication that the pH of rat saliva is as high in vivo as it was shown to be by the in vitro method employed in this investigation. Indicator paper was inserted in the mouths of 12 normal rats equally represented by males and females, susceptibles and resistants, and the pH color change was roughly in the 8.5 zone in all rats tested.

One might expect this slightly alkaline saliva to neutralize, to some extent, the acids produced by the acidogenic

TABLE 10. pH of whole, parotid, and submaxillari-sublingual saliva from caries-resistant and caries-susceptible rats

Type of Saliva	Strain of Rat	Number of Rats	pH
Whole	Resistant	24	8.65
	Susceptible	30	8.68
Parotid	Resistant	7	8.77
	Susceptible	12	8.77
Submaxillari-Sublingual	Resistant	16	8.75
	Susceptible	22	8.61

microorganisms on the teeth and, thus, prevent the demineralization of the tooth enamel (inhibition of the cariogenic process). This would be true provided that the saliva, and the ions contained in it, had free access to the acids produced by the bacteria. This does not always seem to be the case, however, as studies with humans indicate. That is, at the onset of carious lesion formation, many acidogenic microorganisms are enmeshed in an amorphous, mucinous mat, called the plague, which is attached to the tooth surface. When fermentable carbohydrates are ingested, they diffuse into the plague to supply the acidogenic microorganisms with the necessary substrate. The rate of acid production within the plague is rapid.

Perhaps the caries-resistant and caries-susceptible rats differ, therefore, in the degree and quality of plague formation on the teeth, and thereby, in the extent to which the microorganisms are encouraged to form acids. This could possibly be checked in a future study.

BUFFER CAPACITY

Literature Survey

Although there has been some controversy concerning the question of whether the buffering action of saliva was related to caries activity, a large majority of investigators have shown in various ways, but mainly by titratable alkalinity, that caries-resistance is associated with a high buffering action than is caries-susceptibility. These investigators include Rõse (1905), Marshall (1917 a, b), Pickerill (1924a, b) Hubbell (1933), Grove and Grove (1934), Gore (1935), Hanke (1937, 1939), Hawkins (1939), Fosdick, Campaigne and Faucher (1941), Dreizen, Mann, Cline and Spies (1946), Fosdick (1947), Muracciole (1955), Rovelstad, et al., (1958) and Englander, Shklair and Fosdick (1958).

Turner, Scribner and Bell (1953, 1954) reported that titratable alkalinity of children's saliva was associated statistically with caries incidence, but that titratable acidity was less consistently associated. Turner and Anders (1956) indicated that caries-free children as a group show lower titratable acidity and higher titratable alkalinity than those cases with extensive decay, which show higher titratable acidity and lower titratable alkalinity. Forbes and Gurley (1932) and Burrill and Fosdick (1944) were able to demonstrate only a tendency toward a relationship between buffer capacity and caries incidence.

Dreizen, et al., (1946) found that the lactobacillus count of saliva varied inversely with its buffer capacity; however, this was not confirmed by Roe and Clegg (1949).

Although Marshall (1915, 1916a, b, 1917a, b) reported that no relation existed between dental caries and titratable alkalinity or titratable acidity in either stimulated or "unstimulated" saliva, he maintained that his "salivary factor" appeared to be indicative of immunity or susceptibility to caries. He defined the "salivary factor" as the quotient of the neutralizing power of "normal resting" saliva divided by that of stimulated saliva multiplied by 100. The neutralizing power was a function of the titratable alkalinity plus the titratable acidity. He obtained a salivary factor of 43 to 80 for caries-immune saliva and a factor of 80 to 132 for caries-susceptible saliva. Bunting and Wixon (1917) confirmed these findings when applied to caries-immune saliva, but not when applied to caries-susceptible saliva. Shepard and Gies (1916), Gies (1916a, b, 1917) and Gies, Lowenstein, Heft and Noland (1917), on the other hand, vigorously criticized Marshall's "salivary factor" as invalid and not associated with dental caries. Other workers who were unable to demonstrate a relation between dental caries and buffer action of saliva were Karshan, et al., (1931a, b), Belding and Belding (1939) and Rovelstad (1957).

Rõse (1905) reported that carbonates contributed in part to the alkalinity of saliva, but thought organic constituents of saliva were also important in this regard.

Marshall (1917a, b) thought the inorganic constituents of saliva rather than its organic ones were the source of the buffering capacity of saliva. Later it was amply demonstrated by Sellman (1949), Wah Leung (1951), Dreizen, Reed, Neidermeier and Spies (1953) and Lilienthal (1953, 1955a, b) that the buffer system mainly responsible for the buffering capacity of stimulated saliva at "physiologic" pH is the carbonate-bicarbonate system. They also found that phosphates constitute the next most important buffer system while salivary proteins, as well as bacteria, contribute little, if any, to the total buffering capacity of saliva. Knox and Still (1953) also found mucoid, the major salivary protein, to be ineffective as a buffer in vitro.

Related to the carbonate-bicarbonate system is what has been referred to as the CO_2 capacity of saliva, which is the ability of saliva to absorb CO_2, and a measure of its ability to neutralize acid. Carbon dioxide capacity has been shown to be higher in saliva of caries-free persons than in caries-active persons by Hubbell (1933), Karshan (1936, 1939), Karshan, Rosebury and Waugh (1939), and Karshan, Siegel and Waugh (1940).

Dreizen, Spies, Dreizen and Spies (1957) found that the salivary buffers acting to protect against dental caries originate in the gland rather than the serum.

Krasnow and Oblatt (1933) demonstrated a greater interdependence of pH and titratable alkalinity in caries-susceptible saliva that was particularly marked in "unstimulated"

morning saliva. Similarly, Sullivan and Storvick (1950a, b) and Dreizen, et al., (1953) reported a significant positive correlation between salivary pH and buffer capacity, but made no attempt to relate this to caries activity.

Deakins, et al., (1941) studied the acid neutralizing power, base neutralizing power, and total buffer capacity of saliva from 20 subjects. No definite correlation was found between any of these properties and rate of flow, either on an amount-per-cc basis, or on an amount-per-cc-per-minute basis. Englander, Mau, Hoerman and Chauncey (1958) detected no difference in titratable alkalinity in parotid saliva of caries-free and caries-rampant males, but found titratable alkalinity to vary directly with rate of flow. Chauncey, Lisanti and Winer (1958) indicated a significant positive correlation between the rate of flow of parotid saliva and its bicarbonate content.

Grove and Grove (1934) did not believe that titratable alkalinity estimations in themselves were indicative of either susceptibility or resistance, but are of value as an indication of the amount of alkaline salts in saliva, including salts of ammonia. Ammonia is a solvent for dental plaques and salivary mucin, so that in mouths where pH is above 7, plaque formation is obstructed by the action of ammonia, and caries thereby retarded. However, ammonia must be in the form of its alkaline salts to be effective.

Methods and Materials

In order to establish the degree of buffering capacity of rat saliva, the titratable alkalinity was determined. This was accomplished by diluting submaxillari-sublingual and whole saliva 1-5 with distilled water, then titrating the diluted saliva sample with 0.0235N hydrochloric acid until the pH reached 4.5 ± 0.2. As may be seen in figure 6, rat saliva diluted 1-5 has definite buffering capacity in the approximate pH range of 7.5 to 6.0, whereas, distilled water does not impart any buffering action to the saliva. Adding only 0.05 ml of 0.02N HCl to 4 ml distilled water resulted in a precipitous drop in pH.

The pH was measured at room temperature with a Beckman Glass Electrode pH Meter (Model H2), using saliva that had been stored in the refrigerator ($5^{o} \pm 1^{o}$ C) up to 7 days. It was found that no significant change in buffering capacity of rat saliva occurs if it is held at a low temperature for this period of time.

The number of parotid saliva samples that were of sufficient volume for use in this test, was inadequate for presentation in this study. Consequently, the titratable alkalinity of only submaxillari-sublingual and whole saliva will be considered.

A correlation analysis was made between the titratable alkalinity of whole saliva from susceptible rats and caries age in these same rats.

Results

The data for buffering capacity is presented in table 11. Saliva from caries-susceptible rats had a greater capacity to buffer hydrogen ions than did saliva from caries-resistant rats. This was evident in both whole and submaxillari-sublingual saliva. Whole saliva from resistant rats had a titratable alkalinity of 2.05 $\pm$ 0.042 ml, and that from susceptible saliva was 2.25 $\pm$ 0.037 ml. The difference of 0.20 $\pm$ 0.056 ml between these two is highly significant ($P < 0.01$). The titratable alkalinity of submaxillari-sublingual saliva from susceptible rats was 2.25 $\pm$ 0.052 ml, which was 0.41 $\pm$ 0.150 ml greater than that from resistant animals. This difference is significant at a level of 2 percent.

When a correlation analysis was made of the titratable alkalinity of whole susceptible saliva and caries age of these same rats, the correlation coefficient (r) was found to be +0.192, which is not significant ($P > 0.05$). The data and formula used in this analysis is presented in table 23 of the Appendix.

Discussion

The difference of 0.20 $\pm$ 0.056 ml between resistant and susceptible whole saliva, and the difference of 0.41 $\pm$ 0.150 ml between resistant and susceptible submaxillari-sublingual saliva is statistically significant (table 11). These differences are meaningful not only statistically, but when

TABLE 11. Buffering capacity of whole and submaxillari-sublingual saliva from caries-resistant and caries-susceptible rats

Type of Saliva	Strain of Rat	No. of Rats	Titratable Alkalinity*	Difference	t	P
Whole	Resistant	23	2.05 ± 0.042	0.20 ± 0.056	3.62	<0.01
	Susceptible**	33	2.25 ± 0.037			
Submaxillari-Sublingual	Resistant	7	1.84 ± 0.141	0.41 ± 0.150	2.78	<0.02
	Susceptible	13	2.25 ± 0.052			

* Titratable Alkalinity=Milliliters of 0.0235N HCl required to adjust 1 ml saliva, diluted 1-5, to pH 4.5 ± 0.2.

** Correlation coefficient (r) with caries age=+0.192 (P > 0.05).

small increments (0.05 ml) of 0.02N HCl were added to saliva at a pH below 6.0, the pH decreased by about 0.3 (figure 6). Therefore, differences of 0.2 or 0.4 ml of 0.02N HCl are probably meaningful.

The greater buffering capacity of saliva from susceptible rats than of saliva from resistant rats was rather unexpected, since this would suggest that this salivary property contributes to caries-susceptibility, when almost all reports based on human saliva correlated buffering capacity with caries resistance (no reports based on animal saliva were found). But the correlation analysis of the data from these rats indicates that probably no important relation exists between this property and incidence of caries, and therefore, caries susceptibility. This may be another instance of two unrelated traits (i.e., caries-susceptibility and buffer capacity) being selected for as a result of inbreeding.

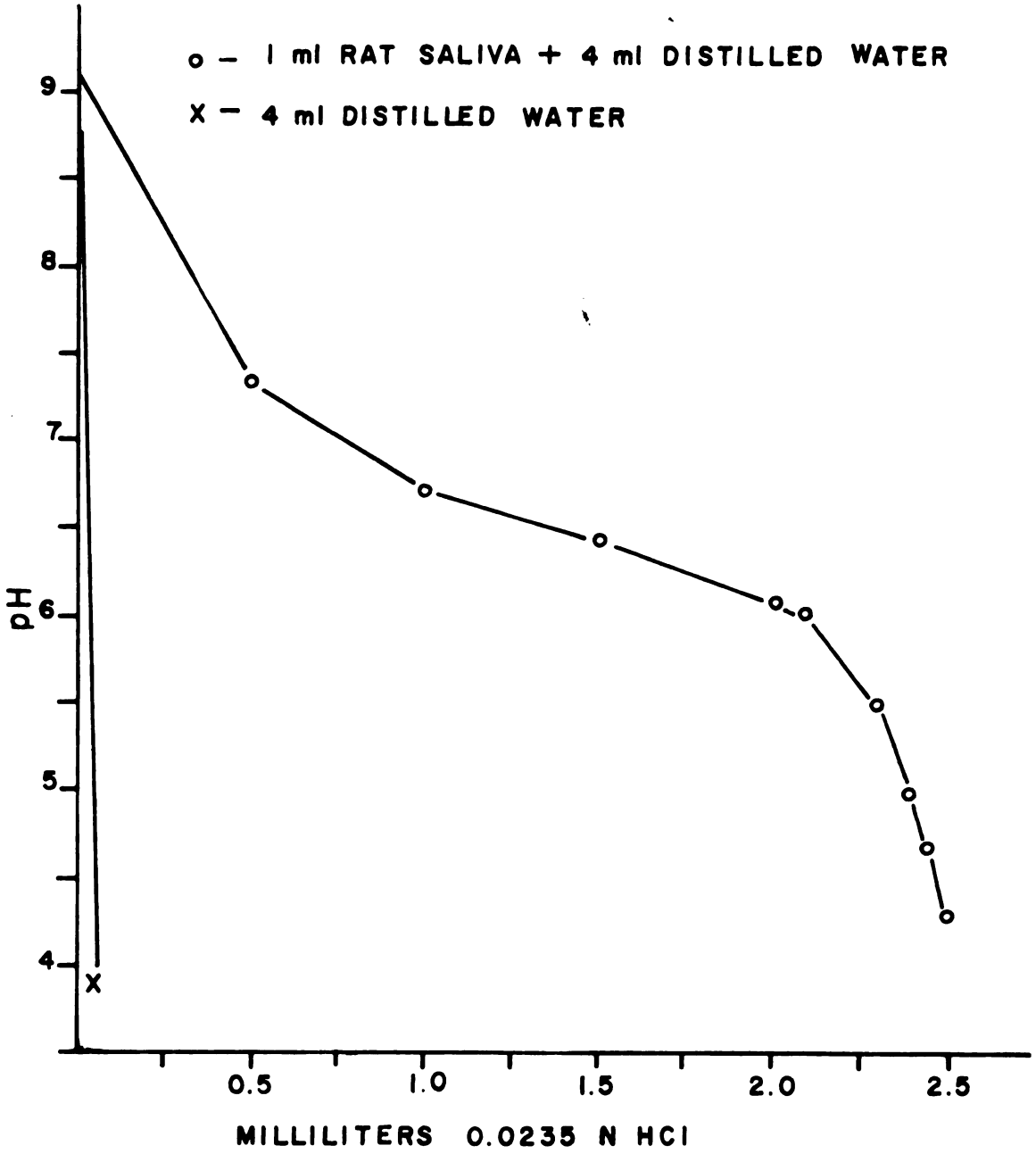

Figure 6. A typical curve illustrating the buffering capacity of diluted rat saliva compared to that of distilled water.

GENERAL DISCUSSION

The saliva from caries-susceptible rats stimulated the growth of rat oral streptococci to a greater extent than the saliva from caries-resistant rats. The saliva from both strains of rats supported, but did not stimulate, the growth of five out of six strains of rat oral lactobacilli.

The stimulation of the streptococci could have been due to a supply of nutrients made specifically available to these organisms. A carbon and nitrogen source could be provided, for example, by the large quantity of mucin, which is a glycoprotein and an important component of saliva. Knox (1953a, b) demonstrated a lysis of mucin in human saliva, which he attributed to the partially purified enzyme, mucinase. This may actually be the expression of more than one enzyme, as the following discussion will suggest. Knox found several salivary microorganisms capable of elaborating an exocellular mucolytic enzyme. He concluded that the mode of action is one of a primary depolymerization of salivary mucin by the activity of a proteolytic enzyme, and subsequently the liberation of the components of the mucopolysaccharide by a hyaluronidase-like enzyme. This conclusion is based on his experiments, which showed that trypsin (an endopeptidase) decreased the viscosity of salivary mucin, and hyaluronidase liberated reducing sugar from mucinase or trypsin depolymerized mucin. If hyaluronidase does, indeed, mediate in the breakdown of the

polysaccharide moiety of mucin (hyaluronic acid), acetylglucosamine and glucuronic acid would be formed. He further suggests that other enzymes are probably required before complete depolymerization results. Simmons (1941) had previously found a similar two-stage mucolytic activity in human saliva.

The saliva of rats used in this study seems to be equipped for such mucolytic activity. Willett (1955) found that the salivary glands of these rats, particularly susceptibles, elaborate a protease; and hyaluronidase is elaborated by microorganisms contained in the oral cavity of these animals.

Growth factors other than those possibly made available by the mucolytic activity in saliva, may be present to a greater extent in susceptible saliva than in resistant saliva. These growth factors could be in the form of metallic ions, as well as other inorganic ions, or vitamins, or the purines and pyrimidines, for example. These may be present in the rat's parotid saliva, and act in a complimentary manner with other nutrients found in the submaxillari-sublingual saliva, since the latter saliva did not stimulate the streptococci but whole saliva did.

Another possible source of nutrients usable by the streptococci is the food ingested by the rats. Although all rats were on the same diet, their teeth brushed, their mouths rinsed, and finally the saliva filter sterilized, there could still have been sufficient residual food not rinsed

out before saliva collection to influence the growth of the streptococci. The residual material might then be washed out of the mouth during active salivation. Since susceptible rats contain 2½ to 3 times more protease in their saliva than do the resistants, it is conceivable that more protein (casein) ingested as food would be hydrolyzed to simpler compounds in susceptible rats. Thus, the growth of streptococci would be favored more in the mouths of susceptible rats if the hydrolyzed protein would be a more readily utilizable form for these organisms.

Another possibility might account for the difference in the degree to which the susceptible and resistant whole saliva stimulated the growth of streptococci. Instead of the susceptible saliva containing more of the stimulatory substance than the resistant saliva, perhaps they both had equal quantities of the stimulatory substance, but that the resistant saliva contains, in addition, something else acting as an inhibitor to this substance. The resistant saliva would then show a diminished stimulation of the streptococci.

It might be desirable to investigate rat saliva further for the presence of an inhibitory agent active against rat oral streptococci and lactobacilli, with the technique used in this study modified to employ a minimal growth medium that would be less apt to mask inhibition by saliva, rather than employing a maximal growth medium.

Still to be established is whether the streptococci, whose growth is stimulated <u>in vitro</u>, are also favored <u>in vivo</u>.

Furthermore, it remains for a future study to determine whether some strains of rat oral streptococci are stimulated in the mouths of susceptible rats, but not in the mouths of resistant rats; and if so, to determine their relative numbers and cariogenic potential.

This greater stimulation of streptococci but not lactobacilli by whole saliva from susceptible than from resistant rats supports the concept that streptococci rather than the lactobacilli are primary etiologic agents in dental caries in rats. Evidence contributed by others suggesting this hypothesis is as follows: Dental caries are induced in germ-free rats by an enterococcus, but not by lactobacilli (Orland, et al., 1955 and Fitzgerald, et al., 1960). Dental caries are induced in "caries-inactive" hamsters by single or pooled cultures of streptococci from a carious hamster; not so by strains of lactobacilli from "caries-active" hamsters, or strains of streptococci from "caries-inactive" hamsters (Fitzgerald and Keyes, 1960). There are different strains of streptococci in caries-resistant and caries-susceptible rats (Rosen, et al., 1955), as well as in "caries-active" and "caries-inactive" hamsters (Fitzgerald and Keyes, 1960).

If this greater stimulation of the streptococci in susceptible whole saliva is to be interpreted as supporting the hypothesis of the streptococci being the etiologic agent for dental caries in rats, certain findings relating to sialoadenectomized rats should be explained. When saliva

from all the major glands (i.e., parotid, submaxillary, and major sublingual) was interrupted, the incidence of caries increased in the resistant rats. Since the stimulatory effect of the saliva for the streptococci was absent, why did the incidence of caries not diminish, instead? Stimulation of the streptococci is only one of the many interrelated factors which may account for the difference between the resistant and susceptible rats. The adverse effects, that are introduced when saliva is prevented from entering the oral cavity and thereby inducing a greater degeree of caries development, far outweigh the beneficial effect of depriving the streptococci of their original stimulatory source (saliva).

Some adverse effects that are possibly involved by removal of the major glands are: the accumulation of ingested food in the rat's mouth; lack of buffering action by saliva of acid produced by microorganisms; and a possible symbiotic or commensalistic relationship between the streptococci and the lactobacilli (the population of the latter increases significantly in a sialoadenectomized rat, as shown by Rosen, et al., 1958, 1959a, b).

The amylase study demonstrated that both resistant and susceptible rats have adequate stores of this enzyme to account for the hydrolysis of starch that might be lodged in the tooth fissures. This would provide large amounts of fermentable carbohydrates for acidogenic organisms. However, even though whole saliva of susceptible rats has

significantly more amylase activity than resistant rats, both have high levels. In addition, amylase activity was not correlated with caries age in susceptibles alone, or when susceptible and resistant rats were considered together. Finally, rats deprived of the secretion from all major salivary glands, and therefore also amylase from these glands, show a greater incidence of caries. Thus, it seems that salivary amylase activity does not play a major role in the etiology of dental caries in rats.

Viscosity and rate of flow are related salivary characteristics, since they both regulate the movement of saliva over the teeth. The greater this movement, the more the various other salivary factors can influence the state of dental health, such as the washing and lubricating action of saliva, its stimulatory properties for certain bacteria, and its buffering action. Relative viscosity and rate of flow of whole, parotid, and submaxillari-sublingual salivas do not appear to be significant factors in accounting for the difference between the two lines of rats. The significant difference in viscosity noted between resistant and susceptible whole saliva when collected at room temperature no longer existed when the saliva was collected at a lower temperature.

This is not to say that these two salivary properties have no influence on the state of dental health in rats. Rather, if the values for viscosity increased and the rate of flow decreased significantly, an adverse effect upon the

teeth could be expected. On the other hand, a beneficial effect could be expected if the values for viscosity decreased and the rate of flow increased significantly.

Of interest in this regard is the work of Muhler, Bixler and Shafer (1957), who reduced caries significantly in rats with daily administrations of 2 mg of pilocarpine (a sialogogue), although 6 and 12 mg did not. Moreover, Shafer, Clark and Muhler (1957) found that higher levels of thyroxine adminstered to rats for two months resulted in a lower incidence of caries and a less viscous saliva that was stimulated by pilocarpine.

Selye, Vielleux and Cantin (1961) were able to induce selective growth of rat's salivary glands to about five times their normal size, by chronic treatment with isoproterenol. This suggests a study to determine whether the increased rate of flow resulting from mitotic proliferation and hypertrophy of the parenchymatous cells will influence significantly the caries incidence in susceptible and resistant rats. Caries inhibition due to increased size and function of the salivary glands should be evident to a greater degree with the susceptible rats.

Buffering capacity and pH are two other salivary characteristics closely related to one another. Together they tend to minimize the harmful effect that the acid produced by microorganisms has on the inorganic constituents of the teeth. According to the acidogenic theory of dental caries, the factors determining whether a tooth will be eroded are,

ultimately, the rate of acid formation and the rate of acid neutralization.

In this study, the buffering capacity was found to be greater in the saliva of caries-susceptible rats. This does not support the acid theory of dental caries. However, that saliva has definite buffer action at all, and that caries becomes more rampant when salivary glands are removed, supports the concept that the buffering capacity of saliva contributes to caries-resistance.

Although no difference was found between the pH of salivas of resistant and susceptible rats, and the buffering capacity was not correlated with caries age (even though the saliva from susceptible rats showed a greater buffering capacity) the data revealed an efficient buffer system in whole and submaxillari-sublingual salivas of these animals. That is, the pH (average 8.70) is decidedly higher than the upper range of the buffer zone (approximately 7.5 to 6.0). See figure 5. Therefore, saliva from these rats maintains its maximum titratable alkalinity.

SUMMARY

Whole, parotid and submaxillari-sublingual saliva from caries-resistant and caries-susceptible rats were studied from various points of view to ascertain the salivary factors that might contribute to resistance or susceptibility to dental caries. These studies were the effect of saliva on the growth of microorganisms, amylase activity, viscosity, rate of flow, pH and buffering capacity.

Whole and submaxillari-sublingual saliva from the two lines of rats did not materially affect the mean rates and maximum limits of growth of five out of six strains of rat oral lactobacilli when compared with a saline control.

Rat oral streptococci, when cultured in the presence of whole and submaxillari-sublingual saliva from resistants and susceptibles, showed an increase in the maximum amount of growth, but not in the rate of growth as compared with a saline control. Whole susceptible saliva supported a greater maximum amount of growth than did whole resistant saliva. No difference in the submaxillari-sublingual saliva from the two lines of rats existed in this respect.

More than 99 percent of the amylase activity of saliva originated in the parotid glands of these animals. Parotid and whole salivas from susceptible rats showed greater amylase activity than these salivas from resistant rats. However, no significant correlation existed between amylase activity and caries activity.

When the various salivas were collected at room temperature, whole and submaxillari-sublingual salivas from resistant rats were more viscous than these salivas from susceptible rats (no real difference in the relative viscosity was found in the parotid secretions between the two lines of rats). However, when whole saliva was collected in tubes submerged in ice, the difference between resistants and susceptibles no longer was observed. Comparison of salivas within the lines of rats showed parotid saliva to be less viscous than submaxillari-sublingual saliva; whereas, whole saliva gave intermediate values. A correlation analysis between caries experience and relative viscosity revealed no significant relation between these two traits.

The mean rate of flow and pH of the parotid, submaxillari-sublingual, and whole secretions were not essentially different in resistant and susceptible rats. Within each line of rat, however, the rate of flow of each saliva differed materially. Whole saliva from unoperated rats showed the greatest flow rate, parotid saliva was slowest, and submaxillari-sublingual saliva was intermediate.

Buffering capacity, determined as titratable alkalinity, was significantly greater in susceptible whole and submaxillari-sublingual salivas, but this salivary property was not correlated with caries activity.

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APPENDIX

TABLE 1. Effect of caries-resistant and caries-susceptible rat salivas on the growth of several bacteria

ORGANISM	NO. OF TRIALS	RESISTANT SALIVA				SUSCEPTIBLE SALIVA				SALINE CONTROL	
		WHOLE		S-S[a]		WHOLE		S-S			
		SLOPE	MAX.[d]	SLOPE	MAX.	SLOPE	MAX.	SLOPE	MAX.	SLOPE	MAX.
Lacto. 1	3	0.99	0.35	0.84	0.28	0.91	0.29	1.06	0.36	0.70	0.29
Lacto. 4	2	1.34	0.26	1.42	0.22	1.26	0.28	1.24	0.19	1.35	0.32
Lacto. 9	2	0.94	0.38	1.16	0.46	1.01	0.36	1.18	0.38	1.28	0.33
Lacto. 10	2	1.02	0.42	0.86	0.29	0.87	0.34	0.56	0.12	0.92	0.27
Lacto. 14	4	1.01	0.34	1.07	0.38	0.92	0.32	1.05	0.35	0.96	0.38
Mean		1.06	0.35[b]	1.07	0.33	0.99	0.32[b]	1.02	0.28	1.04	0.32[b]
Strep. 6	1	1.76	1.10	1.78	0.95	2.46	1.50	1.91	1.10	1.82	0.54
Strep. 1, 2	2	3.06	1.20	3.63	0.96	2.95	1.75	2.50	1.20	2.60	0.64
Strep. 3	2	2.21	1.15	2.24	0.92	2.38	1.65	2.14	0.94	2.80	0.70
Strep. 5	2	4.31	0.88	3.78	0.78	3.99	1.60	4.28	0.74	3.36	0.40
Strep. 7, 8	2	4.04	1.02	3.86	0.86	4.40	1.15	3.74	0.92	5.24	0.61
Mean		3.08	1.07[b]	3.06	0.89	3.24	1.53[b]	2.91	0.98	3.16	0.58[b]
Lacto. 11	4	3.56	1.52[b]	3.67	1.06	3.51	1.55[b]	3.32	0.92	2.65	0.42[b]
S. aureus	3	3.07	1.23[c]	-	-	3.38	1.13[c]	-	-	2.85	0.79[c]
E. coli	3	4.50	0.74[c]	-	-	4.80	0.70[c]	-	-	4.18	0.75[c]
B. subtilis	3	3.80	0.80[c]	-	-	3.32	0.77[c]	-	-	2.69	0.59[c]

a = S-S = Sublingual and submaxillary salivas.
b = Maximum limits of growth in optical density after 25±4 hours incubation at 37° C.
c = Maximum limits of growth in optical density after 13±1 hours incubation at 37° C.
d = Maximum limits of growth.

TABLE 2. Paired data analysis of the effect of caries-resistant and caries-susceptible whole rat salivas on the growth of rat oral streptococci

Streptococcus Culture Number	Maximum Growth in O.D.		Difference (d)
	Susceptible Saliva	Resistant Saliva	
1	1.70	1.00	0.70
2	1.80	1.40	0.40
3	1.90	1.20	0.70
3	1.40	1.10	0.30
5	1.90	0.90	1.00
5	1.30	0.85	0.45
6	1.50	1.10	0.40

$$S_{\bar{d}} = \sqrt{\frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{(n-1)\ (n)}} = 0.0931$$

$$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 6.061$$

Degrees of freedon = 6

$P = < 0.01$

$\Sigma d = 3.95$

$\bar{d} = 0.5643$

$(\Sigma d)^2 = 15.6025$

$\frac{(\Sigma d)^2}{n} = 2.2289$

$\Sigma d^2 = 2.5925$

TABLE 3. Paired data analysis of the effect of caries-resistant and caries-susceptible whole rat salivas on the growth of rat oral streptococci

Streptococcus Culture Number	Slope of Growth Curve		Difference (d)
	Susceptible Saliva	Resistant Saliva	
1	2.13	2.50	-0.37
2	3.77	3.63	0.14
3	2.74	2.42	0.32
3	2.01	2.00	0.01
5	4.11	4.12	-0.01
5	3.87	4.50	-0.63
6	2.46	1.76	0.70
7	4.59	4.13	0.46
8	4.22	3.94	0.28

$$S_{\bar{d}} = \sqrt{\frac{d^2 - \frac{(\Sigma d)^2}{n}}{(n-1)(n)}} = 0.1368$$

$$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 0.731$$

Degrees of freedom = 8

P = >0.5

$\Sigma d = 0.90$

$\bar{d} = 0.1000$

$(\Sigma d)^2 = 0.8100$

$\frac{(\Sigma d)^2}{n} = 0.0900$

$\Sigma d^2 = 1.4360$

TABLE 4. Paired data analysis of the effect of caries-resistant and caries-susceptible submaxillari-sublingual rat salivas on the growth of rat oral streptococci

Streptococcus Culture Number	Maximum Growth in O.D.		Difference (d)
	Susceptible Saliva	Resistant Saliva	
1	1.20	1.00	0.20
2	1.20	0.92	0.28
3	0.98	0.97	0.01
3	0.90	0.88	0.02
5	0.75	0.79	-0.04
5	0.72	0.76	-0.04
6	1.10	0.95	0.15

$$S_{\bar{d}} = \sqrt{\frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{(n-1)\ (n)}} = 0.0546$$

$$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 1.52$$

Degrees of freedom = 6

P = >0.2

$\Sigma d = 0.58$

$\bar{d} = 0.0829$

$(\Sigma d)^2 = 0.3364$

$\frac{(\Sigma d)^2}{n} = 0.0481$

$\Sigma d^2 = 0.1446$

TABLE 5. Paired data analysis of the effect of caries-resistant and caries-susceptible submaxillari-sublingual rat salivas on the growth of rat oral streptococci

Streptococcus Culture Number	Slope of Growth Curve		Difference (d)
	Susceptible Saliva	Resistant Saliva	
1	2.19	3.08	0.89
2	2.80	4.18	1.38
3	2.50	2.36	-0.14
3	1.79	2.12	0.33
5	4.07	3.76	-0.31
5	4.49	3.79	-0.70
6	1.91	1.78	-0.13
7	3.77	4.12	0.35
8	3.71	3.60	-0.11

$$S_{\bar{d}} = \sqrt{\frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{(n-1)(n)}} = 0.2138$$

$$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 0.8106$$

Degrees of freedon = 8

P = > 0.4

$\Sigma d = 1.56$

$\bar{d} = 0.1733$

$(\Sigma d)^2 = 2.4336$

$\frac{(\Sigma d)^2}{n} = 0.2704$

$\Sigma d^2 = 3.5626$

TABLE 6. Paired data analysis of the effect of caries-resistant whole rat saliva and saline control on the growth of rat oral streptococci

Streptococcus Culture Number	Maximum Growth in O.D.		Difference (d)
	Resistant Saliva	Saline Control	
1	1.00	0.70	0.30
2	1.40	0.58	0.82
3	1.20	0.67	0.53
3	1.10	0.72	0.38
5	0.90	0.38	0.52
5	0.85	0.41	0.44
6	1.10	0.54	0.56

$$S_{\bar{d}} = \sqrt{\frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{(n-1)\ (n)}} = 0.0627$$

$$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 8.088$$

Degrees of freedom = 6

$P = < 0.01$

$\Sigma d = 3.55$

$\bar{d} = 0.5071$

$(\Sigma d)^2 = 12.6025$

$\frac{(\Sigma d)^2}{n} = 1.8004$

$\Sigma d^2 = 1.9653$

TABLE 7. Paired data analysis of the effect of caries-resistant whole rat saliva and saline control on the growth of rat oral streptococci

Streptococcus Culture Number	Slope of Growth Curve		Difference (d)
	Resistant Saliva	Saline Control	
1	2.50	3.20	0.70
2	3.63	1.99	-1.64
3	2.42	3.13	0.71
3	2.00	2.47	0.47
5	4.12	3.08	-1.04
5	4.50	3.63	-0.87
6	1.76	1.82	0.06
7	4.13	5.19	1.06
8	3.94	5.29	1.35

$$S_{\bar{d}} = \sqrt{\frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{(n-1)(n)}} = 0.3464$$

$$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 0.257$$

Degrees of freedom = 8

$P = > 0.5$

$\Sigma d = 0.80$

$\bar{d} = 0.0889$

$(\Sigma d)^2 = 0.6400$

$\frac{(\Sigma d)^2}{n} = 0.0711$

$\Sigma d^2 = 8.6928$

TABLE 8. Paired data analysis of the effect of caries-resistant submaxillari-sublingual rat saliva and saline control on the growth of rat oral streptococci

Streptococcus Culture Number	Maximum Growth in O.D.		Difference (d)
	Resistant Saliva	Saline Control	
1	1.00	0.70	0.30
2	0.92	0.58	0.34
3	0.97	0.67	0.30
3	0.88	0.72	0.16
5	0.79	0.38	0.41
5	0.76	0.41	0.35
6	0.95	0.54	0.41

$$S_{\bar{d}} = \sqrt{\frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{(n-1)(n)}} = 0.0323$$

$$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 10.040$$

Degrees of freedom = 6

$P = < 0.01$

$\Sigma d = 2.27$

$\bar{d} = 0.3243$

$(\Sigma d)^2 = 5.1529$

$\frac{(\Sigma d)^2}{n} = 0.7361$

$\Sigma d^2 = 0.7799$

TABLE 9. Paired data analysis of the effect of caries-resistant submaxillari-sublingual rat saliva and saline control on the growth of rat oral streptococci

Streptococcus Culture Number	Slope of Growth Curve		Difference (d)
	Resistant Saliva	Saline Control	
1	3.08	3.20	0.12
2	4.18	1.99	-2.19
3	2.36	3.13	0.77
3	2.12	2.47	0.35
5	3.76	3.08	-0.68
5	3.79	3.63	-0.16
6	1.78	1.82	0.04
7	4.12	5.19	1.07
8	3.60	5.29	1.69

$$S_{\bar{d}} = \sqrt{\frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{(n-1)(n)}} = 0.3709$$

$$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 0.302$$

Degrees of freedom = 8

P = >0.5

$\Sigma d = 1.01$

$\bar{d} = 0.1122$

$(\Sigma d)^2 = 1.0201$

$\frac{(\Sigma d)^2}{n} = 0.1133$

$\Sigma d^2 = 10.0165$

TABLE 10. Paired data analysis of the effect of caries-susceptible whole rat saliva and saline control on the growth of rat oral streptococci

Streptococcus Culture Number	Maximum Growth in O.D.		Difference (d)
	Susceptible Saliva	Saline Control	
1	1.70	0.70	1.00
2	1.80	0.58	1.22
3	1.90	0.67	1.23
3	1.40	0.72	0.68
5	1.90	0.38	1.52
5	1.30	0.41	0.89
6	1.50	0.54	0.96

$$S_{\bar{d}} = \sqrt{\frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{(n-1)(n)}} = 0.1039$$

$$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 10.312$$

Degrees of freedon = 6

P = < 0.01

$\Sigma d = 7.50$

$\bar{d} = 1.0714$

$(\Sigma d)^2 = 56.2500$

$\frac{(\Sigma d)^2}{n} = 8.0357$

$\Sigma d^2 = 8.4878$

TABLE 11. Paired data analysis of the effect of caries-susceptible whole rat saliva and saline control on the growth of rat oral streptococci

Streptococcus Culture Number	Slope of Growth Curve		Difference (d)
	Susceptible Saliva	Saline Control	
1	2.13	3.20	-1.07
2	3.77	1.99	1.78
3	2.74	3.13	-0.39
3	2.01	2.47	-0.46
5	4.11	3.08	1.03
5	3.87	3.63	0.24
6	2.46	1.82	0.64
7	4.59	5.19	-0.60
8	4.22	5.29	-1.07

$$S_{\bar{d}} = \sqrt{\frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{(n-1)(n)}} = 0.3271$$

$$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 0.034$$

Degrees of freedom = 8

$P = > 0.5$

$\Sigma d = 0.10$

$\bar{d} = 0.0111$

$(\Sigma d)^2 = 0.0100$

$\frac{(\Sigma d)^2}{n} = 0.0011$

$\Sigma d^2 = 7.7100$

TABLE 12. Paired data analysis of the effect of caries-susceptible submaxillari-sublingual rat saliva and saline control on the growth of rat oral streptococci

Streptococcus Culture Number	Maximum Growth in O.D.		Difference (d)
	Susceptible Saliva	Saline Control	
1	1.20	0.70	0.50
2	1.20	0.58	0.62
3	0.98	0.67	0.31
3	0.90	0.72	0.18
5	0.75	0.38	0.37
5	0.72	0.41	0.31
6	1.10	0.54	0.56

$$S_{\bar{d}} = \sqrt{\frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{(n-1)\ (n)}} = 0.0188$$

$$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 21.654$$

Degrees of freedom = 6

$P = < 0.01$

$\Sigma d = 2.85$

$\bar{d} = 0.4071$

$(\Sigma d)^2 = 8.1225$

$\frac{(\Sigma d)^2}{n} = 1.1604$

$\Sigma d^2 = 1.3095$

TABLE 13. Paired data analysis of the effect of caries-susceptible submaxillari-sublingual rat saliva and saline control on the growth of rat oral streptococci

Streptococcus Culture Number	Slope of Growth Curve		Difference (d)
	Susceptible Saliva	Saline Control	
1	2.19	3.20	1.01
2	2.80	1.99	-0.81
3	2.50	3.13	0.63
3	1.79	2.47	0.68
5	4.07	3.08	-0.99
5	4.49	3.63	-0.86
6	1.91	1.82	-0.09
7	3.77	5.19	1.42
8	3.71	5.29	1.58

$$S_{\bar{d}} = \sqrt{\frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{(n-1)(n)}} = 0.3342$$

$$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 0.855$$

Degrees of freedom = 8

$P = > 0.5$

$\Sigma d = 2.57$

$\bar{d} = 0.2856$

$(\Sigma d)^2 = 6.6049$

$\frac{(\Sigma d)^2}{n} = 0.7339$

$\Sigma d^2 = 8.7761$

TABLE 14. Data used in the correlation analysis between amylase activity of whole saliva from caries-susceptible rats (first experiment) and caries age

Rat Number	Amylase Activity* (x)	Caries Age** (y)
14890	1140	109
14978	2470	109
15127	1270	93
15130	1260	79
15131	1470	79
15122	876	93
15123	3420	79
15124	2040	93
15128	1900	93
15125	1260	67
15129	1560	107
15579	2340	78
15582	1180	78
15800	1340	71
15803	792	71
15804	1970	71
15878	843	78
15879	1320	78
15885	2260	64
15886	1140	64

* Amylase activity = mg glucose per ml saliva.
** Caries age = days of age when a carious lesion first appeared.

$$r_{xy} = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\sqrt{\left[\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right]\left[\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right]}} = +0.082$$

TABLE 15. Data used in the correlation analysis between amylase activity of whole saliva from caries-susceptible rats (second experiment) and caries age

Rat Number	Amylase Activity* (x)	Caries Age** (y)
16036	2450	76
16037	1680	76
16034	3080	90
16035	1860	76
16038	2000	76
16111	1970	55
16112	1610	55
16113	2530	55
16114	1960	55
16115	3520	55
16116	3300	55
16117	2180	55
16118	3040	55
16144	1180	58
16147	2540	58
16148	2190	58
16141	648	58
16142	838	58
16143	570	58

* Amylase activity = mg glucose per ml saliva.
** Caries age = days of age when a carious lesion first appeared.

$$r_{xy} = \frac{\sum xy - \frac{(\sum x)(\sum y)}{n}}{\sqrt{\left[x^2 - \frac{(\sum x)^2}{n}\right]\left[y^2 - \frac{(\sum y)^2}{n}\right]}} = +0.105$$

TABLE 16. Data used in the correlation analysis between amylase activity of whole saliva from caries-resistant rats (second experiment) and caries age

Rat Number	Amylase Activity* (x)	Caries Age** (y)
16043	1460	488
16044	1470	366
16046	1250	289
16039	1170	366
16041	1270	355
16056	829	346
16057	792	304
16058	1660	318
16049	956	165
16066	964	328
16068	2080	343
16069	810	277
16072	621	207
16065	474	154

* Amylase activity = mg glucose per ml saliva.
** Caries age = days of age when a carious lesion first appeared.

$$r_{xy} = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\sqrt{\left[\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right]\left[\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right]}} = + 0.651$$

Correlation analysis between amylase activity and caries age using combined data of susceptible and resistant rats used in the second experiment

$$r_{xy} \text{ (average 1 and 2)} = \frac{SP_{xy_1} + SP_{xy_2}}{\sqrt{(SS_{x_1} + SS_{x_2})(SS_{y_1} + SS_{y_2})}}$$

where

x_1 = Amylase activity of susceptible rats

x_2 = Amylase activity of resistant rats

y_1 = Caries age of susceptible rats

y_2 = Caries age of resistant rats

$$SS_{x_1} = \sum (x_1 - \bar{x}_1)^2 = \sum x_1^2 - \frac{(\sum x_1)^2}{N}$$

$$SS_{x_2} = \sum (x_2 - \bar{x}_2)^2 = \sum x_2^2 - \frac{(\sum x_2)^2}{N}$$

$$SS_{y_1} = \sum (y_1 - \bar{y}_1)^2 = \sum y_1^2 - \frac{(\sum y_1)^2}{N}$$

$$SS_{y_2} = \sum (y_2 - \bar{y}_2)^2 = \sum y_2^2 - \frac{(\sum y_2)^2}{N}$$

$$SP_{xy_1} = \sum (x_1 - \bar{x}_1)(y_1 - \bar{y}_1) = \sum x_1 y_1 - \frac{(\sum x_1)(\sum y_1)}{N}$$

$$SP_{xy_2} = \sum (x_2 - \bar{x}_2)(y_2 - \bar{y}_2) = \sum x_2 y_2 - \frac{(\sum x_2)(\sum y_2)}{N}$$

TABLE 17. Data used in the correlation analysis between relative viscosity of whole saliva from caries-susceptible rats (first experiment) and caries age

Rat Number	Relative Viscosity (x)	Caries Age (y)
14890	2.51	109
14978	2.02	109
15131	1.69	79
15123	1.70	79
15124	1.44	93
15128	1.31	93
15125	1.50	67
15127	1.61	93
15129	1.39	107
15122	1.60	93
15130	1.59	79
15579	1.80	78
15582	2.02	78
15800	1.71	71
15803	2.00	71
15804	1.72	71
15878	1.36	78
15879	1.50	78
15885	1.49	64
15886	1.69	64
15890	1.50	63
15895	1.94	63
15977	1.88	95
15972	1.45	53
15973	1.30	81

$$r_{xy} = \frac{\sum xy - \frac{(\sum x)(\sum y)}{n}}{\sqrt{\left[\sum x^2 - \frac{(\sum x)^2}{n}\right] \quad y^2 - \frac{(\sum y)^2}{n}}} = +0.251$$

TABLE 18. Data used in the correlation analysis between relative viscosity of whole saliva from caries-susceptible rats (second experiment) and caries age

Rat Number	Relative Viscosity (x)	Caries Age (y)
16036	1.96	76
16037	2.12	76
16034	1.88	90
16035	1.95	76
16038	2.24	76
16111	1.77	55
16112	1.68	55
16113	1.78	55
16114	1.86	55
16115	1.89	55
16116	1.87	55
16117	1.86	55
16118	1.76	55
16141	2.00	58
16142	2.02	58
16143	1.91	58
16147	1.67	58
16148	2.13	58

$$r_{xy} = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\sqrt{\left[x^2 - \frac{(\Sigma x)^2}{n}\right]\left[\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right]}} = +0.452$$

TABLE 19. Data used in the correlation analysis between relative viscosity of whole saliva from caries-resistant rats (second experiment) and caries age.

Rat Number	Relative Viscosity (x)	Caries Age (y)
16043	2.04	488
16044	2.02	366
16046	2.23	340
16039	2.15	366
16040	2.06	355
16041	1.92	355
16049	2.44	148
16056	1.92	246
16057	2.08	304
16058	1.92	318
16065	2.03	159
16066	2.00	328
16068	2.16	343
16069	1.76	277
16070	1.71	249
16072	1.85	207

$$r_{xy} = \frac{\sum xy - \frac{(\sum x)(\sum y)}{n}}{\sqrt{\left[x^2 - \frac{(\sum x)^2}{n}\right]\left[y^2 - \frac{(\sum y)^2}{n}\right]}} = -0.004$$

Correlation analysis between relative viscosity and caries age using combined data of susceptible and resistant rats used in the second experiment

$$r_{xy} \text{ (average 1 and 2)} = \frac{SP_{xy_1} + SP_{xy_2}}{\sqrt{(SS_{x_1} + SS_{x_2})(SS_{y_1} + SS_{y_2})}} + 0.038$$

where

x_1 = Relative viscosity of susceptible rats

x_2 = Relative viscosity of resistant rats

y_1 = Caries age of susceptible rats

y_2 = Caries age of resistant rats

$$SS_{x_1} = \sum (x_1 - \bar{x}_1)^2 = \sum x_1^2 - \frac{(\sum x_1)^2}{N}$$

$$SS_{x_2} = \sum (x_2 - \bar{x}_2)^2 = \sum x_2^2 - \frac{(\sum x_2)^2}{N}$$

$$SS_{y_1} = \sum (y_1 - \bar{y}_1)^2 = \sum y_1^2 - \frac{(\sum y_1)^2}{N}$$

$$SS_{y_2} = \sum (y_2 - \bar{y}_2)^2 = \sum y_2^2 - \frac{(\sum y_2)^2}{N}$$

$$SP_{xy_1} = \sum (x_1 - \bar{x}_1)(y_1 - \bar{y}_1) = \sum x_1 y_1 - \frac{(\sum x_1)(\sum y_1)}{N}$$

$$SP_{xy_2} = \sum (x_2 - \bar{x}_2)(y_2 - \bar{y}_2) = x_2 y_2 - \frac{(\sum x_2)(\sum y_2)}{N}$$

N = Number of rats used

TABLE 20. Body weights in grams of rats with (operated) and without (control) their parotid ducts removed

Male		Female	
Control	Operated	Control	Operated
425	402	252	278
451	456	234	279
472	444	244	250
423	440	225	280
492	523	244	261
421	441	257	230
407	388	250	216
530	413	224	259
395	415	240	240
382	467	236	205
436	449	272	184
412	339	232	197
452	424	236	252
468	420	236	248
422	420	260	204
380	334	236	250
376	396	240	232
372	378	312	244
404	420	232	240
408	448	204	236
432	372	276	230
430	424	260	220
500	380	200	242
332	462	336	264
384	344	372	280
400	364	208	196
348	416	220	232
364	380	252	224
328	324	224	204
356	388	212	230
388	392	220	232
348	332	270	252
380	370	208	200
356	352	232	240
350	328	220	240
340	400	226	240

TABLE 21. Absolute weight in milligrams of the submaxillari-sublingual glands from rats with (operated) and without (control) their parotid ducts removed

Male		Female	
Control	Operated	Control	Operated
654	699	540	541
709	697	493	531
616	709	535	493
650	740	528	578
756	801	494	498
757	704	504	593
631	661	506	513
768	672	440	614
652	707	559	539
647	545	500	560
707	678	556	522
696	509	494	553
670	718	527	611
740	790	446	500
745	686	547	412
641	523	496	500
663	731	524	539
680	685	595	502
719	661	583	544
660	740	486	556
690	648	643	493
868	577	571	520
788	588	505	567
649	802	678	670
771	636	697	608
735	650	510	485
553	980	493	549
549	774	600	597
609	593	534	569
651	670	483	566
638	741	611	633
638	592	616	586
711	525	461	466
619	613	524	546
653	535	486	598
673	761	486	611

TABLE 22. Relative weights* in milligrams of the submaxillari-sublingual glands from rats with (operated) and without (control) their parotid ducts removed

Male		Female	
Control	Operated	Control	Operated
154	174	214	195
157	153	211	190
130	160	219	197
154	168	235	206
154	153	202	181
180	160	196	258
155	170	202	238
145	163	196	237
165	170	233	225
169	117	212	273
162	151	204	284
169	150	213	281
148	169	223	242
158	188	189	202
176	163	210	172
169	157	210	200
176	185	218	232
183	181	191	206
178	157	251	227
162	165	238	236
160	174	233	214
202	136	220	236
158	155	252	234
195	174	202	254
201	185	187	217
184	179	245	247
159	236	224	237
151	204	238	266
186	183	238	279
183	173	228	246
164	189	278	273
183	178	228	232
187	142	222	233
174	174	226	228
187	163	221	249
198	190	215	255

* Relative weight of glands = milligrams of gland weight per 100 grams of body weight.

Formulae used in testing the significance of the differences between the body weights, and absolute and relative weights of the submaxillari-sublingual glands in rats with (operated) and without (control) their parotid ducts removed

Standard error = σ

$$\sigma x = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{N}}{N-1}}$$

$$\sigma y = \sqrt{\frac{\Sigma y^2 - \frac{(\Sigma y)^2}{N}}{N-1}}$$

where x = samples from control rat

y = samples from operated rat

N = number of rats used

Standard error of the means = S.E. = $\frac{\sigma}{\sqrt{N}}$

$$t = \frac{\text{Differnce between means}}{\text{Standard error of the difference between means}} = \frac{\bar{x} - \bar{y}}{\sqrt{(S.E.)_x^2 + (S.E.)_y^2}}$$

TABLE 23. Data used in the correlation analysis between titratable alkalinity of whole saliva from caries-susceptible rats and caries age

Rat Number	Titratable Alkalinity* (ml) (x)	Caries Age** (y)
14890	2.30	109
14978	2.40	109
15127	2.38	93
15130	2.10	79
15131	2.25	79
15122	2.45	93
15124	2.30	93
15129	2.60	107
15579	2.55	78
15582	2.40	78
15800	1.60	71
15804	2.05	71
15879	2.15	78
15885	2.50	64
15890	2.50	63
15977	2.05	95
15973	2.05	81
16036	2.85	76
16037	2.45	76
16034	2.10	90
16035	2.00	76
16038	2.30	76
16111	2.10	55
16112	2.15	55
16113	2.20	55
16114	2.00	55
16115	2.35	55
16116	2.40	55
16117	2.25	55
16118	2.30	55
16147	2.40	58
16141	2.15	58
16142	2.13	58

* Titratable alkalinity = milliliters of 0.0235N HCl required to adjust 1 ml saliva, diluted 1 to 5, to pH 4.5 ± 0.2.

** Caries age = days of age when a carious lesion first appeared.

$$r_{xy} = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\sqrt{\left[x^2 - \frac{(\Sigma x)^2}{n}\right]\left[\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right]}} = + 0.192$$

TABLE 24. Characteristics of the rat oral lactobacilli used to study the effect of rat saliva on the growth of microorganisms

Characteristic	Lactobacilli (Laboratory Culture Number)					
	1	4	9	10	11	14
Dextrose	+[1]	+[2]	+[1]	+[1]	+[1]	+[1]
Dextrin	+[3-5]	-	-	-	+[1]	-
Starch	-	-	-	-	+[2]	-
Arabinose	+[1]	+[1]	+[1]	+[1]	+[3]	+[1]
Lactose	+[3-5]	+[2]	+[2]	+[1]	+[1]	+[2]
Litmus Milk	-	A[14]	-	A[14]	C[3-5] A[2]	A[±14]
Gas	+	+[3-5]	+[3-5]	±[1]	-	+[2]
Micro-aerophilic	+	+	+	+	+	+
Catalase	-	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-
Colony size						
Rogosa SL (Surface)	1.5	1.5	1.5	0.5	NG	0.75
Rogosa SL (Subsurface)	3	2.5	3	3	1	3
Growth in micro inoculum broth	str. cl.	str. cl.	str. cl.	str. cl.	gr.	str. cl.

Superscripts signify day at which positive reaction was first noted.

A = acid; C = curd; NG = no growth; ± = weak or variable reaction; str. = strong; cl. = cloudy; gr. = granular.

TABLE 25. Cultural characteristics of the rat oral streptococci used to study the effect of rat saliva on the growth of microorganisms

	Streptococci (Laboratory Culture Number)					
	1*	2*	3**	5**	6a	6b
Medium	NT	NT	CVI	CVI	CVI	CVI
Location	Surface	Surface	Subsurface	Subsurface	Subsurface	Subsurface
Colony Size (mm)	2.0	3.2	1.0	1.0	2-4	<1.0
Color	Lt. Grey	Lt. Grey	Tan	Tan	White	White
Surface Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Hemolysis	None	None	Alpha	None	None	None

NT = Nivins medium (with tellurite)
CVI = Crystal violet horse infusion agar
* Resembles Streptococcus salivarius
** Resembles Streptococcus fecalis