A COMPARISON BETWEEN THE WIDTHS OF THE CREVICES OF THE LOWER MOLARS OF CARIES RESISTANT AND CARIES SUSCEPTIBLE ALBINO RATS (RATTUS NORVEGICUS)

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By

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INTRODUCTION

> "It is an irony of nature that our teeth, which decay so painfully while we live, stop decaying at our death, and outlast all the rest of us."
>
> --Sir Leonard Woolley (55)

Dental caries is defined as a localized, progressive, molecular disintegration of the teeth (42). This basic definition has been embellished and expanded to include the elements of various causative theories popular from time to time. None has ever satisfactorily and completely explained the cause of caries. Caries is a nonfatal pathological state (43), but it is not a disease in the strict biological sense (44). Caries has been characterized as a process leading finally to necrosis of the affected tissues (18). Finn (17) described it as the ". . . most prevalent chronic disease affecting the human race. . . ."

In 1937 it was estimated that dental caries affected 97 per cent of the population of the North American continent (53). This figure is probably higher today since the benefits of civilization have become more widely available in the past fifteen years. Primitive man did not entirely escape the pangs of decaying teeth, but the incidence of caries is over three times more frequent among modern

man (34). Mummery (5) studied early British skulls and concluded that the incidence of caries among early Britons decreased and increased with the ebb and flow of civilization across the British Isles. A similar relationship between civilization and the incidence of dental caries is reported wherever primitive peoples existing today come in contact with civilization (7). This relationship suggests that the civilized diet may be a factor in the production of dental caries.

Statement of Problem

As a result of these studies, attempts have been made at relating diet, tooth shape, and caries. Hoppert et al. (21, 22), after studying the particle size factor, reported that lodgement of food particles in the grooves of the teeth appeared necessary for the production of dental caries. Other investigators (below) have hypothesized similar relationships between tooth shape and caries.

This study was initiated as an attempt to make some definite measurements on the widths of the three main crevices of the lower molars and to determine, if possible, whether such a relationship to shape exists in the caries resistant and caries susceptible strains of albino rats developed by the Hunt-Hoppert experiment.

REVIEW OF LITERATURE

Effect of Bacteria

Until the late nineteenth century, theories concerning the causes of dental caries were both plentiful and fanciful. Various causes suggested included, among others, worms in the teeth, evil spirits, and electrical activity (7). In 1883 Miller (40) advanced the theory that the activities of oral microorganisms flourishing on carbohydrates in the mouth produced acids which decalcify the teeth, leading to caries. Most research since that time assumes this theory to be correct. However, Cox (11) stated that there is ". . . abundant evidence that bacteria enlarge cavities, but there is only hypothesis that bacteria are involved in bringing about the condition in enamel which permits acid decalcification to proceed."

No specific microorganism has been identified with caries to date. Another disturbing feature has been the finding of rampant caries in individuals whose mouths contain relatively few bacteria. The opposite has also been found--only minor caries present in conjunction with high bacteria counts (5).

The search for a specific organism has centered on Lactobacillus acidophilus. (L. acidophilus was referred to as Bacillus acidophilus in the literature prior to 1930.) Much of this work was done by Bunting, Jay, and their co-workers at the University of Michigan. Bunting (6) summarized the role of L. acidophilus in this manner:

> . . . the presence or absence of B. acidophilus constitutes a definite criterion of the activity of dental caries . . . more accurate than any clinical estimation . . . . there was a spontaneous cessation of caries coincident with the disappearance of B. acidophilus from the mouth . . . .

In 1936, Jay reviewed the previous work and stated that while many do not agree that causal relationship exists between the lactobacilli and dental caries, ". . . these organisms afford an excellent means of estimating caries activity. The total disregard of bacteriology in caries experimentation is sheer extravagance" (32).

Cox (11) concluded a survey of the effects of bacterial action by saying:

> There has been no conclusive demonstration that a single bacterial species as an excavating agent is associated with dental caries, but rather it is likely that cavity formation is due to the activities of any organisms that form acids and of those that can destroy the organic substance, particularly of dentin.

Bacterial Studies on the Hunt-Hoppert Strains

Jay, Hunt, and Hoppert (33) found more lactobacilli in the mouths of the susceptible rats than in the mouths of the resistants. It was suggested that some chemical characteristic of the mouth affecting the growth of acidogenic bacteria might be inherited.

More recently, Rosen (47) has studied the microflora of the mouths of susceptible and resistant rats. Lactobacilli have been found to occur more frequently and in greater numbers in the susceptible animals. This concurs with the study by Jay et al. (above). Streptococcus salivarius has also been found more frequently in the susceptible rats. A third, unidentified microorganism occurs in all resistant animals, but in only 18 per cent of the susceptibles. Although it has some antibiotic activity against L. acidophilus and S. salivarius, it is not thought to be a deterrent.

Effect of Diet

Most investigations of the relationship between nutriton and caries center on the vitamins and minerals.

Adequate Vitamin D intake has been shown to decrease caries incidence in both young animals (46) and growing children (3). Studies of Vitamin A deficiencies in man have not shown any

malformation of the teeth (13). Vitamin C has no effect on the dental caries incidence in various groups of children (39, 19).

Shaw concluded from a review of the effects of nutrition that:

> . . . there is a strong association between diet and susceptibility to caries.
>
> . . . vitamin D supplements . . . decrease . . . caries activity.
>
> [there is] . . . no . . . relation between . . . the vitamin B complex and an increased dental caries incidence.
>
> No incontroversial evidence has been found which indicates that diets partially deficient in calcium or phosphorus or both result in an increased susceptibility to dental caries.

The "civilized" diet has also had to assume a share of the blame for the prevalence of caries in modern societies. Ferguson (16), Waugh (54), and Price (43) have compared caries incidence with diet in both civilized and primitive races. They found a lower incidence among the primitive groups. Steggerda and Hill (49) studied the incidence of caries among both Mayan and Navajo Indians. Although the diets differed, the caries rates were lower than those of modern groups. It was the results of this study which prompted Steggerda to suggest to Hunt and Hoppert a study of the inheritance of caries (below).

In general, the civilized diet with its refined carbohydrates appears to be one of the factors promoting caries.

Effect of Saliva

Saliva, which is constantly bathing the teeth, has interested many caries researchers. Cheyne (8) observed a rapid loss of enamel in rats following removal of salivary glands. Extensive caries in man has been noted in conjunction with a low rate of secretion (38). A higher rate of saliva flow was found in caries free children (compared to caries susceptible) by Ericson (15). Ericson also reported an increased buffering capacity of saliva from caries resistant individuals. No direct relationship between the pH of saliva and caries has been demonstrated.

Pooled saliva has been shown to have an antibacterial action (20). Toverud (51) commented that:

> Not very much is known of the saliva as a whole and of its various components in regard to the preservation of the teeth. There is, however, strong evidence for an increased acid-neutralizing power by caries resistant people compared with others.

Cox (11) says:

> Saliva contains substances which are inhibitory of the development of microorganisms, particularly of those which are not normal inhabitants of the mouth . . . . some reduction of caries activity may be due to anti-bacterial substances of saliva.

Keller (36) has studied the role of salivary gland secretion in dental caries in the Hunt-Hoppert strains of rats. He found that

severing both parotid ducts in resistant and susceptible animals did not produce any significant effects. The secretion of the parotid gland is apparently not of great importance in the carious process in these animals.

Effect of Tooth Shape

The theory has been frequently advanced that the shape of a tooth is directly related to its susceptibility or resistance to caries. The flatter teeth, those with shallow grooves on the occlusal surface or no grooves at all, have been considered the most resistant.

The following statements, made in 1939, are from Dental Caries (1), a book sponsored by the American Dental Association which attempted to bring together all the then known facts relating to caries. Bassert (1) stated:

> Teeth with deep valleys and high cusps show the greatest susceptibility to caries; the flatter teeth, or those with shallow valleys and low cusps, are relatively nonsusceptible.

Mead (1), Mellanby (1), and Hawkins (1) made similar statements. Breese (1) made the statement "Food lodgement is an indispensable factor in the production of caries." Hoppert et al.

hold a similar view (prev. cited). Rosebury (1) also concurred, adding that forcible impaction was to be considered.

Cotton (1) added his belief that:

> Lodgement of food debris can be prevented, to a large extent, by polishing all rough surfaces, and filling every flaw, groove, pit, or fissure where stagnation occurs. . . . not 100% effective, but will save all permanent teeth of children.

Kronfeld (1) dissented slightly, saying: "The high incidence of caries in fissures in modern man does not prove the fissures are defects." He does not say that there is no relation between shape and caries, however.

Bodecker (2), in a discussion of the pathology of dental caries points out that "very frequently, however, the grooves are so deep and narrow that they are highly food retentive . . . The early treatment of these areas is now an established procedure."

Although this theory has been considered for some time, apparently no corroborative work has been done. No reports have been found in the literature relating crevice width, as determined by actual measurement, to dental caries.

Effect of Heredity - The Hunt-Hoppert Experiment

Bunting (7) states that the tendency to dental caries in humans may be inherited, but that the manner in which the trait is conferred

is not clear. Klein (37), after observing 1,700 married couples of Japanese ancestry, stated that the offspring of caries susceptible parents have three times more caries than do the offspring of caries resistant parents. Bodecker (2), noting the wide variations of the degree of caries susceptibility, commented that "susceptibility or immunity may be inherited." Shaw (48), after reviewing twenty-one studies on dental caries in humans, concluded that: ". . . at present there are no data that point to an absolute relation in human beings between heredity and dental caries, uninfluenced by other factors."

In experimental animals the inheritance factor has been demonstrated. In 1937, at the suggestion of Dr. Morris Steggarda, Hunt and Hoppert began an investigation of the inheritance of dental caries in the albino rat.

Previously, Hoppert, Webber, and Canniff (21, 22) had shown that the coarseness of the particles of grain in the diet was a factor in the initiation of dental caries in the rat. Using the Hoppert diet, both caries susceptible and caries resistant lines of rats have been obtained by phenotypic selection, brother x sister inbreeding, and progeny testing (24-28).

In addition to the main study on the mechanism of the inheritance of dental caries, a number of subsidiary studies have been conducted. The results of these, briefly summarized, follow.

In 1948, Braunschneider, Hunt, and Hoppert (4) reported that age was a factor in resistance to caries in the susceptible line. These susceptible rats were more resistant at 100 to 150 days of age than they were at 35 days.

Hunt and Hoppert (29) reported that sex was not an important factor in resistance to caries in rats. Occlusion with the upper molars favored the production of caries in the lower molars (30).

The distribution of gross carious cavities of the lower molar teeth was reported by Hunt and Hoppert in 1950 (31). The most frequent sites of lesions in the susceptible rats were the second fissure of the first molar and the single fissure of the second molar. In the caries resistant rats, the lesions were most frequent on the posterior part of the second molar. They also noted more cavities on the right jaw than on the left jaw in both strains.

Hoppert and Shirley (23) observed no significant difference in the rates of deposition and removal of radioactive phosphorus in the teeth of susceptible and resistant rats.

Keller et al. (35) reported that caries never developed earlier in the upper teeth than in the lower teeth of the susceptible strain. Ninety-six per cent of the resistant rats did not develop caries in the upper molars. In the susceptible animals 42 per cent developed caries in the upper molars.

Nakfoor et al. (41) showed that caries in the susceptible and resistant rats was not due primarily to fracturing. He believed that natural fracturing is not an important factor in the formation of caries in the susceptible animals. A similar conclusion was reached by Van Huysen (52).

Clise and Hunt (9) studied the growth rate and pilosity of the susceptible and resistant animals. Growth rates were not significantly different until the age of forty-four weeks. After this age, the susceptible rats of both sexes showed lower weights. He also found that both male and female susceptible rats had significantly less hair density than the resistants.

Stewart et al. (50) showed that variation in the diet (from the Hoppert diet) did not significantly affect the manifestation of hereditary difference between the two strains of rats. A diet containing a large proportion of granulated sucrose increased the variability of the incidence of caries in the susceptible rats. Conflicting

with other data on the effects of finely ground diets, he found that a finely powdered sucrose diet produced early caries in the susceptible animals.

Epstein (14) found that lingual erosion of a serious nature occurs sooner in susceptible molars than in resistant molars. This erosion was produced in rats fed a noncariogenic diet in conjunction with a sweetened, phosphorylated drink.

In a study of the thyroid glands, Keller (36) discovered that susceptible rats have larger thyroids and larger follicles in the thyroids. He found a slower turnover rate of radioactive iodine (I^{131}) in the susceptible strain. No apparent difference was found in the rate of use of oxygen by the two strains. This would seem to indicate that the metabolic levels of the two strains are about the same.

METHODS AND MATERIALS

The resistant animals used in this experiment were produced from matings of nineteenth generation resistant adults from Hunt's and Hoppert's experiments. The susceptible animals used were from matings of twenty-third generation susceptible adults. All were products of brother and sister matings.

All animals were housed in the Zoology animal house. Thus, they were reared in the same environment as the main breeding lines of the Hunt-Hoppert experiment. Galvanized steel cages, 12" x 14" x 20", were used. These cages have for a floor a removable metal tray which contains wood shavings. The cages were cleaned and the wood shavings replaced every ten days to two weeks, depending on the number of animals in a cage. Except on the hottest summer days, the temperature of the animal house was maintained automatically at 78° F.

The animals received the same diet used for the Hunt-Hoppert experiment. It consists of 66 per cent finely ground hulled rice, 30 per cent whole milk powder, 3 per cent alfalfa leaf meal, and 1 per cent sodium chloride. The rice was ground so that approximately 2 per cent would be retained on a 20 mesh screen. The animals were

given all of this diet they would consume. Water from the college water supply was constantly available in drip bottles.

Upon showing signs of pregnancy, a female was removed and placed in an isolation cage. Each was supplied with paper strips which served as both nesting material and screen. Once isolated, a female was observed daily. The date the young were born was recorded. Twenty-five days later the young, now eating the rice-containing diet supplied, were separated from the parent and were left relatively undisturbed until they were forty days old. A similar routine in handling the resistant animals was followed.

Records of the matings, dates of isolation of females, dates of birth, separation dates of young from parent, and sex of the young rats were carefully kept.

Since it was desired that the teeth of the susceptibles be free of caries, they were killed at forty days after birth. The resistant animals were also killed when forty days old. The lower jaws of the animals were removed, tagged, numbered, and temporarily stored in 95 per cent alcohol. When all of the lower jaws (from 45 susceptible and 40 resistant rats) had been collected, they were dried, cleaned, and the excess jawbone trimmed off with a pair of stout scissors. The remaining segment, a roughly rectangular piece

of bone containing the three lower molars, was placed in a small coin envelope. This envelope was marked with the strain of the rat and the number and sex of the individual. It was further identified as containing a left or right mandibular remnant.

Mounting and Grinding

The general method of Cox and Dixon (10) for mounting and grinding teeth for the observation of fissure caries was followed. In this case, the object of the observation was not the caries, but the crevice itself.

Two blocks of clay, one for the resistant jaw segments and the one for the susceptible, approximately 2'' x 7'' x 1'', and having a plane surface on top, were prepared. Mounting of the jaw segments was accomplished in the following manner. The jaws were arrayed on the top of the clay block in four rows. Right and left jaws from the same rat were kept together. They were placed buccal side down. The buccal side has a flat surface in that area which assisted in the alignment. The lingual side has a projection on it which was to serve as an anchor later. Each jaw segment was then carefully pushed into the clay until the buccal cusps of the first and second molar were imbedded to the same extent. The highest

elevation of the buccal cusps was used as a depth marker to insure even imbedding. It should be mentioned here that the lower third molar was not considered. The fissure of the third molar is ordinarily incomplete or absent. Therefore, it was decided to confine the observations to the large crevices of the first and second molars (Figure 1). When all the segments were in place, they were examined with a binocular microscope, using low power. Any necessary adjustments in position were made. A small diagram of the location of jaw segments was prepared for future reference.

A mold, consisting of four pieces of glass cut to fit the exterior of the clay block, was put in place. Small bits of clay served to hold the glass sides in place quite satisfactorily. The upper edges of the glass sides were about 2 inches higher than the surface of the clay block. A mixture of three parts of dental stone ("Castone") to one part of water, in sufficient quantity to make a block 1-1/2 inches thick, was then poured over the mounted jaws. When the dental stone had set, the mold was removed. The clay peeled off easily, leaving the teeth securely mounted in the dental stone. The exposed teeth were then covered with a similar mixture to give additional support during the initial grinding. The end product was one block

FIGURE 1

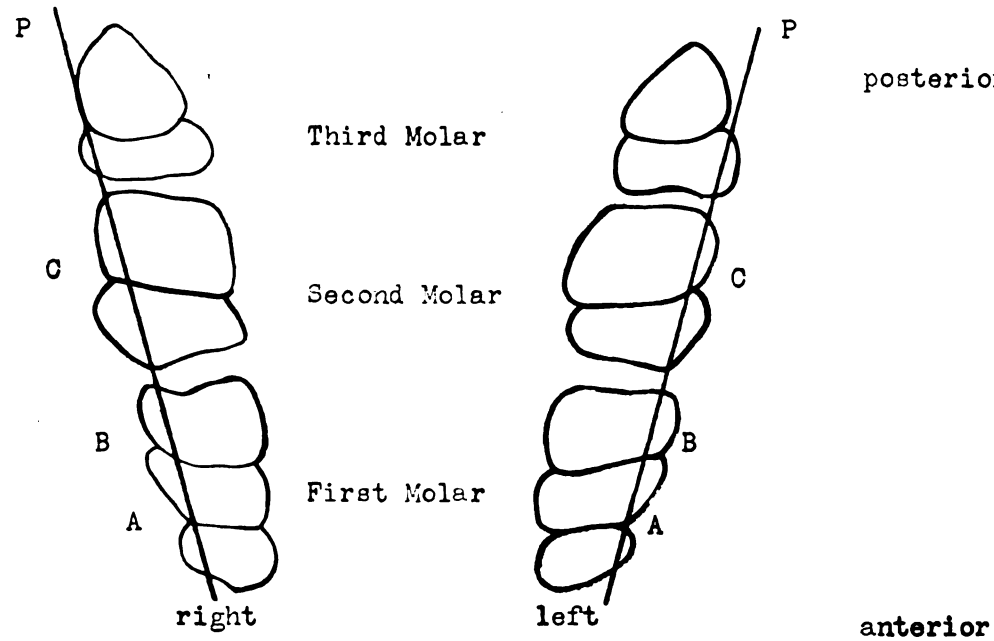
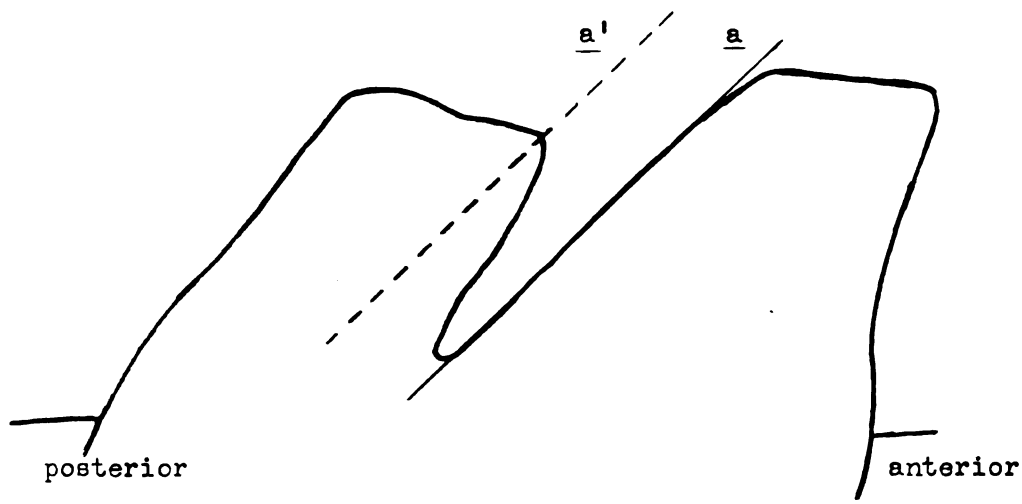


Diagram of the lower molars of the albino rat (slightly separated for clarity). A, B, and C are the crevices measured. The approximate plane of measurement is designated P.

FIGURE 2



Side view of second molar. The measurement of crevice width was from a to a'.

containing all of the resistant jaw segments and one containing all of the susceptible jaw segments.

The initial grinding was done on a machine designed by Dr. G. J. Cox (12) for that purpose. Using linear motion, it is capable of grinding six 2'' x 7'' blocks simultaneously on rectangular carborundum stones. Any one of the six or any combination can be ground. The two prepared blocks were placed in position on the grinding stones, teeth side down. A rectangular lead weight placed on top of each block insured even and constant contact. A metal container around each grinding stone held water which insured a moist surface during grinding.

Grinding was continued until the teeth were partially exposed on the surface of the block of dental stone. By then a plane surface had been established on the plaster block.

At this point the large blocks were cut into smaller pieces. Each piece contained the jaw segments from ten animals. This allowed more convenient handling and an improperly mounted jaw segment was not so apt to be overlooked. (Jaw segments of two animals were found to be so mounted as to make grinding and observation of the fissures impossible.)

Subsequent grinding was done by hand. A piece of number 400A grit waterproof sandpaper (Behr-Manning, Troy, N. Y.) was affixed to a smooth surface, grit side up. A block was selected and was rubbed carefully to and fro on the sandpaper. It was examined frequently. When, in the judgment of the experimenter, the high point of the buccal cusps had been reached, grinding was discontinued (see Figure 1). The high point of the buccal cusps was selected because it was a definite and relatively easily located area. Approximately half of the blocks required a supplementary grinding to bring one or two jaw segments to the desired level. This was done after measurements and camera lucida drawings had been made of the other molars in the block.

When the molars had been ground down to the desired level, they were examined under a low-powered microscope. A camera lucida drawing of each of the crevices marked A, B, and C in Figure 1 was made. Sample drawings are included (Plates I to IV). Each crevice was then measured, using an ocular micrometer. An accurate measurement of an object as irregular as the crevice of a rat molar was difficult to obtain. The following procedure was adopted. The movable hairline of the ocular micrometer was placed on and parallel to the forward slope of the crevice (position a in Figure 2).

The setting of the micrometer scale was observed and recorded. The movable hairline was then moved to the point on the rear slope which most accurately, again in the judgment of the observer, represented the edge of the crevice (position a' in Figure 2). The setting of the scale was again recorded and the difference was the width of the opening of the crevice. At first this seemed like a crude measurement, but experience and repeated measurements of the same sulcus showed a difference in subsequent readings of only a few hundredths of a unit. It is felt that any error, though present, is quite small and is relatively constant for both the resistant and susceptible groups.

In Tables I and II, the measurements are expressed in ocular micrometer units. If it is desired to convert the measurements to fractions of a millimeter, the conversion factor is: 1.00 units = 0.195 mm.

Terminology

Throughout this thesis the terms "sulcus," "groove," "crevice," and "fissure" are used synonymously. All of the terms appear in the literature.

In referring to a particular crevice, the letter designation (Figure 1) is given. Frequently a crevice is further identified as being in a lower left molar or a lower right molar. It should be noted from Figure 1 that crevices A and B are in the first lower molar. Crevice C is in the second lower molar.

PRESENTATION OF DATA

Table I shows the individual crevice widths recorded for the resistant rats used. Table II shows similar data for the susceptible animals. The crevices are lettered to correspond with Figure 1. In all instances (except in the bottom lines of Tables III and IV), the measurements and calculations are in ocular micrometer units. The bottom lines of Tables III and IV show the mean width of each crevice converted to millimeters. For convenience the conversion factor (to millimeters) is listed at the bottom of each table. It was originally planned to have equal numbers of susceptible and resistant rats. However, several crevices in the susceptible group were damaged by caries and secondary fracturing following the carious process. These could not be measured with any degree of accuracy. For the same reason no measurements of crevice depth in any of the susceptibles were possible.

In Tables III and IV are the ranges and frequency distributions of the crevice widths of the resistant and susceptible molars. The calculations are given in the lower section of each table. Frequency polygons based on Tables III and IV are included (Figures 3 to 8). In each of these figures the corresponding resistant and susceptible

TABLE I

CREVICE WIDTHS OF RESISTANT RATS
(in ocular micrometer units)[1]

Rat No.	Left Molars			Right Molars		
	Crevice A	Crevice B	Crevice C	Crevice A	Crevice B	Crevice C
1	0.48	0.76	0.99	0.51	0.80	0.81
2	0.41	1.00	0.91	0.52	1.09	1.12
3	0.44	1.00	0.98	0.72	1.27	0.97
4	0.34	0.93	1.06	0.45	0.84	0.99
5	0.43	1.08	1.11	0.41	1.14	1.23
6	0.59	1.06	1.06	0.57	1.05	0.97
7	0.47	0.90	1.10	0.34	1.16	0.95
8	0.45	1.10	1.11	0.37	1.12	1.18
9	0.44	1.03	1.18	0.50	1.20	1.11
10	0.29	1.05	1.14	0.35	1.08	1.15
11	0.53	1.21	1.03	0.41	1.13	1.17
12	0.49	0.97	1.33	0.54	0.90	1.10
13	0.60	1.24	1.30	0.52	1.15	1.11
14	0.58	1.04	1.09	0.54	1.29	0.92
15	0.45	0.96	1.11	0.34	1.27	0.94
16	0.41	0.82	0.95	0.58	1.01	0.99
17	0.48	1.22	1.40	0.60	1.16	0.88
18	0.61	1.00	1.10	0.35	0.99	0.95
19	0.49	1.30	0.84	0.74	1.19	0.98
20	0.28	0.90	1.05	0.26	1.11	1.03

TABLE I (Continued)

Rat No.	Left Molars			Right Molars		
	Crevice A	Crevice B	Crevice C	Crevice A	Crevice B	Crevice C
21	0.77	1.15	1.22	0.46	1.18	1.05
22	0.62	1.26	0.60	0.85	1.56	0.75
23	0.27	1.07	1.00	0.33	0.90	1.28
24	0.36	0.95	0.89	0.48	0.82	0.91
25	0.41	1.05	1.19	0.30	1.25	1.23
26	0.44	0.74	1.08	0.48	0.95	1.08
27	0.28	1.00	1.27	0.47	0.97	1.26
28	0.42	0.90	1.11	0.56	1.04	1.09
29	0.37	1.05	1.10	0.38	1.02	1.00
30	0.46	0.92	1.05	0.83	1.13	1.22
31	0.55	0.95	1.18	0.44	0.98	1.12
32	0.49	1.00	1.13	0.78	0.96	0.94
33	0.82	1.30	1.21	0.44	1.24	0.86
34	0.73	1.22	0.92	0.63	1.10	1.11
35	0.50	1.11	1.03	0.40	1.06	1.16
36	0.54	1.05	1.13	0.55	1.07	1.11
37	0.71	1.00	0.94	0.29	1.22	1.02
38	0.85	0.94	0.97	0.27	1.03	1.08
39	0.48	1.03	1.15	0.51	1.10	1.25
40	0.40	0.87	0.87	0.20	1.02	1.10

[1] Conversion factor: 1.00 unit = 0.195 mm.

TABLE II

CREVICE WIDTHS OF SUSCEPTIBLE RATS
(in ocular micrometer units)[1]

Rat No.[2]	Left Molars			Right Molars		
	Crevice A	Crevice B	Crevice C	Crevice A	Crevice B	Crevice C
6	0.67	1.33	1.39	0.42	1.13	1.48
7	0.59	1.57	1.17	0.89	1.37	1.34
8	0.82	0.92	1.02	1.59	1.23	0.87
9	0.59	1.11	*	0.39	1.30	*
10	0.69	1.30	*	0.47	1.00	*
11	0.37	1.42	*	0.40	1.59	1.41
12	0.56	1.18	*	0.50	*	*
13	0.74	1.40	1.64	0.56	1.43	1.44
14	0.49	1.40	1.44	0.47	1.25	*
15	0.41	1.75	1.91	0.57	1.54	*
16	0.80	1.37	*	*	*	*
17	x	x	x	x	x	x
18	0.45	1.40	1.19	0.69	1.32	1.39
19	1.01	1.28	1.72	1.02	1.28	1.47
20	0.70	1.30	1.33	0.70	1.46	1.24
21	0.68	1.42	1.68	0.55	1.59	1.40
22	0.56	1.71	1.33	0.64	1.53	1.55
23	0.56	1.52	1.69	0.36	1.51	*
24	0.61	1.22	1.72	0.29	1.71	1.38
25	0.75	1.42	1.51	0.41	1.21	1.13

TABLE II (Continued)

Rat No.[2]	Left Molars			Right Molars		
	Crevice A	Crevice B	Crevice C	Crevice A	Crevice B	Crevice C
26	0.72	1.17	1.65	0.48	1.40	1.62
27	0.54	1.18	1.23	0.80	*	1.38
28	0.97	1.30	*	0.78	1.01	1.58
29	0.58	1.06	*	1.06	1.16	1.39
30	1.17	1.18	1.22	0.62	0.90	1.63
31	0.62	1.21	*	0.61	1.32	1.60
32	0.40	1.08	*	0.26	1.19	1.37
33	0.45	0.98	*	0.46	1.18	1.75
34	0.35	0.99	1.30	0.48	1.18	*
35	0.69	1.25	1.21	0.42	1.56	1.52
36	0.60	1.43	1.22	0.60	1.12	*
37	0.67	1.57	1.58	0.80	1.22	1.34
38	0.75	1.32	1.61	0.79	1.36	1.40
39	0.68	0.98	*	0.77	1.18	*
40	0.51	1.46	1.31	0.82	1.19	1.34
41	1.12	2.01	1.09	0.51	1.59	1.62
42	0.55	1.40	1.40	0.64	1.71	1.48
43	0.54	1.54	*	0.62	1.75	*
44	x	x	x	x	x	x
45	0.48	1.31	1.34	0.59	1.18	1.32

[1] Conversion factor: 1.00 unit = 0.195 mm.

[2] Rats nos. 1-5 omitted; damaged with probe.

* Not measured because of caries or fracture due to caries.

x Inaccurately mounted; no measurements possible.

TABLE III

RANGE AND FREQUENCY DISTRIBUTION OF CREVICE WIDTHS OF RESISTANT RATS
(in ocular micrometer units)[1]

Class Range and Mid-Point	Left Molars			Right Molars		
	Crevice A	Crevice B	Crevice C	Crevice A	Crevice B	Crevice C
0.195-0.295 (0.245)	4			4		
0.295-0.395 (0.345)	3			8		
0.395-0.495 (0.445)	19			10		
0.495-0.595 (0.545)	6			11		
0.595-0.695 (0.645)	3		1	2		
0.695-0.795 (0.745)	3	2		3		1
0.795-0.895 (0.845)	2	2	3	2	3	3
0.895-0.995 (0.945)		10	7		7	11
0.995-1.095 (1.045)		16	9		11	7
1.095-1.195 (1.145)		3	14		11	12
1.195-1.295 (1.245)		5	3		7	6
1.295-1.395 (1.345)		2	2			
1.395-1.495 (1.445)			1			
1.495-1.595 (1.545)					1	

TABLE III (Continued)

Class Range and Mid-Point	Left Molars			Right Molars		
	Crevice A	Crevice B	Crevice C	Crevice A	Crevice B	Crevice C
Total (N)	40	40	40	40	40	40
Mean ($\bar{x}$)	0.493	1.028	1.072	0.482	1.089	1.054
Standard Deviation (s)	0.150	0.143	0.152	0.156	0.139	0.131
Standard Error ($s/\sqrt{N}$)	±0.024	±0.023	±0.024	±0.024	±0.022	±0.021
Mean ($\bar{x}$)[1] Expressed in mm.	0.096	0.201	0.209	0.094	0.212	0.206

[1] Conversion factor: 1.00 unit = 0.195 mm.

TABLE IV

RANGE AND FREQUENCY DISTRIBUTION OF CREVICE WIDTHS OF SUSCEPTIBLE RATS
(in ocular micrometer units)[1]

Class Range and Mid-Point	Left Molars			Right Molars		
	Crevice A	Crevice B	Crevice C	Crevice A	Crevice B	Crevice C
0.195-0.295 (0.245)				2		
0.295-0.395 (0.345)	2			2		
0.395-0.495 (0.445)	6			9		
0.495-0.595 (0.545)	10			6		
0.595-0.695 (0.645)	9			7		
0.695-0.795 (0.745)	5			4		
0.795-0.895 (0.845)	2			4		1
0.895-0.995 (0.945)	1	4			1	
0.995-1.095 (1.045)	1	2	2	2	2	
1.095-1.195 (1.145)	2	5	2		9	1
1.195-1.295 (1.245)		4	4		5	1
1.295-1.395 (1.345)		7	6		5	9
1.395-1.495 (1.445)		9	2		3	7
1.495-1.595 (1.545)		4	2	1	7	3
1.595-1.695 (1.645)			5			4

TABLE IV (Continued)

Class Range and Mid-Point	Left Molars			Right Molars		
	Crevice A	Crevice B	Crevice C	Crevice A	Crevice B	Crevice C
1.695-1.795 (1.745)		2	2		3	1
1.795-1.895 (1.845)						
1.895-1.995 (1.945)			1			
1.995-2.095 (2.045)		1				
Total (N)	38	38	26	37	35	27
Mean ($\bar{x}$)	0.643	1.327	1.419	0.622	1.332	1.423
Standard Deviation (s)	0.194	0.239	0.233	0.246	0.208	0.184
Standard Error ($s/\sqrt{N}$)	±0.031	±0.039	±0.046	±0.040	±0.035	±0.035
Mean ($\bar{x}$)[1] Expressed in mm.	0.125	0.259	0.277	0.121	0.260	0.278

[1] Conversion factor: 1.00 unit = 0.195 mm.

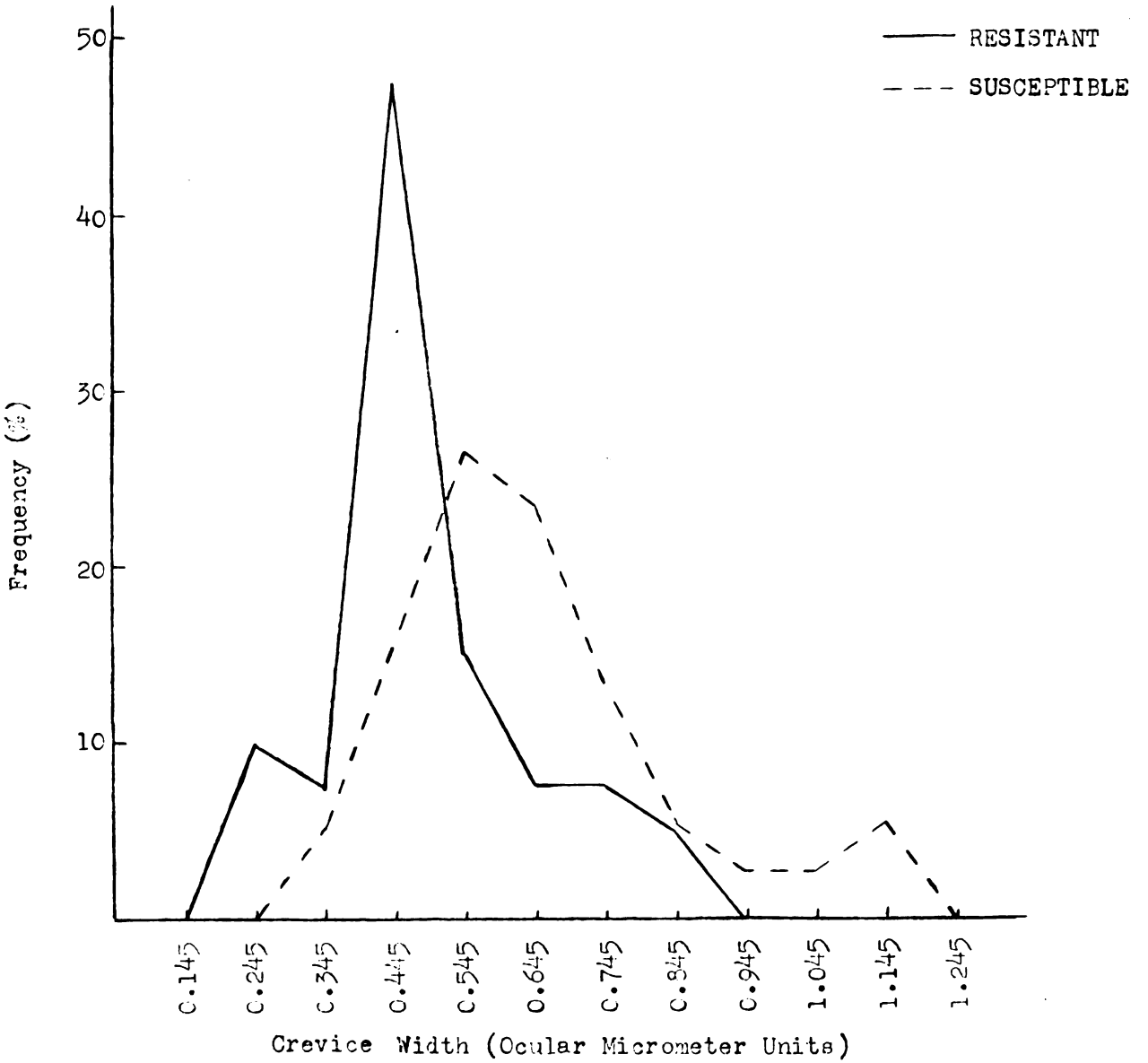


FIGURE 3. CREVICE A (LEFT)

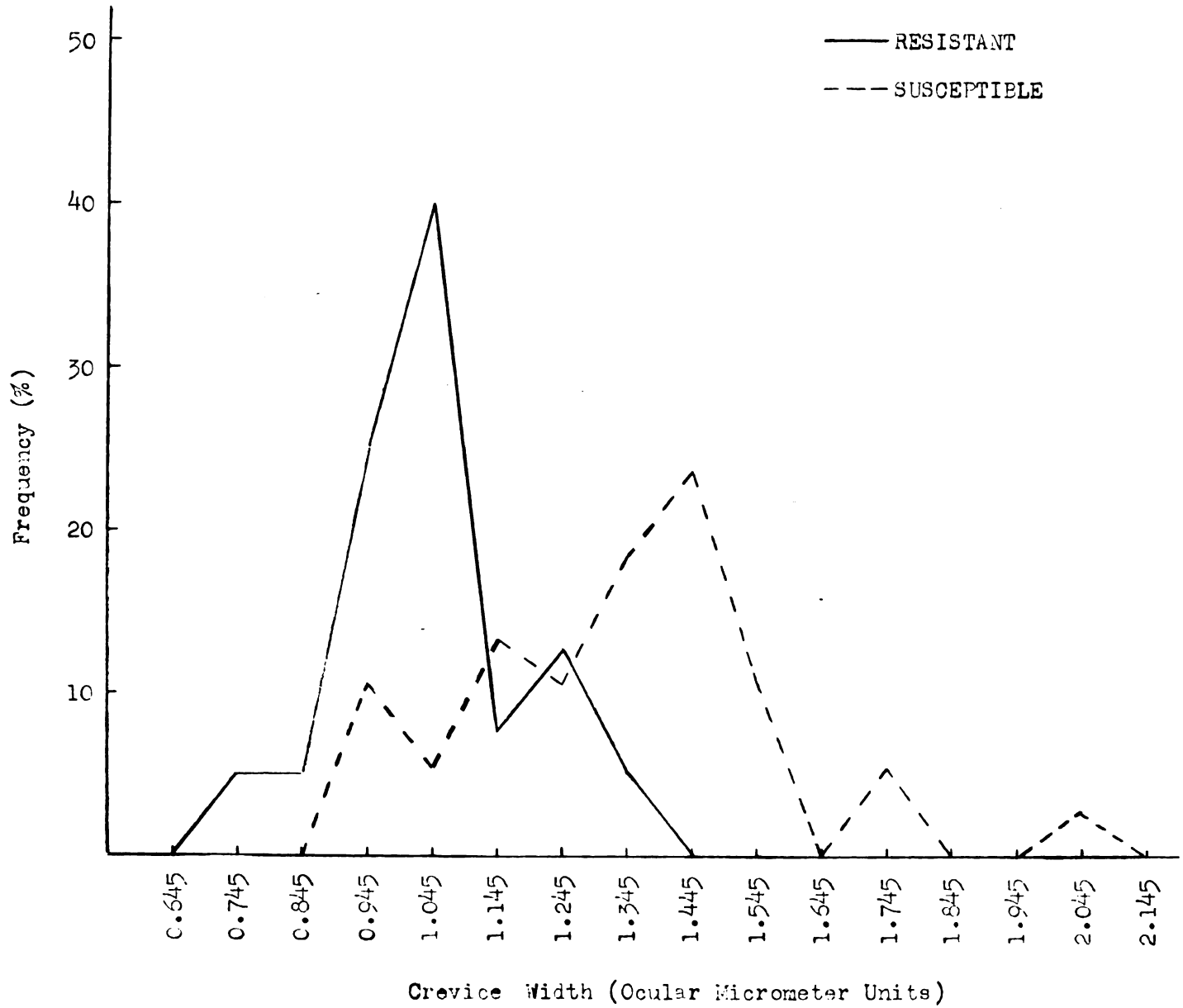


FIGURE 4. CREVICE B (LEFT)

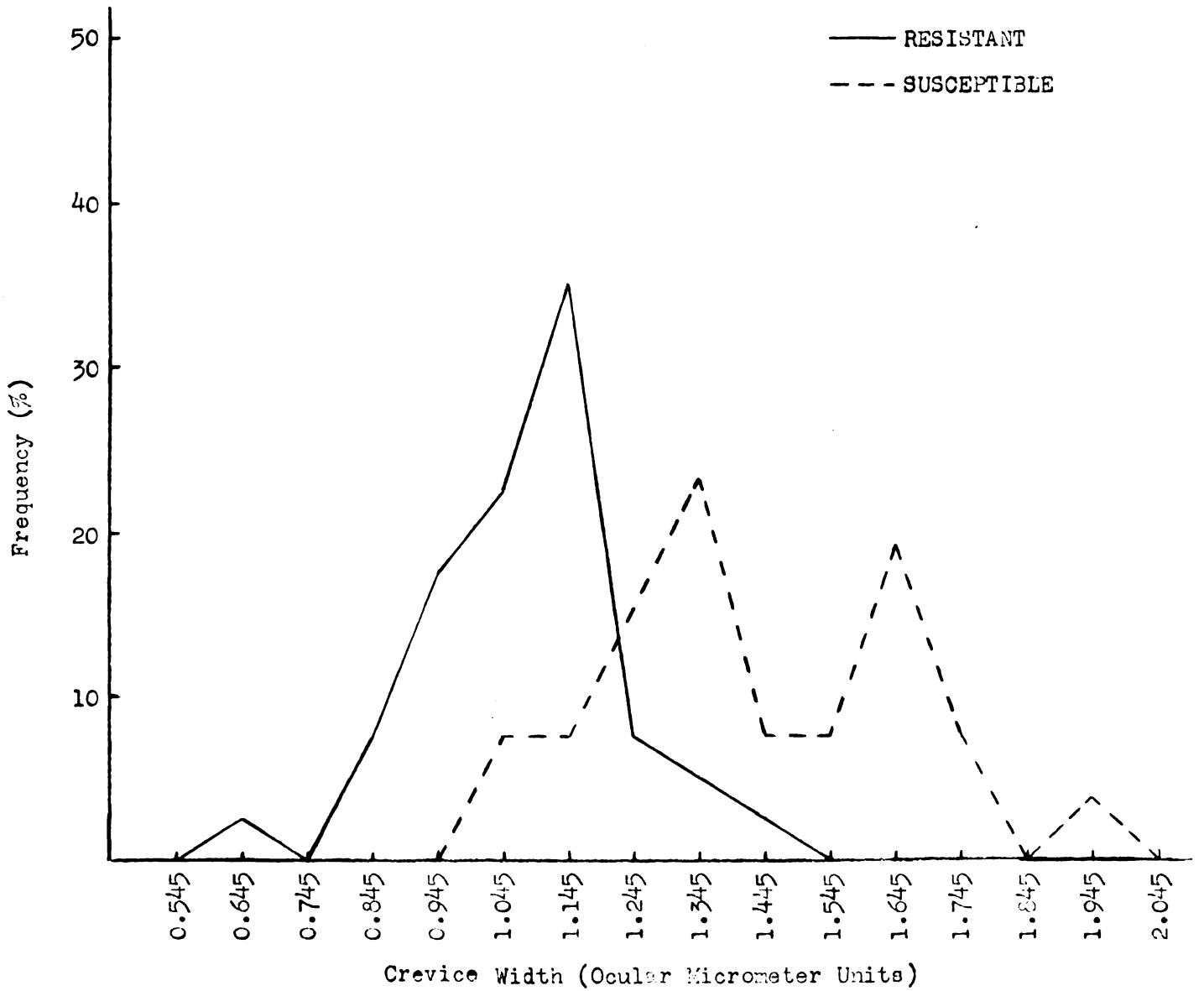


FIGURE 5. CREVICE C (LEFT)

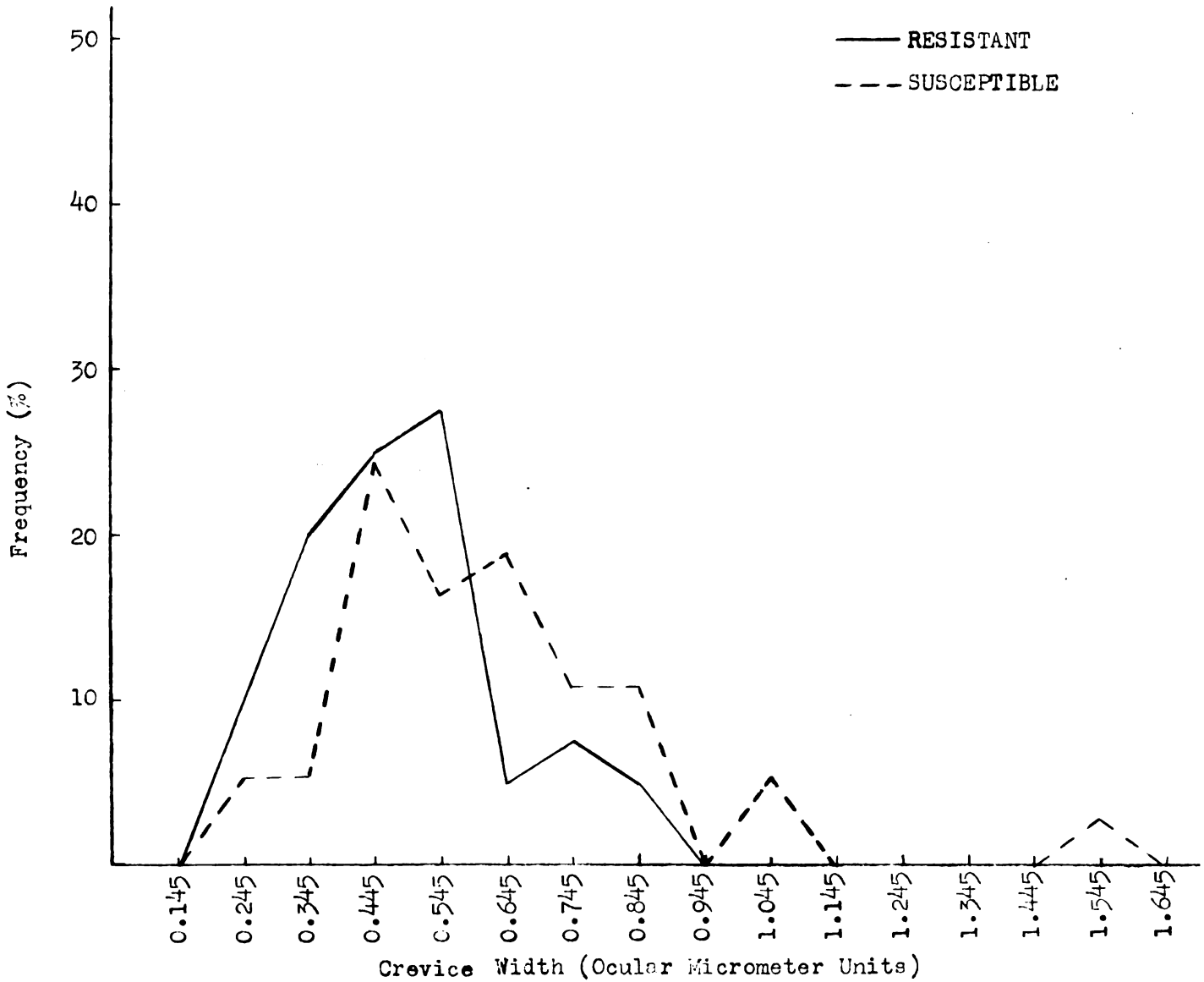


FIGURE 6. CREVICE A (RIGHT)

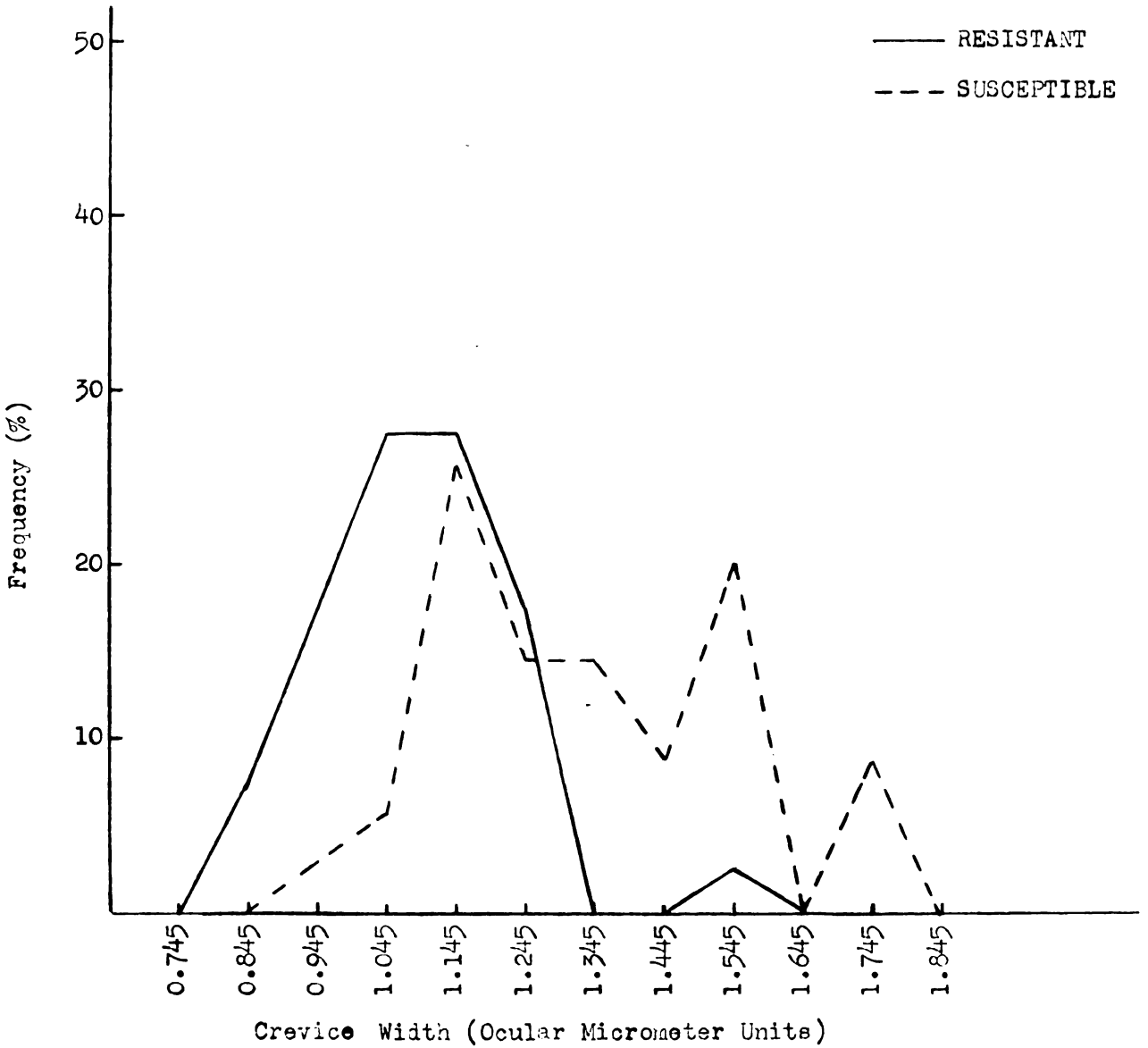


FIGURE 7. CREVICE B (RIGHT)

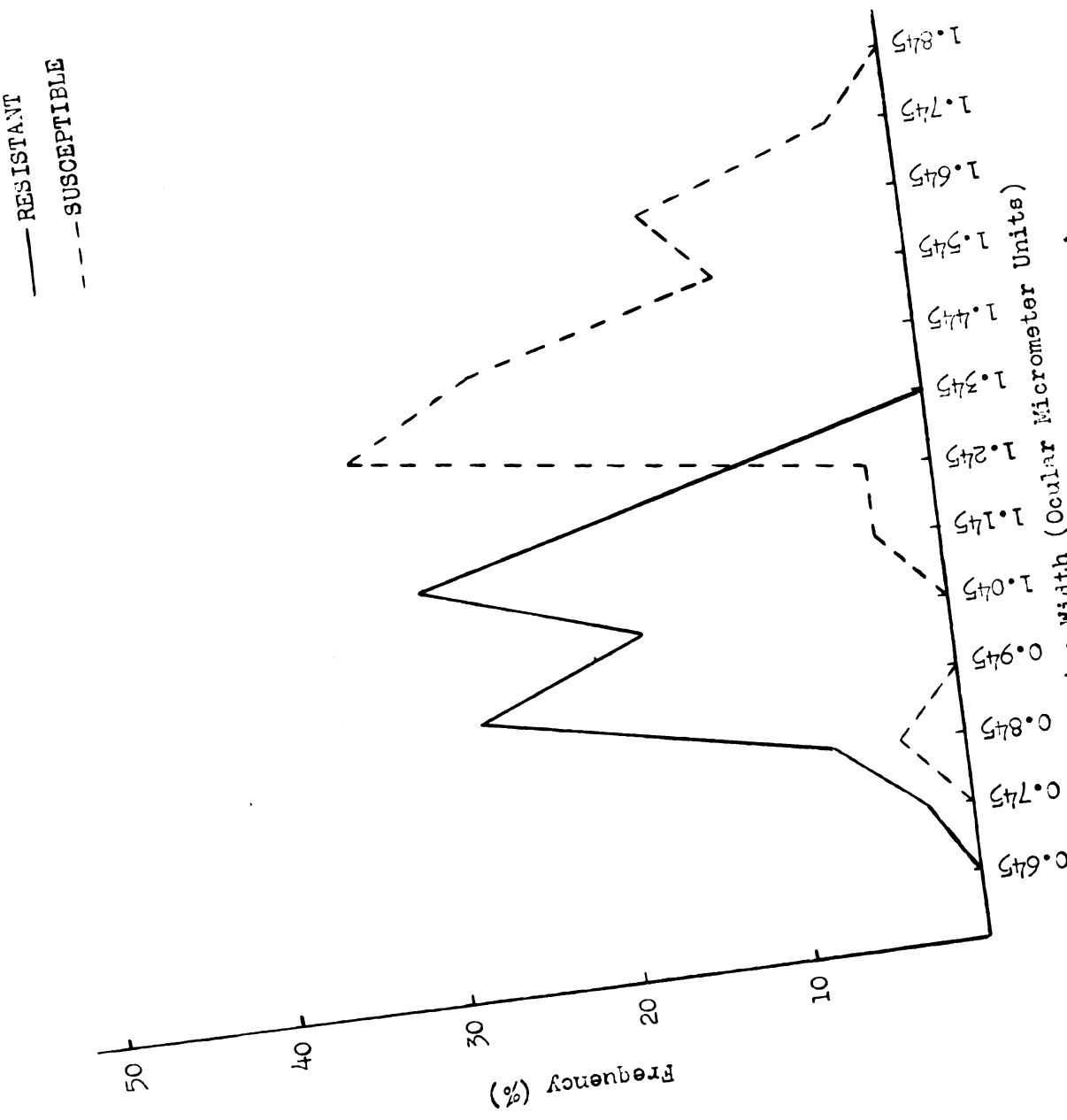


FIGURE 8. CREVICE C (RIGHT)

crevices are compared. For example, in Figure 3, crevice A of the lower left molar of the resistant rats is compared with crevice A of the lower left molar of the susceptible rats. These graphs show clearly that, although the shape varies, the curve for the susceptible animals is usually the flatter. The range of the curve for the susceptible animals is always greater. Figure 6 shows the only instance where the narrowest susceptible crevices are in the same class as the smallest resistant crevices. However, the frequency of resistant crevices in that class is twice that of susceptible crevices.

Since sex is not a factor (29), the only criterion of classification which need be considered is strains. Both groups of rats originally contained twenty-four females and sixteen males. Although some crevices were eliminated from the susceptible group, both males and females were involved. There were always more females than males. The ratios (females:males) for the susceptible animals follow:

	Left Molars	Right Molars
Crevice A	22:16	21:16
Crevice B	22:16	20:15
Crevice C	16:10	16:11

In the resistant group the sex ratio for all crevices was 24:16.

The "t" test, based on standard errors, was chosen as a means of testing the significance of the differences between average crevice widths of the susceptible and resistant groups of animals. The "t" values were determined by the following formula:

$$t_{(d.f.)} = \frac{\bar{x}_s - \bar{x}_r}{\sqrt{(\sigma_{\bar{x}_s})^2 + (\sigma_{\bar{x}_r})^2}}$$

where: $\bar{x}$ is the mean.

$\sigma_{\bar{x}}$ is the standard error.

$d.f. = n_r + n_s - 2$

The subscript r or s refers to the resistant or susceptible strain. An example of the calculations for crevice A, left molar, follows.

Substituting the necessary values from Tables III and IV in the above formula:

$$t_{(76)} = \frac{0.643 - 0.493}{\sqrt{(0.031)^2 + (0.024)^2}} = 3.8^*$$

(At the 1% level, a value of 2.64 is significant.)

Table V contains the comparisons between the various sulcus widths. In both right and left lower molars the differences between the susceptible and resistant rats in the mean widths of all the crevices were significant at the 1 per cent level.

TABLE V

COMPARISON OF CREVICE WIDTHS[1]

Crevice	Strain	Average Width ($\bar{x}$)	Standard Error ($\sigma_{\bar{x}}$)	"t" Value
		Left Molars		
A	Susceptible	0.643	0.031	3.8*
	Resistant	0.493	0.024	
B	Susceptible	1.327	0.039	6.6**
	Resistant	1.028	0.023	
C	Susceptible	1.419	0.046	6.8**
	Resistant	1.072	0.024	
		Right Molars		
A	Susceptible	0.622	0.040	3.0*
	Resistant	0.482	0.024	
B	Susceptible	1.332	0.035	5.9**
	Resistant	1.089	0.022	
C	Susceptible	1.423	0.035	9.0**
	Resistant	1.054	0.021	

[1] In ocular micrometer units.
Conversion factor: 1.00 unit = 0.195 mm.

* Significant at the 1% level.

**Highly significant at the 1% level.

Discussion

One question which arises is: Were all the crevices measured in comparable planes? Without doubt there is some variation between teeth in the level at which the final measurements were made. The buccal crests were used as markers during both mounting and grinding. During the later stages of the grinding, the plaster was carefully scraped away from the cusps of the teeth. This enabled the experimenter to see the level at which he was working. The variation in the plane of sectioning is believed to be approximately the same for both susceptible and resistant groups. The average widths are very similar for corresponding crevices of left and right molars in the same strain (Table V). This would indicate that the measurements were made in corresponding planes.

Subsequent to the accumulation of the statistics reported, an attempt was made to eliminate the effects of some of the crevices of the caries susceptible group. The wider crevices were arbitrarily assumed to be improperly mounted or improperly ground. The corresponding measurements were removed and new means calculated. The greatest reduction of any mean was 0.08 units. Since elimination of these crevices did not affect the significance of the data, they were used in the final calculation.

As previously mentioned, extensive microscopic caries was observed in the sectioned teeth of the susceptible animals. Dr. C. J. Witkop of the United States Public Health Service examined the sectioned teeth. Of the 228 crevices in the susceptible group, only 5 (2.2 per cent) did not show either caries or decalcification of the enamel. Three of these were A crevices, one a B crevice, and one a C crevice. In the resistant group (240 crevices), five A crevices showing slight decalcification of the enamel were found. One other doubtful case was recorded. Three C crevices showed a slight decalcification of the enamel. Only one other instance of caries was found in the resistant group. This animal had fractured the cusps of the lower molars, resulting in secondary caries. A small piece of metal was firmly impacted in one lower crevice. This suggests that the fractures resulted from the animal chewing the wire of the cage. Such action by the rats has been observed.

As noted in Table II, the data reported are from those crevices in which damage due to caries was not extensive enough to invalidate the measurements.

The first examination for caries by Hunt and Hoppert is at the age of thirty-five days. "Caries time," as defined by them, is the difference between thirty-five days of age and the age at which caries

is observed. For the twenty-fourth susceptible generation, caries time was twenty-three days. This was the generation corresponding to the animals used in the measurement of crevice width. Thus, gross carious lesions were observed in the main experiment of Hunt and Hoppert at the age of fifty-eight days. Both microscopic caries and secondary fracture due to caries were present at forty days in this experiment. This suggests that the caries time observed by Hunt and Hoppert might actually be "fracture time." It should be emphasized that the type of fracture referred to here (in the susceptible animals) is secondary fracture, following undermining of the enamel by the carious process.

In an earlier study, Hunt and Hoppert (31) reported on the distribution of gross carious cavities in these strains of rats. Their study was based on the first appearance of a gross cavity. Table VI summarizes comparable data from this study and that of Hunt and Hoppert.

It is evident that width of sulcus is only one factor in the causation of caries. Only 39.5 per cent of the caries on the right side of susceptibles were found at sulci, and 20.9 per cent of the caries on the left side of susceptibles. Corresponding frequencies

TABLE VI

CORRELATION BETWEEN OBSERVED CREVICE WIDTHS AND FREQUENCIES OF CARIES IN PAPER BY HUNT AND HOPPERT (31)

Author's Designation	Hunt-Hoppert Designation	Susceptible Series			Resistant Series		
		Crevice Width (mm.)	Incidence of Caries		Crevice Width (mm.)	Incidence of Caries	
Right A	Right A	0.121	0		0.094	0.03%	
Right B	Right B	0.260	16.4%	39.5%	0.212	4.4%	11.4%
Right C	Right F	0.278	23.1%		0.206	7.0%	
Left A	Left A	0.125	0		0.096	0	
Left B	Left B	0.259	5.3%	20.9%	0.201	1.4%	4.2%
Left C	Left F	0.277	15.6%		0.209	2.8%	

for resistant rats were 11.4 per cent and 4.2 per cent. Hunt and Hoppert showed that proximal caries was important also (31).

Hunt and Hoppert did not find frequent initial carious lesions at crevice A. Table V shows that there is a significant difference in crevice width at this location (between susceptible and resistant strains). Dr. Witkop, examining the ground sections, found caries with equal frequency at all crevices. A possible explanation of this discrepancy is that the B and C crevices are deeper than the A crevices. Examination of the camera lucida drawings included (Plates I to IV) shows that all crevices are inclined forward. Possibly the longer, unsupported, rear crevice wall is more susceptible to fracture after undermining by caries. Such secondary fracture would result in a gross lesion, easily seen in macroscopic examination.

For the resistant animals, a slightly different concept is suggested. The average caries time for resistant animals is currently about five hundred days. An animal which has lived relatively longer has incurred a correspondingly increased hazard of fracturing the lower molar teeth. It is suggested (though the point is not proved) that caries in some, but not all, of the resistant animals may have been initiated by a fracture, followed by true caries as a

secondary phenomenon. Against this hypothesis is the observed fact that among 954 resistant rats of the fourteenth to seventeenth generations, inclusive, only 3.2 per cent of the resistant rats showed mechanical damage to the upper molars, even at an advanced age.

From the data presented, it appears that crevice width is one of the factors involved in the causation of dental caries in rats. The mechanism is not clearly demonstrated. Since the particle size of the diet is important, it seems probable that the teeth with wider crevices offer a more favorable site for lodgement of food particles.

SUMMARY

1. The crevices of the left and right lower molars of caries susceptible rats are significantly wider than corresponding crevices in caries resistant rats at the age of forty days.

2. The presence of extensive microscopic caries in forty-day-old caries susceptible rats is discussed.

3. Crevice width may be one of the factors involved in the etiology of caries.

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PLATES

Plate

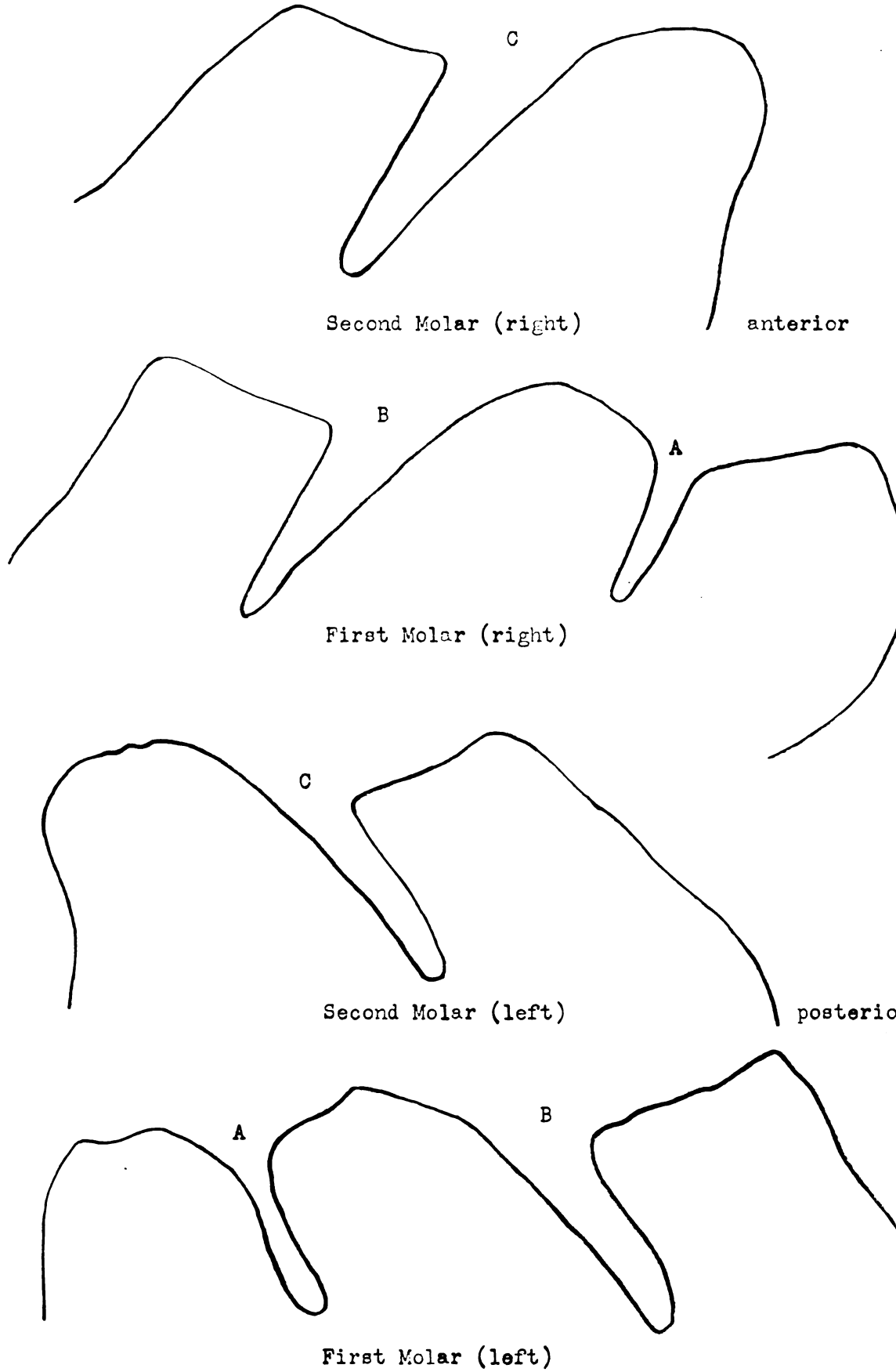
1. Side View of the Lower Molars of Resistant Rat No. 9 (Camera lucida drawing).

2. Side View of the Lower Molars of Resistant Rat No. 39 (Camera lucida drawing).

3. Side View of the Lower Molars of Susceptible Rat No. 13 (Camera lucida drawing).

4. Side View of the Lower Molars of Susceptible Rat No. 25 (Camera lucida drawing).

PLATE 2



Side View of the Lower Molars of Resistant Rat #39. (Camera lucida drawing).

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