ATTEMPTS TO IMMUNIZE HUNT-HOPPERT CARIES RESISTANT AND CARIES SUSCEPTIBLE RATS WITH RAT ORAL LACTOBACILLI

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Charles Joseph Sylvester

1954

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by

Charles Joseph Sylvester

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in Partial Fulfillment of the Requirements for a Degree of

Master of Science

DEPARTMENT OF BACTERIOLOGY AND PUBLIC HEALTH

1954

ACKNOWLEDGMENTS

The author wishes to acknowledge with sincere appreciation the guidance and technical advice so generously offered to him by Dr. Samuel Rosen, Research Assistant, Department of Zoology.

He also wishes to express his gratitude to Dr. Walter L. Mallmann, Professor, Department of Bacteriology and Public Health for his helpful criticisms. To Nathan Kraut, the author is indebted for offering his knowledge of the electrophonetic techniques and the time he devoted to those aspects of the study. Grateful acknowledgement is due to Dr. Carl A. Hoppert, Department of Biochemistry, for examining the rats' teeth regarding the development of dental caries.

A PILOT TO INITIATE MULTI-DISCIPLINE

CARIES RESISTANT AND CARIES

SUSCEPTIBLE RATS WITH

RAT ORAL LACTOBACILLI

ATTEMPTS TO IMMUNIZE HUNT-HOPPERT CARIES RESISTANT AND CARIES SUSCEPTIBLE RATS WITH RAT ORAL LACTOBACILLI

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AN ABSTRACT

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Curriculum in Bacteriology

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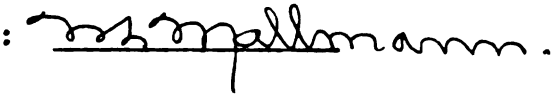
Approved: 

As part of a larger project concerned with the influence of heredity as a factor in the development of dental caries in the Hunt-Hoppert caries resistant and caries susceptible rats, this study was undertaken in an attempt to induce a lactobacillus agglutinin titer above the natural occurring titer in these rats, and to determine its effect upon the oral lactobacilli and the development of caries.

The vaccine consisted of a living suspension of four strains of rat oral lactobacilli. Two caries resistant and two caries susceptible litters were used, injecting one half of each litter every other day for two weeks.

To determine the initial agglutinin titer of all rats prior to the inoculations, the uninoculated rats were bled by cardio-puncture and the sera assayed by the tube agglutination test. Ten to 13 days after the last inoculation, all rats were bled and the sera assayed to determine what effect the inoculations would have on the lactobacillus agglutinin titer. Eighteen to 21 days after the last inoculation, saliva samples were collected from all rats and assayed as the serum samples.

The resistant and susceptible control rats showed similar agglutinin titers before the inoculations and again after three or four weeks. No significant differences were observed in the agglutinin titer of sera and saliva of inoculated and uninoculated rats of all litters.

The mouths of all rats were examined for a "total" bacterial count and lactobacillus count. The results showed no difference in the "total" numbers of organisms or numbers of lactobacilli between the inoculated and uninoculated animals.

There was no difference in caries time between inoculated and uninoculated caries susceptible rats.

Since the agglutination test showed no difference in the natural occurring agglutinin titer, and no difference between the inoculated and uninoculated rats, an electrophoretic analysis was carried out on the pooled serum of one resistant litter and one susceptible litter. This was done in order to detect any changes in the serum proteins as a result of inoculations with the oral lactobacilli. The results of these analyses showed no significant differences in serum proteins between inoculated and uninoculated rats.

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INTRODUCTION

This study was part of a larger project concerned with the influence of heredity as a factor in the development of dental caries in the Hunt-Hoppert caries resistant and caries susceptible rats.

The close correlation between the presence or absence of lactobacilli in the mouth, and the presence or absence of dental caries respectively, has been recognized for some time. It has been shown that lactobacilli are recovered more frequently and in greater numbers in the caries susceptible rats (Rosen, et al. 1954a).

A study has been made for agglutinins against lactobacilli in the sera and saliva of these animals. Although the data were variable, high natural occurring titers were found more frequently in the sera and saliva of caries resistant rats (Benarde, 1954).

The purpose of this study was an attempt to induce a titer of lactobacillus agglutinins above the natural occurring titer in rats, and to determine its effect on the oral lactobacilli and the development of caries.

REVIEW OF LITERATURE

In their attempt to determine the etiology of dental caries, McIntosh, James and Lazarus-Barlow (1925) were the first to observe natural occurring Lactobacillus acidophilus agglutinins in a body fluid. In this study they assayed human saliva, but they did not try to correlate the small quantities of agglutinins found to be present (in the range of 1:5 to 1:40) with the absence of the carious lesion. As a part of the same study, these workers tried to produce dental caries artificially in vivo. They fed a monkey and one of the workers broth cultures of L. acidophilus, and were successful in raising the salivary agglutination titer to 1:160 and 1:320, respectively.

Ross, Krasnow and Lanet (1927) by inoculating rabbits with L. acidophilus were able to demonstrate the presence of L. acidophilus agglutinins in both the blood and saliva of one half the animals inoculated. The agglutinins in the saliva were of much lower concentration than in the blood serum.

Rosebury (1930) found that tests for agglutinins and growth-inhibiting factors in the saliva of caries-free humans and dogs against dental aciduric bacteria isolated from carious teeth were negative in practically all cases. Saliva of two humans were negative for agglutinins, and a third was slightly positive. Saliva of two dogs were negative

or doubtful, and a third dog showed positive agglutination in 1:256 dilution with one of the five strains of organisms used. Therefore, he concluded that immunity and susceptibility to dental caries do not depend on the presence or absence of an inhibiting factor or an immunity factor in the mouth against organisms of the L. acidophilus type.

Blood sera of caries resistant and caries susceptible groups were tested by Jay, Crowley and Dunting (1932a) for L. acidophilus agglutinins. In a group of 23 caries-free individuals, all reactions were positive for the presence of some agglutinins, the highest titer being 1:640. In a group of 25 caries susceptible individuals, the majority gave negative agglutinin reactions; the highest titer was 1:80.

Jay, Crowley and Dunting (1932b) in another study, attempted skin reaction tests on persons harboring lactobacilli in their mouths, as well as on persons who were free of this organism. They injected intradermally a filtrate of 40 strains of L. acidophilus, and found that the majority of those who harbored this organism gave positive tests, whereas, the majority of persons who did not have the organism in their mouths gave negative skin tests.

In the work just cited, the authors also tried to raise the agglutinin titer in those individuals who gave the posi-

tive tests. They did this by administering intradermal injections of a purified form of the filtrate over a period of time, but their attempt failed. However, repeating the experiment using a heat-killed L. acidophilus culture resulted in two persons showing more agglutinins in the blood, as well as exhibiting a negative skin reaction. Therefore, in a minority of cases, negative skin reactions were associated with L. acidophilus agglutinins in the blood serum after the use of polyvalent L. acidophilus vaccines.

In a second paper, Jay, Crowley, Hadley, and Bunting (1933) worked with the rough (R) and smooth (S) phases of L. acidophilus. They divided a series of rats into groups of three each for the purpose of testing R, S, and mixed vaccines. Those that received the R vaccine had no caries, whereas the remaining six had an unusual amount of caries. It seemed at first glance that the R vaccine had successfully protected the rats against caries. However, when the sera of all animals were analysed, no agglutinins could be detected. This would seem to indicate that the vaccine had not been antigenic for rats, and that the failure of some animals to develop dental caries was due to some circumstance other than the use of vaccine.

Following a similar approach as Jay, Crowley and Bunting (1932) in their work correlating presence of agglutinins with caries resistance, Macphee (1935) described a definite corre-

lation between the degree of precipitin reaction and the clinical presence or absence of dental caries in humans. The precipitin titer was more pronounced in sera from individuals free from carious teeth. This was demonstrated using an antigen made from filtered saline emulsions of carious dentin.

Canby and Burnier (1942) demonstrated that by inoculating 20 persons with heat-killed L. acidophilus, 19 of them showed a reduction of this organism in their mouths, and also an increase in their agglutinin titer. The authors hesitate to draw conclusions from this study, because of the small number of cases studied.

In their studies using sera from 15 caries resistant and 15 caries susceptible patients, Dietz, Williams and Lawton (1943) demonstrated that no significant differences could be observed when the strength of agglutinins for lactobacilli were compared. It was also shown that the highest agglutinin titers were accompanied by a negative salivary lactobacillus count irrespective of the caries experience. The low agglutinin titers in the two groups were not consistantly accompanied by high salivary lactobacillus counts. They, therefore, conclude that the high serum titers appear to serve more directly as indicators of low incidence of lactobacilli in the mouth rather than as an index of caries experience.

METHODS AND MATERIALS

Preparation of vaccine. Four strains of oral lactobacilli, designated by Rosen (1954) as TS 109, TS 116, LS 149 and LS 110 were used to prepare the vaccine. These strains were maintained in Micro Assay Culture Agar (Difco). Cells to be used as vaccine were inoculated into tubes of Micro Inoculum Broth (Difco). The tubes were incubated for 24 hours at 37°C and centrifuged. The centrifugate was washed once in 0.85% NaCl, centrifuged, and resuspended in 0.85% NaCl. Each strain was standardized to a turbidity of No. 4 McFarland's nephelometer. An equal volume was taken from each of the standardized suspensions, and added to a sterile vaccine vial. This mixture was used as a live vaccine, and was frozen until needed.

Inoculation of vaccine. Twenty-four Hunt-Hoppert caries resistant and caries susceptible rats (14 and 10 respectively) were used in this study. Two litters of each strain were chosen, and the animals were three weeks old at the onset of the experiment. The rats were inoculated into the tail intravenously, the inoculation schedule being as follows: Approximately one half of each litter was injected with 0.1cc of the vaccine every other day for the first week, then increased to 0.2cc for the second week.

Table I shows the distribution of rats with respect to kind of litter, as well as the number of inoculated as compared to the number of uninoculated rats of each litter.

Collection and preparation of sera and saliva. The

uninoculated rats were bled on the same day that the inoculations began for their litter mates. The serum from these rats were assayed for agglutinins against the strains of oral lactobacilli using the tube agglutination test, and were used either as separate samples (as from litter 327-) or as pooled samples. This was done in order to determine the initial agglutinin titer of the inoculated rats so that comparisons might be made following the inoculations. It was assumed that all litter mates would have the same titer, if they had any titer at all.

Ten to thirteen days after the last injection was given, all rats were bled by cardiopuncture. The blood was dispensed into plugged, sterile test tubes, and allowed to clot at room temperature for about two hours with the tubes in a slanted position. The blood samples were stored in the refrigerator overnight to complete clotting. The tubes were then ringed, centrifuged and the serum drawn off with a capillary pipette. They were recentrifuged, if necessary, and the serum drawn off as before. These samples were used immediately or stored in the refrigerator until needed.

The saliva samples were always also by the tube agglutination test. Beginning to twenty-one days after the last inoculation, the rats were salivated by pilocarpine stimulation having first been anaesthetized by Nembutol (Demarte, 1954).

To collect the saliva, the rats were placed on an inclined plane, with head down and through a hole in an abut-

ment to prevent any marked movement of the head. The saliva was thus able to drip into small wide-mouth jars. The apparatus for this was designed by Bernrde (1954).

The saliva samples from the inoculated rats, as well as those from the uninoculated rats of each litter were pooled, centrifuged and the centrifugate used immediately for the agglutination test.

Preparation of antigen for the agglutination test. The same strains of oral lactobacilli were used and the same procedure was followed as that used for the preparation of the vaccine, with the following exceptions: The cells were washed in a 1:1000 dilution of 0.85% NaCl. This dilution was determined in a preliminary experiment to be one which minimized the characteristic tendency toward autoagglutination of the four strains of lactobacilli used in this study.

After the second centrifugation, the cells were standardized as before, but resuspended separately in a solution of Sorenson's buffer of pH 6.0 in the case of strain LB 109, and pH 5.0 in the case of LB 116, LB 149 and LB 110. These reactions were also determined in a preliminary experiment to minimize autoagglutination. The standardized cells were used immediately in the agglutination test. A fresh preparation was used in each test.

Agglutination test. The tube agglutination test was used to determine the factors present in the serum and saliva

of rats which could agglutinate the four strains of oral lactobacilli previously mentioned. All strains were used with each sample of saliva and serum.

The initial dilution of the serum and saliva ranged from 1:4 to 1:40 depending upon the volume of serum and saliva available for the test, and was made in distilled water containing 0.5% phenol.

The concentration of saline was kept constant in all tubes. That is, if the initial dilution of the serum or saliva was 1:10, for example, then the stock solution of 0.3% NaCl was also diluted 1:10.

One ml of the various lactobacillus antigens was added to their respective series of tubes. The contents were well mixed by shaking and incubated at 37°C for 24 hours. Readings were taken at two and 24 hours. A tube was recorded as positive if there was sediment and a clear supernatant, or if there was definite granulation when compared with the control tube.

Quantitative and qualitative bacterial analysis. Approximately one week after the final injection of vaccine, and then again after approximately two weeks, the mouths of all rats were examined for a "total" bacterial count as well as a lactobacillus count. This was done by brushing the lower right molars with a moist sterile swab, then returning it to a sterile plugged tube which contained 1.0 ml peptone water. The swab was streaked on one section of each of two plates;

one on bovine fuller's earth (Difco) (with 0.2% sodium azide added), and one on Rogosa SL agar (Difco). Both are selective for the lactobacillus organism. The tip of the swab was broken off into a screw-capped vial which contained 5.0 ml peptone water. The 1.0 ml of peptone water was also poured into the vial. The vial was shaken vigorously 40 times to loosen the cotton and allow any organisms to go into suspension. One in ten and 1:100 dilutions were made of this suspension. One tenth ml portions were plated on the above mentioned media as well as on Trypticase Glucose Extract Agar (containing 4.0% skim milk). The latter medium was used to determine a total count of oral bacteria. The inoculum on each plate was spread evenly over the surface by means of a sterile glass rod bent at an angle. The plates were incubated at 37°C for 72 hours, after which time the number of colonies were counted, and recorded as the number of bacteria per ml. The vaccinated plates inoculated by the cotton swab directly were recorded as positive if 10 or more colonies were found.

Examination of rats. Five susceptible rats were examined microscopically every two weeks for the first appearance of dental caries.

Electrophoretic analysis. Serum samples from both inoculated and uninoculated rats of Litter 527- (resistant), and Litter 570+ (susceptible) were used for this phase of the study. Hence, four electrophoretic runs were made. These rats were bled, and the serum prepared as previously mentioned. Litter 527- was bled six weeks after the last inoculation was administered. Litter 570+ was given a booster injection of antigen nine weeks after the last routine injection, and was bled two weeks later.

The serum samples were used immediately after collecting to minimize changes due to storage. Two ml of sample were diluted with four ml of barbiturate buffer (veronal and sodium veronal) of 0.1 ionic strength, and pH 8.6, made up at room temperature. Each of the diluted samples was then placed in a dialysing membrane of seamless regenerated viscose process cellulose*, and dialysed against 400 ml of buffer at room temperature (Reiner and Fenichel, 1949), followed by two more hours against 500 ml of fresh buffer. The equilibrated serum-buffer, and assembled cells were then placed in a cold room (4°C) overnight, the cells being filled in the cold room just prior to the run made on the following day.

All electrophoretic analysis were carried out with the Perkin-Elmer Model 38 Tiselius Electrophoresis Apparatus.

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*Visking Corp., Chicago, Illinois.

A complete detailed description of the operation of this apparatus is provided in the Perkin-Elmer instruction manual for this model. All analyses were made employing 7.5 milliamperes, approximately 115 volts, at an average bath temperature of 0.5°C. The potential gradients and duration of each run are as specified under each pattern.

The electrophoretic patterns of the ascending and descending limbs of the cell were photographed by the schlieren scanning system as modified by Longsworth (1939). All photographs were made with Kodak M plates (3 1/4" x 4 1/4").

The resistance of the equilibrated buffer was measured at the end of each run, using Leeds and Northrop No. 4760 electrolytic conductivity bridge.

Using the following formula (Longsworth and MacInnes, 1940), the mobilities of the various serum proteins were obtained:

$$u = \frac{dqdp}{itn}$$

The potential gradients were computed by providing data in the following formula:

$$\text{Potential gradient} = \frac{i}{qAcp}$$

Where: d = distance migrated in cm.

q = cross-sectional area of the cell

t = time of migration in seconds

i = current in amperes

n = enlargement factor

K_{sp} = specific conductance of protein

$$= \frac{\text{conductivity cell constant}}{\text{resistance of protein in cms}}$$

The relative percent composition of the serum components was determined in the following way. Two-fold enlargement tracings were made of the descending patterns by projecting from an enlarger upon plain paper. The base line was established by taking a scanning photograph of the cell limbs prior to moving the initial boundary into view, and transposing this base line to the base of the pattern. Areas were assigned to the various components by drawing an ordinate from the lowest point between adjacent maxima to the base line as suggested by Mesilius and Kabat (1939). These areas were measured by employing a planimeter.* The average of five such readings for each component area was used as the final reading. The relative per cent concentration of each component was determined from the size of its area in terms of percentage of the total area.

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*K&E No. 4236 Compensating Polar Planimeter, Keuffel and Esser Co., New York.

DATA AND DISCUSSION

Agglutination test. It may be noted in Table II that both resistant and susceptible strains of rats showed similar titers prior to the inoculations. This was also the case when the rats were examined three or four weeks later. It is apparent, therefore, that rats do not behave in this respect as do humans, since Jay, Crowley and Bunting (1932) found a definite correlation between the presence or absence of agglutinins, and caries resistance and susceptibility, respectively, in their subjects.

To determine any possible increase in titer as a result of inoculation with lactobacillus vaccine, the serum of both inoculated and uninoculated was tested 10 to 13 days after the last inoculation. The results of these agglutination tests are indicated in Table II. There was no significant increase or decrease in titer as a result of inoculation of rats with live lactobacillus vaccine. Since there was, at most, only a two-fold difference in titer in all cases, there was no reason to believe that inoculation with live vaccine will increase the normal lactobacillus agglutinin titer in rats.

This is in agreement with the findings of Jay, Crowley, Malloy and Bunting (1933) who could not induce an agglutinin response in rats by injecting them with rough (R) smooth (S)

and mixed vaccines of Lactobacillus acidophilus. Only the rats that received the K vaccine were protected from dental caries, which could not be accounted for by these workers.

However, Canby and Bunnier (1942), on the other hand, did find humans to show an increase in agglutinin titer as a result of L. acidophilus injections.

Possibly the mechanism for the production of antibodies in the very young rat might not function too well. However, the three week old rat was chosen to allow enough time for the development of lactobacillus antibodies before the onset of caries.

The saliva samples were handled in the same manner as were the serum samples, with the exception that no saliva was collected before the inoculations were begun. In Table III, it may be noted that the saliva agglutinin titers, as the serum agglutinin titers, showed no significant difference between inoculated and uninoculated rats in either the caries susceptible or resistant animals.

It was expected that the saliva would have less of an agglutinin titer than occurred in the serum, as Thesbury (1936) pointed out. This was not found to be the case, since titers of both serum and saliva were approximately the same. It was also expected that the agglutinin titer of the saliva would be proportional to that found in the serum. This too did not turn out to be the case.

In as much as the serum of all rats had the ability to agglutinate the lactobacillus antigen, to more or less the

same degree, but not to increase in titer upon inoculation of lactobacillus antigen, the possible presence of a non-specific factor is suggested. Eagle (1930) found by diluting fresh normal serum, a fraction was precipitated out.

It is also hypothesized that these titers found in both strains of rats are specific agglutinins which were perhaps induced by their normal contact with the oral lactobacilli.

Qualitative bacterial test. It may be observed in Table IV that there was no difference in frequency of recovery of oral lactobacilli between inoculated and uninoculated rats.

Quantitative bacterial test. Tables V and VI show that no consistant increase or decrease in the "total" numbers of organisms, and numbers of lactobacilli was observed in the mouths of inoculated and uninoculated rats. This is true for both the resistant and susceptible strains of rats.

It is perhaps worthy to mention that greater numbers of oral lactobacilli were recovered from the susceptible rats on both media. Also in Table IV it may be noted that lactobacilli were recovered more frequently from caries susceptible rats. While these data support the various claims that lactobacilli may be etiologic agents for dental caries, it could not be correlated with a difference in titer.

Caries time. The term "caries time" refers to the time in days which elapse from the birth of the rat, to the appearance of the first macroscopic carious lesion. It is evident

in Table VII that there is no difference between the uninoculated and inoculated caries susceptible rats. Therefore, it can be concluded that the inoculation of lactobacillus vaccine had no effect upon the caries time of these rats.

This is in agreement with Jay, Crowley, Malley and Bunting (1933), who could not induce agglutinins by injecting smooth and rough strains of L. acidophilus, nor could they inhibit dental caries in these rats with the smooth strains.

Electrophoretic analysis. As there were no differences in titer between inoculated and uninoculated rats, and since there was a high naturally occurring lactobacillus titer in these animals, as determined by the tube agglutination test, electrophoretic analyses were performed in order to detect any changes in the serum proteins as a result of inoculation with the lactobacillus vaccine.

The results of the electrophoretic analysis, with respect to mobilities, relative per cent composition and A/G ratios of the serum protein components (Tables VIII and IX, Figures 1 and 2), do not show any consistant or significant differences. In view of this, and the fact that there was no increase in titer, as determined by tube agglutination tests, inoculation with live lactobacillus vaccine does not appear to alter the serum proteins.

TABLE I

KIND AND DISTRIBUTION OF THE VARIOUS LITTERS USED AS TO THE NUMBER OF RATS INOCULATED AND UNINOCULATED

Litter Number		Strain	No. of Rats in Litter	No. of Rats Inoculated	No. of Rats Uninoculated
Mother#	Cross#				
9510	327-	Resistant	5	2	3
9593	737-	Resistant	9	4	5
9740	573/	Susceptible	6	3	3
9623	541/	Susceptible	4	2	2

TABLE II

AGGLUTINATION TITERS OF SERUM FROM RATS INOCULATED WITH FOUR STRAINS OF ORAL LACTOBACILLI AND UNINOCULATED CONTROLS

Strains of Oral Lactobacilli	Caries Susceptible Rats							Caries Resistant Rats					
	Litter 578+			Litter 541+				Litter 827-*			Litter 785-		
	U		I	U		I		U		I	U		I
	A	B	B	A**	B	B		A	B	B	A	B	B
TS 109	0	0	10	0 0	2560	5120		NR	0 0	0 0	0	1280	1280
TS 116	40	160	320	320 160	160	160		80 80	160 160	160 320	40	80	80
LS 149	80	160	320	320 640	160	160		80 80	160 160	160 320	80	160	160
LS 110	20	160	320	320 640	NR	320		NR	160 160	80 160	20	80	80

U = Uninoculated rats (Controls)

I = Inoculated rats

A = Serum sample taken at onset of experiment

B = Serum sample taken 10-13 days after last inoculation

* = Serum from inoculated and uninoculated rats of this letter were pooled into groups of two each.

** = Serum from the two uninoculated rats of this litter were assayed seperately

NR = No reading taken

TABLE III

AGGLUTINATION TITERS OF POOLED SALIVA FROM RATS INOCULATED WITH FOUR STRAINS OF ORAL LACTOBACILLI AND UNINOCULATED CONTROLS

Strains of Oral Lactobacilli	Caries Susceptible Rats					Caries Resistant Rats			
	Litter 578+		Litter 541+			Litter 827-		Litter 735-	
	U	I	U	I		U	I	U	I
TS 109	16	16	0	16		64	128	16	32
TS 116	32	32	64	64		NR	NR	64	64
LS 149	128	128	128	128		128	16	512	128
LS 110	64	64	128	128		512	512	256	128

U = Uninoculated rats (Controls)

I = Inoculated rats

NR = No reading taken

TABLE IV

FREQUENCY OF RECOVERY OF RAT ORAL LACTOBACILLI
AFTER INOCULATION WITH LACTOBACILLUS ANTIGEN

Time in Days After Inoculations	Caries Susceptible Rats					Caries Resistant Rats				
	Litter Cross Number	Media				Litter Cross Number	Media			
		TJA		LBS			TJA		LBS	
		U	I	U	I		U	I	U	I
7 to 11	541+	2/2	2/2	2/2	2/2	827-	4/4	3/3	4/4	3/3
	578+	3/3	3/3	3/3	3/3	785-	0/5	0/4	0/5	0/4
15 to 25	541+	2/2	2/2	2/2	3/3	827-	3/4	1/2	1/4	1/2
	578+	3/3	2/2	2/3	2/2	785-	5/5	2/2	0/5	0/2

U = Uninoculated rats (Controls)

I = Inoculated rats

TJA = Tomato Juice Agar Special (Difco) with 0.2% sodium azide added

LBS = Lactobacillus Selection Medium (BBL)

Numerator = Number of rats from which 10 or more colonies were recovered

Denominator = Number of rats examined

TABLE V

AVERAGE NUMBERS OF ORAL LACTOBACILLI PER ML OF ORIGINAL SUSPENSION, AND "TOTAL" COUNT IN RESISTANT RATS AFTER INOCULATION WITH LACTOBACILLUS ANTIGEN

Time in Days After Inoculation	Litter Cross Number	Media					
		TGE		TJA		LBS	
		U	I	U	I	U	I
7 to 11	827-	$619{,}000^{4}$	$99{,}480{,}000^{3}$	$4{,}140^{4}$	$1{,}785^{3}$	497^{4}	405^{3}
	785-	$7{,}680^{5}$	$17{,}800^{4}$	$1{,}820^{5}$	0^{4}	25^{5}	0^{4}
15 to 25	827-	$154{,}750^{4}$	$35{,}500^{2}$	$1{,}077^{4}$	150^{2}	265^{4}	310^{2}
	785-	$40{,}830^{5}$	$300{,}000^{2}$	$18{,}500^{5}$	320^{2}	0^{5}	85^{2}

U = Uninoculated rats (controls)

I = Inoculated rats

TGE = Tryptone Glucose Extract Agar (Difco) with 4.0% skim milk added

TJA = Tomato Juice Agar Special (Difco) with 0.2% sodiumazide added

LBS = Lactobacillus Selection Medium (BBL)

Superscripts signify number of rats examined

TABLE VI

AVERAGE NUMBERS OF ORAL LACTOBACILLI PER ML OF ORIGINAL SUSPENSION, AND "TOTAL" COUNT IN SUSCEPTIBLE RATS AFTER INOCULATION WITH LACTOBACILLUS ANTIGEN

Time in Days After Inoculation	Litter Cross Number	Media					
		TGE		TJA		LBS	
		U	I	U	I	U	I
7 to 11	578+	460,000 (3)	139,000 (3)	24,300 (3)	8,670 (3)	620 (3)	1,620 (3)
	541+	181,000 (2)	75,000 (2)	4,245 (2)	2,615 (2)	650 (2)	1,055 (2)
15 to 25	578+	372,000 (3)	300,300 (2)	2,250 (3)	4,600 (2)	0 (3)	155 (2)
	541+	316,500 (2)	309,000 (2)	2,490 (2)	6,250 (2)	660 (2)	1,800 (2)

U = Uninoculated rats (controls)

I = Inoculated rats

TGE = Tryptone Glucose Extract Agar (Difco) with 4.0% skim milk added

TJA = Tomato Juice Agar Special (Difco) with 0.2% sodium azide added

LBS = Lactobacillus Selection Medium (BBL)

Superscripts indicate number of rats examined

TABLE VII

AVERAGE CARIES TIME IN CARIES SUSCEPTIBLE RATS

Litter Number		No. of Rats Surviving Experiment	Caries Time*
578+	Uninoculated	1	145
578+	Inoculated	2	113
541+	Unoculated	2	90
541+	Inoculated	2	85

* Time in days elapsed from birth of rat to first macroscopic carious lesion

TABLE VIII

THE EFFECTS OF INOCULATION WITH LIVE LACTOBACILLUS VACCINES ON THE MOBILITIES OF COMPONENTS OF POOLED RAT SERUM FROM A CARIES RESISTANT AND A CARIES SUSCEPTIBLE LITTER

Litter Cross Number		Mobilities*				
		Albumin	Globulin			
			$Alpha_1$	$Alpha_2$	Beta	Gamma
827-	U	7.14	6.25	6.09	4.02	3.53
827-	I	7.56	6.71	5.61	4.28	3.20
573/	U	7.13	6.25	5.13	4.13	3.14
573/	I	6.22	5.32	4.09	3.12	2.20

U = Uninoculated rats (Controls)

I = Inoculated rats

* = Table figure x $10^{-5} cm^2 volt^{-1} sec^{-1}$

TABLE II

RELATIVE PERCENT COMPOSITION OF EACH COMPONENT IN TERMS OF PERCENTAGE OF THE TOTAL AREA

Litter Cross Number		Relative Percent Composition					
		Albumin	Globulin				A/G Ratio*
			Alpha$_1$	Alpha$_2$	Beta	Gamma	
327-	U	47.6	15.0	13.7	14.0	9.3	.93
327-	I	53.2	8.5	14.1	21.2	4.6	1.10
573/	U	51.3	13.3	8.2	13.1	8.4	1.07
573/	I	45.7	16.1	13.7	11.4	8.2	.92

U = Uninoculated rats (Controls)

I = Inoculated rats

*A/G Ratio = $\frac{\text{Total albumin area}}{\text{Total globulin area}}$

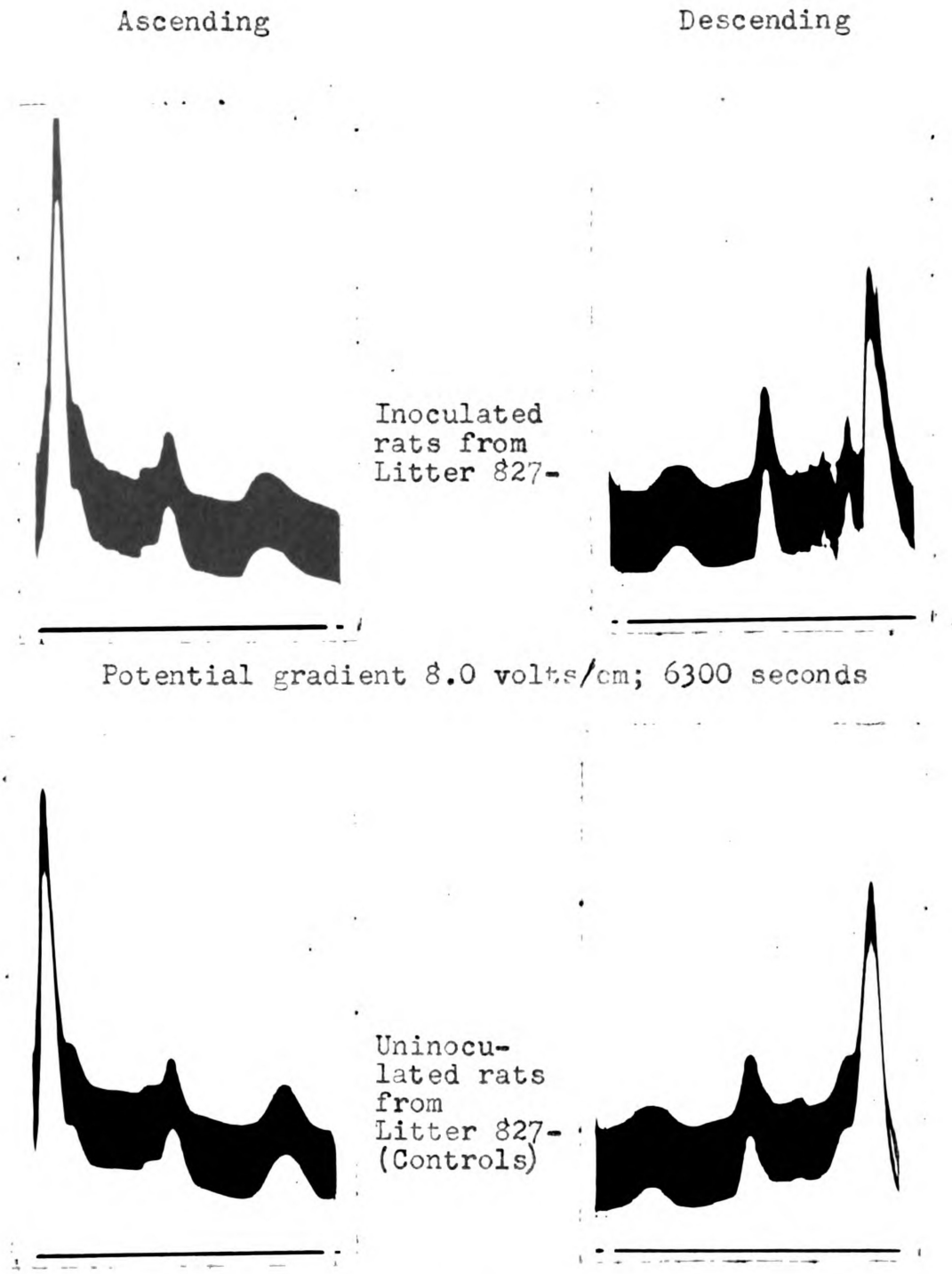

FIGURE I

ELECTROPHORETIC PATTERNS OF RESISTANT RAT SERA SIX WEEKS AFTER ORAL LACTOBACILLUS INOCULATION COMPARED WITH UNINOCULATED RAT SERA

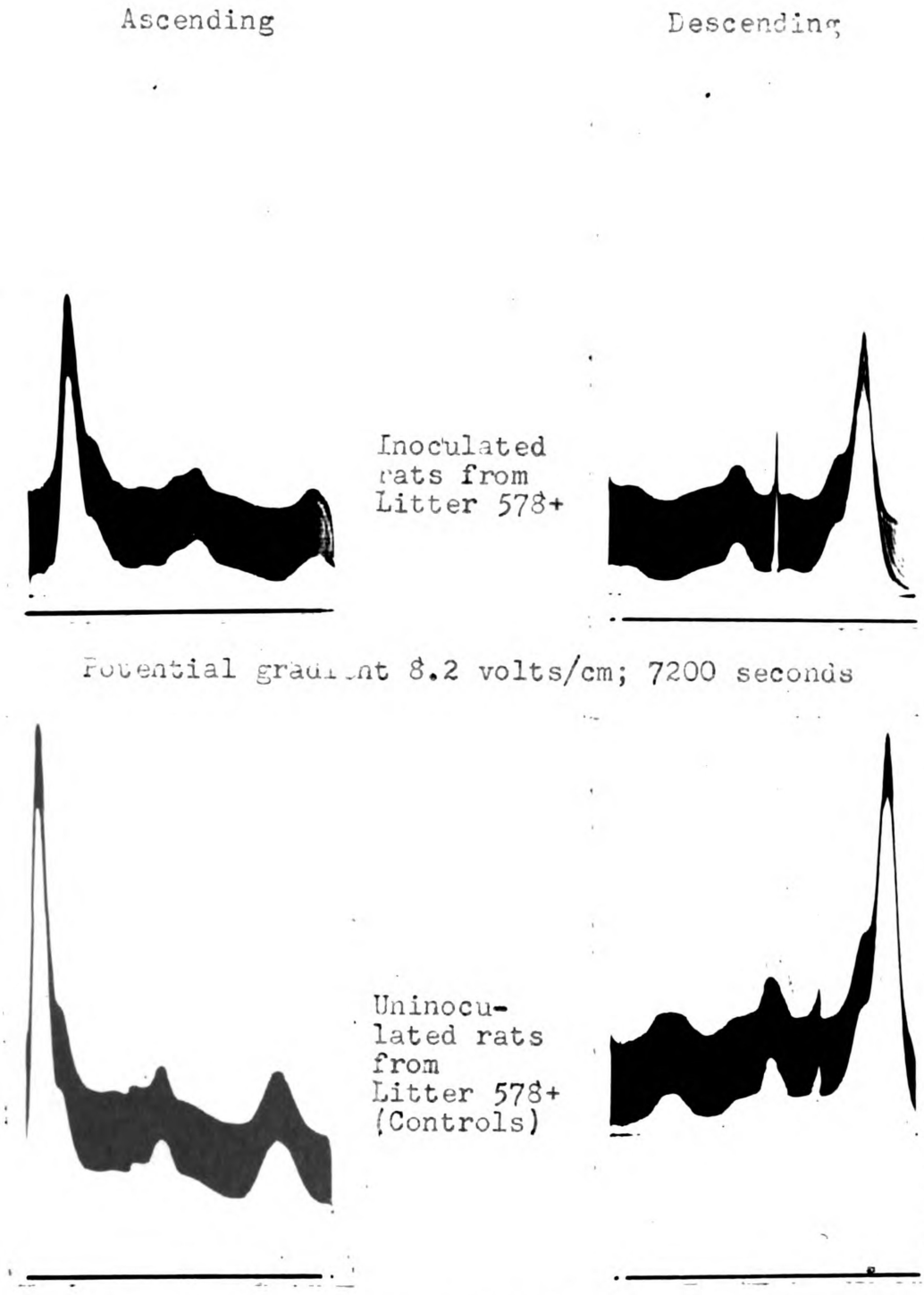

FIGURE 2

ELECTROPHORETIC PATTERNS OF SUSCEPTIBLE RAT SERA TWO WEEKS AFTER ORAL LACTOBACILLUS BOOSTER INOCULATION COMPARED WITH UNINOCULATED RAT SERA

SUMMARY

Serum and saliva samples from Hunt-Hoppert caries resistant and caries susceptible rats were tested for agglutinins following intravenous injections of rat oral lactobacilli, using the tube agglutination reaction. No significant difference in titer was noted between the inoculated and control litter mates of both resistant and susceptible animals.

Electrophoretic analysis of serum samples from two of the litters (one resistant and one susceptible) was carried out in order to determine if inoculation with live lactobacillus vaccine would alter the serum proteins. There were no significant differences in electrophoretic patterns of the inoculated and uninoculated rats.

The caries time of all susceptible rats was observed macroscopically to determine whether the lactobacillus antibodies which might be produced as a result of the inoculations would influence the time usually required for dental caries to occur. There was no change in caries time as a result of the inoculations.

Examining the mouths of all rats of both strains for a "total" bacterial count, and lactobacillus count showed no significant differences between inoculated and uninoculated rats.

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