vived without sequelae and have shown no evidence of bleeding tendency.

Summary. The use of an homologous lung is at present the simplest and most efficient method of providing extracorporeal oxygenation of blood. A simplified method of using such a lung experimentally has been described. In this experimental study of 12 animals there were 7 survivors, the 5 fatalities being caused by severe bleeding resulting from contaminated equipment.

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Relationship of Salivary Tryptophane to Lactobacillus Colony Morphology and Dental Caries.* (22084)

GORDON E. GREEN, MATTHEW C. DODD, AND ARTHUR W. RADIKE.

Dental Research Laboratory and Department of Bacteriology, Ohio State University, Columbus, and Procter and Gamble Co., Cincinnati.

In a previous investigation of the salivary microflora of dental caries-free and caries-susceptible humans, an association of smooth colony morphology of lactobacilli with saliva of caries-free subjects has been described(1). Rough colony types predominated in saliva of the caries-susceptible subjects. The observation that a tryptone medium as well as tryptophane induced rough to smooth dissociation in colony morphology of oral lactobacilli prompted the present study of this action of tryptophane on lactobacillus colony morphology, and the possible relationship of salivary tryptophane to caries resistance.

Materials and methods. The lactobacilli used in this study were of human oral origin. The cultures were classified into 3 types, smooth, intermediate and rough on the basis of colony morphology on tomato agar. The rough colony type is flat, translucent, with an indented to serrated edge, and is stable on consecutive passage on tomato agar. Tomato juice agar was prepared according to the Hadley method(2). Rogosa's Lactobacillus Selective Medium(3), used for growth and fermentation studies, was modified for general use as a liquid medium by omission of the agar, glacial acetic acid, and 5 g of sodium acetate per liter.

To test the action of tryptophane the medium was modified to contain 10 g acid hydrolyzed Casamino Acids (Difco) instead of trypticase; 0.013 g dl-tryptophane; and 15 g agar. Additional amounts of dl-tryptophane were added as required. Modifications of the Ehrlich (paradimethylaminobenzaldehyde) and Hopkins-Cole (glyoxylic acid) tests were studied for determination of tryptophane in saliva, and the latter test was selected. The procedure has been described by Block(4), with the essential modification that the concentration of H_2SO_4 was reduced to avoid undesirable reactions with other constituents of saliva.

Three ml of resting and 3.0 ml of stimulated saliva were collected from a subject and mixed. Two ml of the mixture were placed in a 10.0 ml volumetric flask and 0.5 ml of M/25 $CuSO_4$ and 0.5 ml of glyoxylic acid were added. The flask was held in an ice-and-water bath for 5 min., then 5.0 ml of 80% H_2SO_4 were slowly added. The flask was held in the cold bath for 10 min. then placed in boiling water for 5 min. After cooling 2 min. at room temperature the contents were mixed

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by rotating the flask and cooled 8 more min. The contents were diluted to 10 ml volume with 50% H_2SO_4, incubated at room temperature for 15 min., then observed in a Coleman Junior Spectrophotometer at 515 and 570 mu. The reference standard was similarly prepared except that 0.5 ml distilled water was substituted for glyoxylic acid. The characteristic absorption maxima in this test for tryptophane and indol are respectively 570 and 515 mu. The test procedure described by Block, when applied to pure solutions of tryptophane and indol, results in colored reaction mixtures that follow the Lambert-Beer Law; concentration $= -K$ log transmittance. Substitution of 80% H_2SO_4 introduced some error. The error in transmittance at a known concentration of 0.02 mg tryptophane was about 3%, at 0.10 mg about 15%. The values for time, volume, concentration and temperature are all critical. The subjects used as sources of saliva in this study were all young adults classified as caries-free or "immune," and caries susceptible on the basis of clinical and roentgenologic examinations. The susceptibles showed past or present caries, whereas the immunes showed no evidence either past or present.

Results. The original observation was that certain rough colony strains of oral lactobacilli exhibited a previously unobserved change to smooth colony morphology when grown on tryptone glucose extract agar. This change was demonstrable regularly on this medium, and was reversible after 2 or 3 transfers on tomato agar. The addition of 0.5% tryptone to tomato agar also induced the rough to smooth colony variation. In addition, some rough colony lactobacilli, grown on tomato agar containing 0.004% dl-tryptophane, underwent rough to smooth dissociation which did not occur on this medium without added tryptophane. However, 10 rough colony lactobacillus strains cultured on modified Rogosa's lactobacillus selective medium to which 0.0013% dl tryptophane had been added, at pH 5 and 7 showed only slight changes in colony morphology as compared to the changes observed on tomato agar. The addition of 0.02% dl-tryptophane or indol to this medium caused 7 of the strains to vary to smooth morphology. Although the results were variable, colony dissociation induced by tryptophane or indol was more common at pH 5 than at pH 7. Tryptophane and indol were about equally effective and strains of lactobacilli reacted to either, both or neither.

The rough to smooth variation was seen in all colonies which developed on media with added tryptophane, and all colonies which were picked from such media were capable of reversion. Aliquots of broth cultures developed approximately equal numbers of colonies on media with and without added tryptophane. The variation was usually reversed on first passage without added tryptophane, although some strains required 3 to 4 transfers before all smooth characteristics were lost. Successive passages on tryptophane-containing media did not render the smooth variants more resistant to reversal when removed from the influence of tryptophane. Thus the effect of tryptophane was transitory, perhaps suggestive of adaptive enzyme formation. The fermentative capacity of the smooth variants for a number of carbohydrates did not differ from the parent cultures, the original acidogenic and aciduric properties being maintained. Attempts to differentiate smooth variants from rough parents by several common capsule stains, especially by use of Alcian Blue, a carbohydrate specific stain described by McKinney(5), were not successful.

Turner and Crowell(6) have reported an association of tryptophane or tryptophane-like compounds with saliva of caries-free humans. More recently, however, Stack(7) has asserted the independence of salivary tryptophane and caries experience. In view of the above findings chemical analyses of the tryptophane and indol content of caries-immune and susceptible individuals were performed.

Descriptions of the subjects whose salivas were analyzed and results of the analyses are given in Table I. The statistical analyses of the data show a significantly greater amount of both tryptophane and indol in immune salivas. The Hopkins-Cole test is not specific

TABLE I. Analyses of Salivas for Tryptophane and Indol in 31 Subjects.

Caries immune			Caries susceptible		
Solution color	% transmission at: 570 mu	515 mu	Solution color	% transmission at: 570 mu	515 mu
Yellow	100.0	100.0	Yellow	95.0	92.2
Purple	81.3	81.3	"	99.2	97.0
"	40.0	40.0	Pink-gray	71.2	81.0
"	44.0	64.3	Yellow	94.3	95.2
"	25.3	46.0	Pink	52.1	71.2
"	28.2	41.2	Pink-brown	62.0	63.1
"	38.0	41.2	"	66.3	66.2
"	50.3	63.3	Yellow	82.1	77.1
"	36.0	58.1	Purple	53.1	88.3
"	13.1	22.0	"	56.2	106.0
"	45.0	56.1	Pink	29.3	42.2
"	34.0	61.1	"	61.1	66.3
"	51.2	57.1	"	28.0	39.2
"	28.2	50.1	Yellow	29.1	40.0
"	28.3	53.2	"	66.2	52.1
			Pink	40.0	57.2

"t" Tests

Tryptophane, 570 mu: $t = 2.33$ Probability $= 0.038$
Indol, 515 mu: $t = 2.087$ Probability $= 0.05$

Chi-Square Test for Tryptophane Color

	Purple	Non-purple	Totals
Immunes	a 14	b 1	15
Susceptibles	c 2	d 14	16
	16	15	

Chi-square $= 20.26$; Probability $=$ less than 0.001

for free tryptophane alone, as combined tryptophane or compounds having similar configurations may give a color reaction. However, the deep purple color seen with most immune salivas is the same as that given by pure solutions of tryptophane.

The association of smooth colony salivary lactobacilli and high salivary tryptophane in caries immunes prompted an additional study. Several dental students having a mixed flora of salivary lactobacilli were given paraffin containing dl-tryptophane to chew intensively during 6 hours per day for one week. This procedure increased salivary tryptophane content but had no effect on the numbers of smooth and rough colony type lactobacilli isolated from their salivas.

Discussion. Dissociation of colony morphology of a culture of microorganisms due to addition of a single compound is not novel. Rogosa and Mitchell(8) have reported similar results on adding sorbitan monooleate (Tween 80) to a medium. They attributed the change in morphology to alteration of the nutritional environment. Thus, alteration of colony morphology of an entire population was not the result of genetic changes, but rather a difference in phenotypic expression of genetically similar populations in two different environments. It is rather likely that such is the case in tryptophane-induced dissociation.

The finding of significantly greater amounts of tryptophane or tryptophane-like compounds in caries-free salivas compared to caries-susceptible has confirmed the work of Turner and Crowell(6), and seems to strengthen their hypothesis that salivary tryptophane levels are related to the presence or absence of caries. It is not, however, necessarily at variance with the results of Stack (7), since a high salivary tryptophane content was found in one individual (age 14) with clinically rampant caries. In addition, Stack's conclusions might be confirmed by examining selected items of data from Table I, since his study apparently included only 3 caries-free individuals. Comparison of the results with the 2 clinically different populations in the present work, and in that of

Turner and Crowell(6), leads to other conclusions. It is also well to bear in mind that factors which may be correlated with caries immunity may be unrelated to susceptibility.

Obviously, the relationship of high salivary tryptophane or indol and the caries-immune state is not one of cause and effect. Furthermore, the rough to smooth colony dissociation of lactobacilli produced by tryptophane or indol *in vitro* is different both in extent and kind from the similar action of immune saliva(1). The latter is effective on most cultures of lactobacilli and is definitely affected by pH. It appears to be a selection process associated with inhibition of growth and results in organisms with modified physiological activities. As noted above, none of these apply to the tryptophane-indol induced variants. Likewise the low count of predominantly smooth colony lactobacilli in caries-immunes suggests that the above action of immune saliva occurs in the oral cavity, while the present findings indicate that high salivary tryptophane is not present in all immunes and does not affect the number of rough colony lactobacilli in susceptible mouths even when the salivary concentration of tryptophane is increased.

It is equally clear, however, that caries immunity and high salivary tryptophane are probably related in some way when both are present. In such cases, even the rough to smooth dissociation in lactobacillus colonies may be related to tryptophane present in immune saliva. In cases of low salivary tryptophane in immunes, this type of resistance to caries may not be involved.

Summary. 1. Addition of dl-tryptophane or indol to media induces a rough to smooth dissociation in colony morphology of some strains of oral lactobacilli. 2. This dissociation is readily reversible, is apparently not a selection, and is unaccompanied by gross changes in physiology. 3. Chemical analysis of salivas for tryptophane and indol indicates the association of greater amounts of such compounds with the caries-immune group. 4. Tryptophane-induced rough to smooth variation in lactobacilli is different from the similar dissociation which occurs in saliva of caries-immune individuals.

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