

days, reducing sugar was determined in the filtrates by adaptations of Folin's⁴ methods. In 30 experiments the added enzyme preparations caused an increase in reducing sugars followed by their gradual disappearance. An occasional failure to digest occurred. Calculated as dextrose, the digestible carbohydrate in 1 gm. tubercle bacilli varied up to 3 mg., depending upon the digestion period and other factors.

Digestible Fat. Series of May, 1930-June, 1931. Acid liberated from 1 gm. portions of tubercle bacilli by autolytic and by pancreatic enzymes was titrated with N/20 sodium hydroxide, with phenol red indicator. The rapid acid liberation during the first week slowed down in a month, almost to a standstill. A gram of tubercle bacilli undergoing autolysis for one month in the presence of excess of chloroform and toluol, liberated, on the average, 3.8 cc. N/20 acid. The corresponding figure is 6.5 cc., when 50 mg. of pancreatic enzyme had been added. Part of the acid liberated probably was due to protein digestion.⁵ To calculate the weight of fat digested is difficult in the absence of figures for molecular weights of true glycerides in tubercle bacillus. If the above figure, 6.5 cc. N/20 acid is calculated as oleic acid, which involves many assumptions, the result indicates that all or nearly all of the fat was digested.

Physical Changes. In tubes containing the added pancreatic enzymes the bacilli seemed to swell to 2 or sometimes 3 times their original volume. When hand centrifuged for 5 minutes the flocculent bacillary masses were sedimented to their original volume. The bacilli became buttery, and sampling the mixture was impossible. In control tubes the bacilli remained the same in gross appearance although autolysis was going on. Such mixtures could be sampled.

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Aciduric Organisms in Dental Caries.*

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With the recent interest in dietary factors in dental decay the rôle

⁴ Folin, O., *J. Biol. Chem.*, 1926, **70**, 410.

⁵ Sherman, H. C., and Neun, D. E., *J. Am. Chem. Soc.*, 1916, **38**, 2210.

* Work supported by the Commonwealth Fund Grant for the study of the cause of Dental Caries.

of the aciduric bacteria has taken a secondary place. Attention to the possible significance of the acid-resisting, non-sporing, gram-positive bacilli was first drawn by Kligler¹ who found them constantly present on carious teeth, but irregularly on normal teeth. Since then a number of workers, Howe and Hatch,² McIntosh and Lazarus-Barlow,^{3, 4} and Rodriguez,⁵ have isolated these organisms from various depths of carious cavities and have ascribed to them major importance in the production of caries. Bunting and Palmerlee,⁶ Bunting, Nickerson and Hard,⁷ and Jay and Vorhees,⁸ have compared the incidence of the aciduric bacilli in carious mouths and in the mouths of individuals "immune" to caries. They found the presence of these organisms almost perfectly correlated with the occurrence of caries and their absence with non-susceptibility to caries.

In the experiments reported here we have repeated and confirmed the work of the last named authors. Three groups of cases† were studied: (1) Individuals showing active and extensive caries, (2) Individuals with a history of freedom from caries and in whom careful examination revealed no trace of decay. In most cases the examination was confirmed by complete X-ray. (3) A third small group of individuals who had previously had caries but in whom no active caries had been present for some years previous to the experiment. The age distributions in the 3 groups were approximately the same: young adults being in slight preponderance in the immune group and children of school age in the caries group. Cultures obtained by swabbing the entire exposed surfaces of all the teeth were made in pH 5 chopped meat, 1% glucose infusion broth without any air seal. After 48 hours' incubation the broth was inoculated onto the surfaces of pH 7.4 1% glucose infusion

¹ Kligler, I. J., *J. Allied Dent. Soc.*, 1915, **10**, 141.

² Howe, Percy R., and Hatch, R. E., *Dental Cosmos*, 1917, **59**, 961.

³ McIntosh, J., James, W. W., and Lazarus-Barlow, P., *Brit. J. Exp. Path.*, 1922, **3**, 139.

⁴ McIntosh, J., James, W. W., and Lazarus-Barlow, P., *Brit. J. Exp. Path.*, 1924, **5**, 175.

⁵ Rodriguez, F. E., *Milit. Dent. J.*, 1922, **5**, 199.

⁶ Bunting, R. W., and Palmerlee, Faith, *J. Am. Dent. Assn.*, 1925, **12**, 381.

⁷ Bunting, R. W., Nickerson, Gail, and Hard, D. G., *Dental Cosmos*, 1926, **68**, 931.

⁸ Jay, P., and Vorhees, R. S., *J. Am. Dent. Assn.*, 1929, **16**, 2054.

† I am indebted to the Staff of the Columbia University School of Dentistry, especially to Drs. E. C. McBeath and L. B. Stowe, for the examination and classification of the cases.

agar plates and incubated aerobically for 48 hours. The colonies were studied with the naked eye and with the low power microscope and gram stains of the organisms were examined. Pure cultures picked from the plates were grown in pH 7.4 chopped meat, 1% glucose infusion broth.

Except for occasional yeasts and staphylococci the only organisms surviving the acid broth and developing on the plates were gram-positive, non-sporing, pleomorphic rods.

Pleomorphic gram-positive, non-sporing bacilli were obtained in great numbers from 21 of the 24 caries cases and from all 7 of those classified as arrested cases. The organisms grew profusely in the broth cultures and gave rise to numerous typical pin-point colonies on the agar plates. They were obtained from only 6 of the 19 cases classified as "immune" and in 4 of these grew only sparsely in the acid broth, producing only an occasional isolated colony when transferred to the plates. In further transfer to pH 7.4 glucose infusion broth these organisms grew profusely, indicating that their acid-resisting properties were less marked than those of the other organisms. All the colony types and morphological varieties described by Hadley, Bunting and Delves⁹ were isolated; usually 1 or 2 types being obtained from any one case. There was no indication that any type or combination of types was characteristic of immune mouths or of carious mouths.

These results, although not based on a very extensive series of experiments, are very definite and completely confirm the reports of previous workers. It is difficult, however, to assay the possible significance of this degree of correlation between caries and the presence of the aciduric bacilli. The profuse growth from the 2 immune and from the 7 arrested cases indicates that the presence of the organisms is not the only factor in the production of caries. It is hardly likely that all 7 arrested cases, after years of immunity, were about to become active at the time of the cultures. It is equally difficult to believe that the degree of correlation between positive cultures and the presence of caries noted here, and by previous workers, is pure coincidence. Unless it can be shown that cavity formation offers a milieu without which aciduric bacilli of this type cannot survive (that is, in which they occupy the rôle of secondary invaders) the aciduric organisms must be included in any consideration of the etiology of dental caries.

Summary. Aciduric, gram-positive, non-sporing, pleomorphic

⁹ Hadley, Faith, Bunting, E. W., and Delves, Edna A., *J. Am. Dent. Assn.*, 1930, 17, 2041.

bacilli were isolated from the mouths of 21 of 24 individuals with extensive active caries, and from 7 patients in whom caries was arrested. Of 19 individuals who had never had any dental decay, the organisms were isolated from only 6; from 4 of these, organisms were obtained which had less acid-resistance than those from the carious mouths.

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Blood Groups and Susceptibility to Dental Caries.*

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Since it is generally known that in many cases immunity to dental caries is apparently inherited, it was considered possible that this immunity or, conversely, great susceptibility might be linked with the blood group of the individual. To test this possibility bloods from a number of individuals with a history of freedom from decay and shown by dental examination to have absolutely no caries were typed. Another group of individuals in whom caries was very active and extensive was studied in the same manner.

The grouping was done in the usual way by testing the cells of the blood in question with known O, A and B sera, and the serum against known A and B cells. The tests were carried out macroscopically on a glass plate.

The distribution of the blood types in each group of cases is shown in the table.

TABLE I.

	Blood Groups				Total
	O	A	B	AB	
Immune group	11	9	3	1	24
%	45.8	37.5	12.5	4.1	
Caries group	18	12	3	3	36
%	50	33.3	8.3	8.3	

It is obvious that there is no significant difference between the distribution of blood types in the 2 groups of individuals.

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