feeding, whereas insulin hypoglycemia increased the frequency and amplitude of such waves.

It is possible that insulin hypoglycemia is able to liberate pancreozymin and/or secretin from the antrum (or intestinal mucosa) in a way that sham feeding cannot. However, it is also known that antral motility is not essential for release of gastrin(7), and hence the differences in antral motility that occur with insulin hypoglycemia and sham feeding may be irrelevant.

It is of interest to note that in both humans(8) and dogs(9) insulin hypoglycemia has been shown to cause an increase in blood flow to the liver. Tanturi and Ivy(10) published studies on the effect of blood flow to the liver on bile flow. They concluded that in acute experiments an increased blood flow through the liver augmented bile output except when increased blood flow was associated with an increased intrahepatic vascular pressure. They acknowledged that the evidence was inconclusive. We have no knowledge of what the effect of sham feeding is on blood flow to the liver, and we do not know if the blood flow changes that occur with insulin hypoglycemia are relevant to the associated choleresis. Brauer(11) argues that bile flow rates are independent of blood flow or pressure when the oxygen supply is not a limiting factor.

Summary. In contrast to the true choleretic effect of insulin hypoglycemia and feeding in chronic cholecystectomized dogs, sham feeding produced only a slight hydrocholeresis. An increase in gastric secretion in the same dogs indicated effective vagal stimulation. These results add another difference between sham feeding and insulin-hypoglycemia in their effects on the digestive tract.

1. Fritz, M. E., Brooks, F. P., Am. J. Physiol., 1963, v204, 825.
2. Thomas, J. E., Proc. Soc. Exp. Biol. and Med., 1941, v46, 260.
3. Komarov, S. A., Marks, I. N., ibid., 1958, v97, 574.
4. Kocour, E. J., Ivy, A. C., Am. J. Physiol., 1938, v122, 325.
5. Malloy, H. T., Evelyn, K. A., J. Biol. Chem., 1938, v122, 597.
6. Olbe, L., Jacobson, B., Gastroenterology, 1963, v44, 787.
7. Thein, M. P., Schofield, B., J. Physiol., 1959, v148, 291.
8. Bearn, A. G., Billing, B. H., Sherlock, S., Clin. Sci., 1952, v11, 151.
9. Shoemaker, W., Mahler, R., Ashmore, J., Pugh, D., Am. J. Physiol., 1959, v196, 1250.
10. Tanturi, C. A., Ivy, A. C., ibid., 1938, v121, 61.
11. Brauer, R. W., J. Am. Med. Assn., 1959, v169, 1462.

Received September 2, 1964. P.S.E.B.M., 1965, v118.

The Antilactobacillus System of Saliva. Role of Salivary Peroxidase.* (29882)

S. J. KLEBANOFF† AND R. G. LUEBKE‡
USPHS Hospital, Department of Medicine and School of Dentistry, University of Washington, Seattle

More than 70 years ago, W. D. Miller proposed the chemico-parasitic theory of caries production(1). According to this concept, caries is initiated by decalcification of the surface of the tooth by acid and the source of the acid is the fermentation of carbohydrates by oral micro-organisms. Other processes, *e.g.*, proteolysis and chelation, also have been implicated in the pathogenesis of caries (2). However, it is still widely held that

* Supported in part by USPHS grant GM-10310 from Nat. Inst. Health.

† Research Career Development Awardee of Nat. Inst. Health (NIAMD).

‡ Supported by Training Grant 5T1 71-03 from Nat. Inst. Dental Research. Present address: College of Dentistry, Univ. of Kentucky Med. Center, Lexington.

"decalcification by acid is the initial and crucial step of the carious process"(2). Interest for many years has centered around lactobacilli as acid producing agents of possible etiological importance in dental caries. The status of lactobacilli in the etiology of caries is still controversial(2). However, because of its possible implication, there is considerable interest in a factor or factors in saliva which inhibit the growth of certain species of lactobacilli(3,4). The antilactobacillus system of saliva can be divided into a heat labile non-dialyzable component and a heat stable dialyzable component(5). Previous studies have suggested that the heat stable dialyzable component is the thiocyanate ion(6,7). The present study suggests that the heat labile non-dialyzable component is the salivary peroxidase.

Methods and materials. A stock culture of *Lactobacillus acidophilus* ATCC #4357 was maintained on lactobacillus selective medium slants (LBS medium-Baltimore Biological Laboratories). A 24-hour culture grown in lactobacillus selective broth (LBS broth-Baltimore Biological Laboratories) was centrifuged and the bacteria suspended in water to an absorbancy of 0.200-0.300 at 540 mμ. An aliquot of the standard suspension which gave a final bacterial concentration of approximately 1×10^8 cells per ml was employed in the test system.

Paraffin-stimulated whole human saliva was collected, pooled, centrifuged at 10,000 $\times$ *g* for 20 minutes and the supernatant fraction was stored at —4°. The saliva was sterilized prior to use by exposing a thin layer to ultraviolet light at 5 inches for 5 minutes. The saliva was dialyzed against distilled water overnight at 4° where indicated. Lactoperoxidase was prepared in a highly purified form from bovine milk by the method of Morrison and Hultquist(8). A preparation eluted from a column of CG 50 resin type 2 and dialyzed had a 412 mμ to 280 mμ ratio of 0.85. Myeloperoxidase was prepared by the method of Agner(9) to the end of Step 6 at which stage the preparation had a 430 mμ to 390 mμ ratio of 2.0. The peroxidase activity of the saliva and peroxidase preparations was determined by the o-dianisidine method (Worthington Biochemical Corp. Manual).

In the determination of bacterial growth 1 ml of double-strength LBS broth and a 0.2 ml aliquot of the standard suspension of organisms were added to a small test tube (10 $\times$ 75 mm). The reagents under study (see text and figures) were added and the volume made up to 2.0 ml with water. The tubes were incubated at 37° with continuous shaking in a water bath and growth of the organisms was determined turbidometrically by the increase in absorbancy at 540 mμ in a Beckman model B spectrophotometer. Readings were taken at hourly intervals for 6 hours.

Results and discussion. Fig. 1 confirms the inhibition by human mixed saliva of the growth of lactobacillus in complete growth medium (LBS broth). The addition of catalase to the reaction mixture, but not catalase heated to 90° for 10 min, decreased the inhibition of lactobacillus growth by saliva.§

Saliva contains peroxidase activity. A portion of the peroxidase activity of whole saliva is sedimented on centrifugation while the remainder is in the supernatant fraction(10). The peroxidase activity which is sedimented on centrifugation is believed to be present largely in the sedimented white blood cells. The soluble peroxidase is secreted by the salivary glands. A bovine salivary gland extract has been found to contain a protein which reacts immunologically with antisera to pure bovine milk lactoperoxidase(11). In addition, the peroxidase isolated from bovine salivary glands has spectral properties similar to those of lactoperoxidase prepared from milk(11). This suggests that the peroxidase from the bovine salivary gland and the peroxidase from bovine milk are closely similar if not identical enzymes. Lactoperoxidase has been found to have antibacterial properties. The addition of lactoperoxidase to a culture of streptococcus cremoris in skim milk or in a whey ultrafiltrate supplemented with amino acids leads to decreased growth of the organisms

§ The addition of catalase, in the concentration employed in Fig. 1, to a normal growing culture of lactobacillus had no effect on rate of growth of the organisms in the absence of saliva.

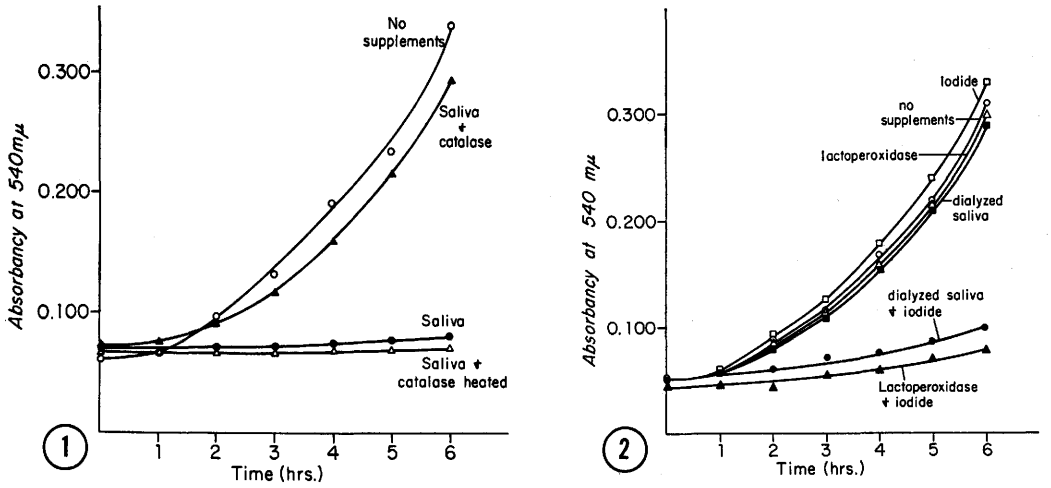

FIG. 1. Effect of catalase on antilactobacillus effect of saliva. Reaction mixture contained 1.0 ml of double-strength LBS broth, 0.2 ml of a suspension of *Lactobacillus acidophilus*, water to a final volume of 2.0 ml and supplements indicated in the Figure as follows: saliva—0.5 ml; catalase (beef liver, cryst. 150,000 U/ml, Worthington Biochemical Corp.)—7500 units; catalase—7500 units heated to 90° for 10 min.

FIG. 2. Inhibition of lactobacillus growth by lactoperoxidase and iodide. As in Fig. 1, except for supplements as follows: dialyzed saliva—0.5 ml; lactoperoxidase—20 o-dianisidine units; iodide—2 μmoles.

(12). H_2O_2 was an additional component of the system.

Saliva dialyzed against water loses its ability to inhibit the growth of lactobacilli. The antilactobacillus effect of dialyzed saliva is restored by addition of an amount of thiocyanate comparable to the amount present in saliva(6,7). This is confirmed in Table I. The antilactobacillus effect of the dialyzed saliva-thiocyanate system was completely lost by pre-heating the dialyzed saliva to 90° for 10 minutes. Table I demonstrates that addition of a purified preparation of bovine lactoperoxidase to the heated dialyzed saliva-thiocyanate system restored the antilactobacillus effect. Indeed lactoperoxidase could replace dialyzed saliva in this system. Lactoperoxidase or thiocyanate alone was without effect at the concentrations employed. Lactoperoxidase can be replaced by myeloperoxidase. The number of o-dianisidine units of peroxidase activity present in the mixed human saliva employed in Fig. 1 was 20. A comparable number of o-dianisidine units of peroxidase activity were present in the lactoperoxidase-thiocyanate system in Table I. Similarly the concentration of thiocyanate employed in the lactoperoxidase-thiocyanate system was of the same order as that present in the salivary system. The normal range of thiocyanate concentration in saliva is 1-27 mg%(7). The amount of thiocyanate employed in Table I is equivalent to the amount which would be added in 0.5 ml of saliva (the amount employed in Fig. 1) if the thiocyanate concentration of the saliva was 26 mg%. The amount of thiocyanate added in the lactoperoxidase-thiocyanate system could be decreased to the equivalent of a salivary con-

TABLE I. Inhibition of Lactobacillus Growth by Lactoperoxidase and Thiocyanate.

As in Fig 1, except for addition of the supplements indicated below as follows: saliva-0.5 ml; dialyzed saliva-0.5 ml; sodium thiocyanate-2.5 μmoles; dialyzed saliva heated to 90° for 10 min.-0.5 ml; lactoperoxidase-20 o-dianisidine units; myeloperoxidase-20 o-dianisidine units.

Supplements	Absorbancy at 540 mμ 0 hr	6 hr
None	.040	.300
Saliva	.045	.065
Dialyzed saliva	.045	.280
Idem. + thiocyanate	.050	.070
Dialyzed saliva heated + thiocyanate	.070	.340
Idem. + lactoperoxidase + thiocyanate	.050	.065
Lactoperoxidase + thiocyanate	.050	.055
Lactoperoxidase	.045	.310
Thiocyanate	.040	.300
Myeloperoxidase + thiocyanate	.045	.050

centration of 0.1 mg% without a complete loss of inhibitory effect.

Of a series of anions tested for antibacterial activity in the presence of dialyzed saliva, only iodide was effective in addition to thiocyanate(6,7). Fig. 2 confirms the antibacterial effect of iodide in the presence of dialyzed saliva and also demonstrates the antibacterial effect of iodide in the presence of an amount of lactoperoxidase equivalent to the amount present in 0.5 ml of saliva. The amount of iodide employed (250 μg per tube) was considerably in excess of the amount present in 0.5 ml of saliva (0.02-0.12 μg taking a range in saliva of 0.0035-0.024 mg/100 ml(7)). No effect of iodide was observed at a concentration equivalent to that of saliva.

The mechanism of the inhibition of lactobacillus growth by saliva is unknown. Thiocyanate is oxidized by peroxidase and H_2O_2 and the relationship of thiocyanate oxidation to microbial growth inhibition is under investigation. The demonstration that myeloperoxidase can substitute for lactoperoxidase in the antilactobacillus system suggests the possibility that myeloperoxidase present in leucocytes might exert an antibacterial effect by a mechanism similar to that reported here.

Summary. It has been previously reported by others that the growth of lactobacillus in complete growth medium is inhibited by saliva and that a component of the antilactobacillus system of saliva is thiocyanate ion. The inhibition of growth of lactobacillus by saliva is decreased by catalase. The heat labile non-dialyzable component of the antilactobacillus system of saliva can be replaced by an amount of lactoperoxidase equivalent to the amount present in saliva. This suggests that the antilactobacillus system of saliva contains at least two components, peroxidase and thiocyanate ions. Iodide at a concentration in excess of that present in saliva can replace thiocyanate in this system.

The excellent technical assistance of Mrs. Aline Alenty and Mrs. Ann Waltersdorph is very gratefully acknowledged.

1. Miller, W. D., The Micro-organisms of the Human Mouth. S. S. White Dental Mfg. Co., Philadelphia, 1890.

2. Burnett, G. W., Scherp, H. W., Oral Microbiology and Infectious Disease. 2nd ed., Williams & Wilkins Co., Baltimore, 1962.

3. Clough, O. W., J. Dent. Res., 1934, v14, 164.

4. Clough, O. W., Bibby, B. G., Berry, G. P., ibid., 1938, v17, 493.

5. Zeldow, B. J., ibid., 1959, v38, 798.

6. Dogon, I. L., Kerr, A. C., Amdur, B. H., Arch. Oral Biol., 1962, v7, 81.

7. Zeldow, B. J., J. Immunol., 1963, v90, 12.

8. Morrison, M., Hultquist, D. E., J. Biol. Chem., 1963, v238, 2847.

9. Agner, K., Acta Chem. Scand., 1958, v12, 89.

10. Nickerson, J. F., Kraus, F. W., Perry, W. I., Proc. Soc. Exp. Biol. and Med., 1957, v95, 405.

11. Morrison, M., Allen, P. Z., Biochem. Biophys. Res. Comm., 1963, v13, 490.

12. Jago, G. R., Morrison, M., Proc. Soc. Exp. Biol. and Med., 1962, v111, 585.

Received September 2, 1964. P.S.E.B.M., 1965, v118.

Effect of Ethionine on Total Blood Ammonia in Rats.* (29883)

K. KOWALEWSKI

Surgical-Medical Research Institute, University of Alberta, Edmonton, Alberta, Canada

Amino nitrogen is normally disposed of in the liver by way of glutamic acid and the production of urea. If the deamination system of the liver is damaged, some of the amino nitrogen appears in the blood as ammonium ion(1). Endogenous ammonia intoxication(2) is due to the inability of the liver to detoxify endogenous ammonia, and exogenous ammonia intoxication is a result of the failure of metabolizing extrahepatic ammonia, produced by gastro-intestinal bacterial activity(3). In both cases there is too much ammonia in the peripheric circulation.

* This project was supported by University of Alberta Medical Research Fund. Mrs. M. Foster and Mrs. E. Dimitrov are thanked for technical assistance.