

53 to 57%. In the experimental period the activity in the control experiments varied from 61 to 56%, while the activity of the morphine experiments varied from 89 to 94%. This great increase in activity continuing throughout the 200 minute period which followed morphine injections is characteristic of each experiment.

Summary. 1. The immediate effect of morphine on colon motility resulting from the subcutaneous injection of $\frac{1}{4}$ grain is essentially a marked increase in the tone, with a diminution, frequently a disappearance of quiet periods. 2. Alternating with the periods of high tone is an exaggeration of Type I components of Type II contractions. 3. As far as the colon motility is concerned the dog shows no increased tolerance to $\frac{1}{4}$ grain of morphine given daily. 4. No evidence of a cumulative effect on colon motility was seen to follow daily injections of $\frac{1}{4}$ grain of morphine for a period of 60 days. 5. Twenty hours after subcutaneous injection of $\frac{1}{4}$ grain morphine the colon motility is practically the same as that prior to the injection.

11595

Inhibitory Effect of Quaternary Ammonium Synthetic Detergent on Metabolism of Dental Plaque Material.*

BENJAMIN F. MILLER, JOHN A. MUNTZ AND SIGMUND BRADEL.
(Introduced by G. F. Dick.)

From the Walter G. Zoller Memorial Dental Clinic and the Department of Medicine, University of Chicago.

The carious lesion begins almost invariably on the outer surface of the tooth, *i.e.* under plaque material, and works its way toward the interior. It appears likely that the metabolism of dental plaque material† is intimately associated with the pathogenesis of dental caries. Therefore, it would seem possible to prevent or retard the formation of early lesions if the metabolism of the plaque material could be inhibited completely, or even if it could be reduced to a sub-

* A preliminary report of these experiments was presented before the Subsection on Dentistry of the American Association for the Advancement of Science, Columbus, Ohio, December 30, 1939 (Abstracted in *J. Am. College of Dentists*, 1940, 7, 80).

† "Plaque material" is the thin film, composed of bacteria embedded in organic substance, which remains on the surface of the teeth after swabbing with cotton and washing with a stream of water.

clinical level. This paper presents some experiments to that end.

It was shown previously by one of us¹ that experimental rat caries is inhibited markedly by the addition of fluoride or iodoacetate to the food and water. Presumably, these enzyme inhibitors interfere with the metabolic activities of the surface flora of the rat molars. However, the local or systemic toxicology of these compounds for man is not fully understood. Therefore, it has seemed desirable to look for compounds, capable of producing the same effects, which are less toxic and which possess the additional property of rapid penetration through the dense mass of human plaque material. The quaternary ammonium type of germicidal, wetting and detergent compound described by Domagh² appeared to possess these qualities. This compound, alkyldimethylbenzyl ammonium chloride,[‡] reduces the surface tension of water even at very low concentrations, acts as a wetting and cleansing agent, and has relatively low toxicity for tissues.³ Also, as shown by Miller, Baker and Harrison⁴ it exhibits a remarkably potent inhibitory action on

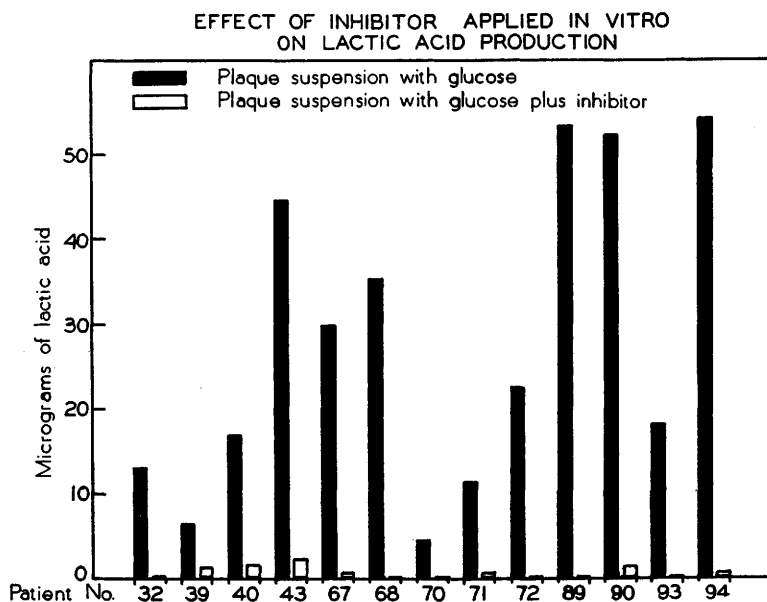


FIG. 1.

¹ Miller, B. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 389.

² Domagh, G., *Deutsche Med. Wochenschr.*, 1935, **61**, 829.

[‡] We are indebted to the Alba Pharmaceutical Co. for samples of this compound.

³ Wright, L. T., and Wilkinson, R. S., *Am. J. Surg.*, 1939, **44**, 626.

⁴ Miller, B. F., Baker, Z., and Harrison, R. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 705.

the metabolism of pure cultures of microorganisms usually found in dental plaque material and lesions of early dental caries. These authors demonstrated that 2 to 25 μg of the compound inhibit the glycolysis or respiration of a billion bacterial cells within 5 to 10 minutes, and that the full effect could be obtained in dilutions as low as 1 part in 30,000.

In this paper, the action of alkyl-dimethylbenzyl ammonium chloride on the metabolism of the total plaque material is studied by *in vitro* and *in vivo* experiments.

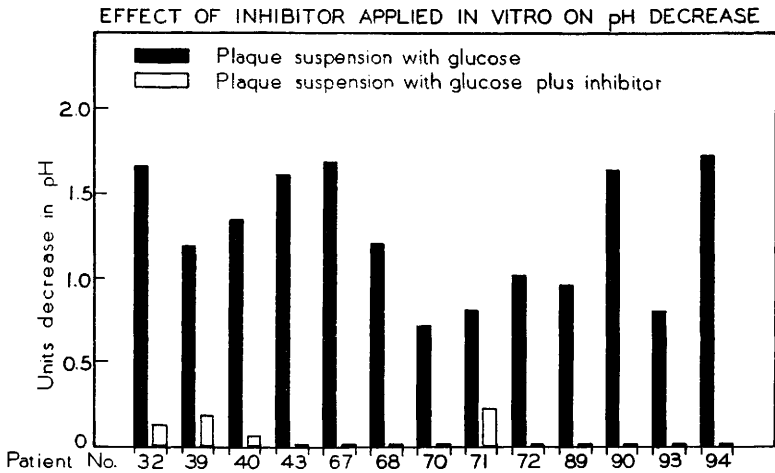


FIG. 2.

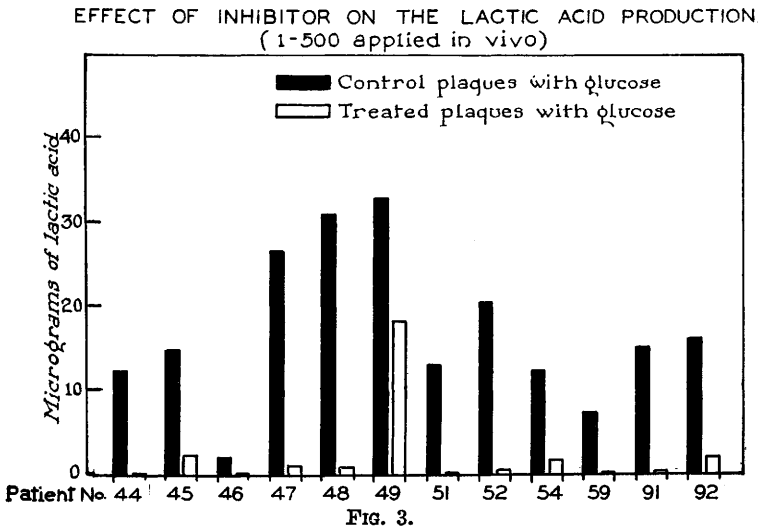


FIG. 3.

In view of the present state of our knowledge of dental caries, it must be emphasized that no definite conclusions concerning the prevention or treatment of the disease can be drawn from this report. The efficacy of any agent or procedure proposed for the cure, prevention or mitigation of dental caries can be determined only on the basis of results obtained from clinical studies.

Experimental. For the *in vitro* experiments, plaque material§ from 10 to 15 areas on the buccal surfaces of premolar and molar teeth was removed and homogenized in 0.6 to 0.8 cc of water. The plaque suspension was divided into 3 portions. Initial pH and lactic acid content were determined on one portion; the other 2 were incubated at 37°C for 30 minutes, one containing 0.5% glucose alone, and the other 0.5% glucose plus sufficient aqueous detergent to give a final concentration of one part in 3000 (approx. M/1000). Lactic acid was determined by the method of Miller and Muntz;⁵ the pH drop was measured with a micro glass electrode. (It was demonstrated that neither determination was affected by the quantities of detergent employed.)

The lactic acid production, with and without the alkyldimethylbenzyl ammonium chloride, is shown in Fig. 1. It is apparent that this compound, in the concentration employed, suppresses very effectively aerobic glycolysis by the plaque material. Similarly, at the same concentration, it prevents any significant decrease in pH, as demonstrated in Fig. 2.

That alkyldimethylbenzyl ammonium chloride reacts with the plaque material very rapidly and tenaciously is demonstrated by the results of cases 89 and 90 in Fig. 1. In these experiments the compound was added to the homogenized plaque substance in the usual way; the suspension was centrifuged immediately, and the precipitated plaque material resuspended and incubated in fresh glucose solution which did not contain the inhibitor. It was found that the lactic acid production was completely suppressed.

In vivo application of alkyldimethylbenzyl ammonium chloride. In these experiments control plaque material was removed from 5 to 6 areas from one lower quadrant of the mouth, and incubated for 30 minutes in 0.5% glucose. The lactic acid and pH drop were determined as before. To corresponding areas on the other side of the subject's mouth, a 1:500 solution of alkyldimethylbenzyl ammonium chloride (approximately 0.006 molar) was applied with

§ The plaque material was obtained from both caries-free and caries-active patients.

⁵ Miller, B. F., and Muntz, J. A., *J. Biol. Chem.*, 1938, **126**, 413.

a cotton swab directly to the plaque areas for 2 minutes. When the swab was removed the subject was permitted to talk and salivate for 8 to 10 minutes. The treated plaques were then removed, placed immediately in 0.5 cc of the glucose solution, and incubated in the same manner as the untreated controls. The results in Fig. 3 demonstrate that the application of the detergent *in situ* reduced the capacity of the plaque material to produce lactic acid upon subsequent incubation in glucose solution. In 11 of the 12 cases the inhibition was essentially complete.

To ascertain the extent to which the alkyldimethylbenzyl ammonium chloride becomes fixed to the plaque material before removal of the plaques, the following experiment was performed: plaque substance was treated *in vivo* as above, removed and suspended uniformly in water, and immediately thereafter centrifuged at high speed. The precipitate was then resuspended in fresh glucose solution and incubated. The results, Nos. 91 and 92 in Fig. 3, show that the inhibition is as complete as in the other experiments. It was shown further that the supernatant fluid from the first suspension did not contain an inhibitory concentration of the detergent. This experiment demonstrates that the effect observed *in vivo* is extremely rapid, and is due apparently to a tenacious adsorption of the inhibitory compound on the plaque material.

Summary. Dental plaque material was suspended in glucose solution and incubated with the quaternary ammonium wetting and detergent compound, alkyldimethylbenzyl ammonium chloride. One part of this compound in 3,000 (approximately M/1000) completely inhibited the production of acid by the plaque material.

In another type of experiment, a 1:500 solution of the detergent was applied to the plaque material *in situ* for 2 minutes. The plaque material was removed 10 minutes later, and did not produce appreciable quantities of acid when it was then incubated in glucose solution.