Experimental Dental Caries. XIV. Further Studies on Effect of Certain Quinones.* (17537)

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The investigations on the inhibitory action of certain quinones, with and without vitamin K activity, on acid formation *in vitro*, as well as the contradictory findings on the effect of vitamin K in the prevention of dental caries have been recently reviewed by Dam.(1) In order to study further this problem we(2) carried out an experiment in which was studied the effect of 3 quinones with, and 3 without vitamin K activity, on dental caries activity in hamsters. The results showed that none of the compounds tested induced any significant decrease of dental caries. However, in the discussion of the results obtained we indicated the advisability of testing in a future experiment the effect on caries activity of various quinones and related compounds having different ability to inhibit acid production *in vitro*, and using larger amounts of the compounds than those previously used. It was stated that although in case of obtaining favorable results the implications of such an experiment would not favor the use of quinones, due to their toxicity, in the prevention of dental caries, a study of this kind should indicate how far the ability of certain quinones to inhibit acid production *in vitro* would be paralleled by a corresponding ability of these compounds to decrease caries activity. Therefore the findings thus obtained should contribute to a proper evaluation of the actual relationship between acid production and the development of caries. We are here reporting the results of such an experiment, which was carried out after the inhibitory power of certain quinones on acid production *in vitro* had been determined.

Experimental. First, an *in vitro* experiment was carried out in order to determine the inhibitory power of a series of quinones and related compounds, with and without vitamin K activity, on acid formation by certain oral bacteria. As a source of acidogenic bacteria was used pure cultures of 2 strains of lactobacilli (L_1 and L_2) and streptococci (S_1 and S_2) isolated from the deepest portion of a human carious lesion. The substrate used for the growth of streptococci consisted of casein-peptone broth plus 2% glucose. For the lactobacilli was used the same medium supplemented with yeast autolysate. The effect of 13 quinones and related compounds, 6 with and 7 without vitamin K activity, on the growth of the isolated bacteria was tested. 2-methyl-1,4-naphthoquinone (menadione) was used in the amount of 2 mg per 100 cc of substrate; the other substances were tested in the same concentration calculated on a molecular basis. In order to avoid the autoclaving of the compounds to be tested, they were added to the sterilized substrate in the tubes, dissolved either in sterile water or in an easily evaporable organic solvent such as acetone. The tubes were inoculated and then all were incubated with occasional shaking at 37°C for 18 to 20 hours, at which times the pH of the tubes, including the non-inoculated and inoculated controls, was determined with a glass-electrode potentiometer. The results obtained are presented in Table I.

It can be seen from this table that of the quinones derivatives with vitamin K activity methylnaphthoquinone, methylnaphthohydroquinone and methylnaphthohydroquinone disuccinate markedly inhibited acid formation by lactobacilli, and afforded complete inhibition of acid production by streptococci. On the other hand, the tetrasodium and dicalcium salts of methylnaphthohydroquinone diphosphate, as well as the disodium salt of methylnaphthohydroquinone disulfate, all of which have the same vitamin K activity as the first 3 substances, did not inhibit acid production by any of the bacteria tested. This marked inhibitory power of methylnaph-

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1. Dam, H., *Vitamins and Hormones*, 1948, VI, 27.

2. Granados, H., Glavind, J., and Dam, H., *Acta Path. et Microbiol. Scand.*, 1949, v26, 597.

TABLE I.
Comparison of Inhibition by the Compounds Tested of Acid Formation by Lactobacilli and Streptococci.

Kind of acidogenic bacterium		Lactobacillus L_1	Lactobacillus L_1	Lactobacillus L_2	Streptococcus S_1	Streptococcus S_1	Streptococcus S_2
No. of bacteria inoculated per tube (10 cc)		1,200	200,000	400	4,400	20,000	10,000
Incubation time (hr)		20	18	20	20	18	18
pH of non-inoculated tubes		6.95	6.86	6.95	6.89	6.89	6.89
Compounds added to inoculated tubes	Vit. K activity	pH of inoculated and incubated tubes					
Control		4.70	4.4	4.5	4.2	4.3	4.3
2-methyl-1,4-naphthoquinone (menadione)	+		6.3	6.4	6.9		
2-methyl-1,4-naphthohydroquinone	+		5.7		6.9		
2-methyl-1,4-naphthohydroquinone diphosphate tetrasodium salt (32.02% H_2O)	+		4.6		4.2	4.3	4.3
2-methyl-1,4-naphthohydroquinone diphosphate dicalcium salt (9.5% H_2O)	+	4.72	4.6	4.5	4.2	4.3	4.3
2-methyl-1,4-naphthohydroquinone disulfate disodium salt, $2H_2O$	+		4.6		4.2		
2-methyl-1,4-naphthohydroquinone disuccinate	+		5.6	6.4	6.9		
Benzoquinone	—	4.75	4.5	4.7	4.2	4.4	4.3
Hydroquinone	—	4.80	4.8	4.8	4.2	4.4	4.3
Quinhydrone	—	5.41		4.5		4.5	4.3
1,2-naphthoquinone	—	4.69		4.3		4.6	4.4
2-hydroxy-1,4-naphthoquinone	—	4.50		4.1		5.3	5.1
2,3-dichloro-1,4-naphthoquinone	—	5.29	4.5	6.4	4.3	6.0	4.8
Anthraquinone-2-sulphonic acid	—	4.62	4.7	4.5	4.2	4.4	4.3

TABLE II.
Compounds Added and Caries Activity in the 5 Groups.

	Group 1 5 +4	Group 2 5 +5	Group 3 5 +5	Group 4 5 +4	Group 5 5 +5
Kind and Amt. of each substance added to 100 g of cariogenic diet	Control	280 mg 2-methyl-1,4-naphthohydroquinone diphosphate dicalcium salt (9.5% H_2O)	223 mg 2-methyl-1,4-naphthohydroquinone disuccinate	168 mg anthraquinone-2-sulphonic acid	148 mg 2-3-dichloro-1,4-naphthoquinone
Vit. K. activity		+	+	—	—
Inhibits acid production *in vitro*		—	+	—	+
Av. No. of molars affected	9.6	9.6	9.2	8.4	8.3
Stand. dev.	±0.6	0.3	0.4	0.6	0.1
Av. No. of carious lesions	15.9	15.4	15.5	12.6	13.8
Stand. dev.	±2.0	1.0	2.0	1.6	1.1
Avg caries scores	8.6	8.7	13.4	15.2	7.8
Stand. dev.	±1.1	0.8	4.3	6.2	0.2

thoquinone, methylnaphthohydroquinone and methylnaphthohydroquinone disuccinate on acid production agree with the marked antibacterial effects *in vitro* of the same compounds reported by other investigators.(3,4) Of the 7 compounds with none or negligible vitamin K activity, only dichloronaphthoquinone showed in some cases a considerable inhibitory power on acid production, and hydroxynaphthoquinone inhibited slightly acid formation by streptococci.

Considering the results of these *in vitro* studies, in order to compare the effects of quinone derivatives, with and without vitamin K activity, having marked or none inhibitory action on acid formation), 3. anthraquinone-the following compounds were used in the caries experiments: 1. dicalcium salt of 2-methyl-1,4-naphthohydroquinone diphosphate (with vitamin K activity and without inhibitory action on acid production), 2. 2-methyl-1,4-naphthohydroquinone disuccinate (with vitamin K activity and with marked inhibitory action on acid formation), 3. anthraquinone-2-sulphonic acid without vitamin K activity and without inhibitory action on acid formation, and 4. 2-3-dichloro-1,4-naphthoquinone (without vitamin K activity and with inhibitory action on acid formation).

Fifty newly weaned hamsters from an inbred colony were littermate distributed into 5 groups (5 males and 5 females in each), and were reared in screen bottom cages without bedding for 100 days on the following basal diet to which was added in each case the quinone derivative indicated in Table II: Salt mixture† 1%, alfalfa meal 2%, Brewer's yeast 5%, powdered whole milk 22%, finely ground yellow corn 25%, and finely powdered sucrose 45%. All the quinone derivatives were supplied in equal molecular concentrations. The groups were given water *ad libitum*, and were weighed weekly. On completion of the 100-day experimental period the animals were sacrificed and autopsied. The molars were prepared for examination and the carious lesions were recorded and scored as previously.(5)

Results. At the levels given most of the

† The salt mixture used was McCollum's Salt Mixture No. 185, supplemented with 13.5 mg KI, 139 mg $CuSO_4$, $5H_2O$, and 556 mg $MnSO_4$, $4H_2O$ per 100 g.

3. Armstrong, W. D., Spink, W. W., and Kahnke, J., PROC. SOC. EXP. BIOL. AND MED., 1943, v53, 230.

4. Atkins, P., and Ward, J. L., *Brit. J. Exp. Path.*, 1945, v26, 120.

5. Granados, H., Glavind, J., and Dam, H., *Acta Path. et Microbiol. Scand.*, 1948, v25, 453.

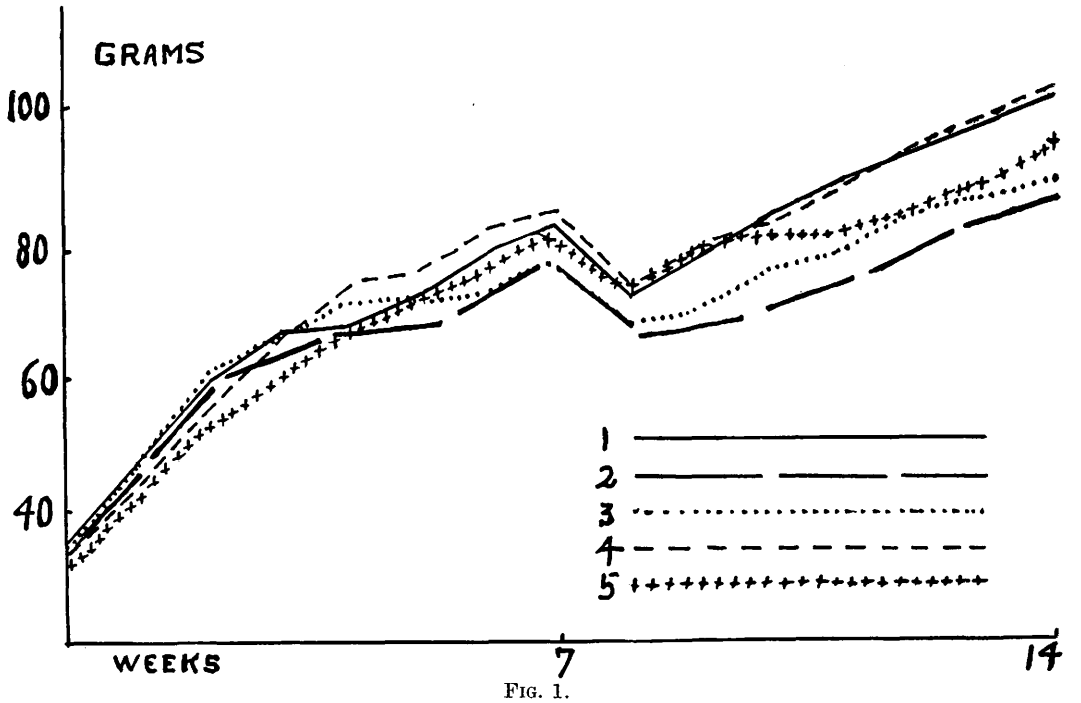

FIG. 1.

Average growth curves of the 5 groups. Group 1, control. Group 2, dicalcium salt of 2-methyl-1,4-naphthohydroquinone diphosphate. Group 3, 2-methyl-1,4-naphthohydroquinone disuccinate. Group 4, anthraquinone-2-sulphonic acid. Group 5, 2-3-dichloro-1,4-naphthoquinone.

quinones derivatives used showed to be toxic through an inhibition of growth. Fig. 1 shows that in increasing order dichloronaphthoquinone (group 5), methylnaphthohydroquinone disuccinate (group 3), and dicalcium salt of methylnaphthohydroquinone diphosphate (group 2) were toxic. On the other hand, anthraquinone sulphonic acid (group 4) did not induce any inhibition of growth as compared with the control (group 1). Table II shows, besides the compounds used, their vitamin K activity and their ability to inhibit acid production, the caries activity of the 5 groups. Beneath the average number of carious molars, carious lesions, and caries scores are presented the standard deviations of the means. The results presented in this table show that none of the quinone derivatives tested decreased the incidence or extent of caries. On the other hand, the higher average caries scores exhibited by groups 3 and 4 are not significant since these increased caries scores were due only to the exceptionally high caries activity of one animal in each of these groups. This is clearly seen from the much higher standard deviations of the caries scores in these two groups.

Discussion. This experiment confirms the negative findings of our previous study on this subject:(2) dicalcium salt of methylnaphthohydroquinone diphosphate and dichloronaphthoquinone were used in both experiments, but in none of them these compounds were able to decrease caries activity, in spite of the fact that in the present study the substances were used in amounts four times higher than those used in the first experiment. Furthermore, although most of the quinone derivatives used in the previous(2) and present studies have shown to inhibit considerably acid formation(6-10) and to have antibac-

6. Fosdick, L. S., Fancher, O. E., and Calandra, J. C., *Science*, 1942, v96, 45.

7. Armstrong, W. D., and Knutson, J. W., PROC. SOC. EXP. BIOL. AND MED., 1943, v52, 307.

8. Fancher, O. E., Calandra, J. C., and Fosdick, L. S., *J. D. Res.*, 1944, v23, 23.

9. Calandra, J. C., Fancher, O. E., and Fosdick, L. S., *J. D. Res.*, 1944, v23, 31.

terial effects(3-4) *in vitro,* none of them decreased caries activity. Likewise, except dichloronaphthoquinone, all the other substances used in both experiments are readily soluble in water, while methylnaphthoquinone is very slightly soluble. Moreover, of the compounds tested in the present experiment methylnaphthohydroquinone disuccinate deserves special mentioning; it is easily soluble in water, inhibits markedly acid production, and has a definite antibacterial effect.(4) However, in spite of these properties it did not decrease at all the incidence or extent of carious lesions.

Assuming that the quinones tested should have had *in vivo* the same action as they showed *in vitro,* the results of the present studies might be interpreted in various ways. 1. Presuming that the initial lesion in dental caries is primarily due to disintegration of the mineral part of the enamel by acids produced by bacteria in the dental plaque, it could be said that the quinone derivatives tested did not decrease caries activity because they were unable to penetrate the dental plaque, and there exert their inhibitory action on acid formation. However, there have been made only a few studies on the degree of, and factors influencing the penetration of acid-formation inhibiting compounds into the dental plaque *in vivo.* Muntz and Miller(11) have studied the inhibition by certain compounds of the bacterial metabolism of intact dental plaques and of homogenized plaque material, as well as the degree of permeability of the plaques to various substances. They found that the metabolic activity of the intact plaque is inhibited more slowly than that of an equivalent quantity of homogenized plaque material, and that certain very diffusible substances such as urea and glucose penetrated intact plaques slowly. On the other hand, in a previous study on the effect of dietary lactic acid on dental caries activity (given in the food or in the water) we(12) found clear signs of acid action, combined or not with caries, at the very bottom of the occlusal fossae. This showed that the dietary lactic acid at the concentration given had actually penetrated the intact dental plaques. These facts indicate the complexity of the problem and the need for more fundamental research on the ability to penetrate the intact dental plaque of various substances known to influence the development and/or progress of caries.

2. Another factor which should be considered is that the acid-formation inhibiting power of the quinone derivatives used may have been annulled by the action of some other substances, either of dietary or systemic origin. A 3rd possibility is that the quinone compounds at the levels given, both in the present and in the preceding experiments,(2) may not have encountered in the oral environment sufficient water available for their dissolution and subsequent action. A 4th possibility is that the quinone derivatives which inhibited acid production *in vitro* may have also, at the concentrations given, inhibited acid production in the mouth, inside and outside the dental plaque. In such a case, since there was no decrease of caries activity in any of the groups which were given quinone compounds, this would mean that acid formation should not play any primary role in the development of dental caries since its inhibition would not decrease the incidence of the disease. However, none of the foregoing considerations goes beyond the field of speculation since most of the fundamental processes related to dental caries which take place in the organism, inside and outside the oral cavity, have not yet been adequately and sufficiently studied, and therefore they are not as yet well understood.

Thus, the various studies(6-10) *in vitro* carried out so far, using saliva-glucose mixtures, have demonstrated beyond doubt the acid-formation inhibiting action of a good number of quinone derivatives, with and without vitamin K activity. The first part of the present studies has shown once more such an inhibitory power of certain quinone compounds. Furthermore, the antibiotic effects of various quinone derivatives on a good number of bacteria permanently found in the

10. Fosdick, L. S., and Calandra, J. C., *J. D. Res.,* 1947, v26, 309.

11. Muntz, J. A., and Miller, B. F., *J. D. Res.,* 1943, v22, 73.

12. Granados, H., Glavind, J., and Dam, H., *J. D. Res.,* 1949, v28, 282.

oral cavity have also been demonstrated. (3,4,13,14) On the other hand, the few clinical studies on the effect of 2-methyl-1,4-naphthaquinone incorporated into chewing gum, on caries activity have been contradictory since the decrease in incidence of new carious lesions reported by Burrill and coworkers(15) could not be substantiated by the U. S. Professional Service Schools.(16) Moreover, the previous experimental investigations carried out in rats(17) and hamsters,(2) as well as the present studies, on the effect of quinone derivatives with and without vitamin K activity, in the control of dental caries have all been negative. Therefore, an impartial analysis of the results obtained in clinical and experimental studies on this subject, demonstrate that vitamin K active compounds or other quinone derivatives do not show any promise of controlling caries, and therefore the use of these compounds as a control measure against human dental caries should be discouraged. Certain previous suggestions on the possible beneficial effect of vitamin K in the control of caries(18) were the product of theoretical considerations which have not been substantiated by actual experiments.

Summary. Further studies on the effect of different quinone derivatives with varying degrees of acid-formation inhibiting power, as determined *in vitro,* and with or without vitamin K activity, on dental caries activity were carried out in hamsters. In these studies the following quinone derivatives were tested: Dicalcium salt of methylnaphthohydroquinone diphosphate, methylnaphthohydroquinone disuccinate, anthraquinone sulphonic acid, and dichloronaphthoquinone. The compounds were used in higher amounts than those used in the previous experiment.

The results showed that none of the quinone derivatives tested exerted beneficial effect against dental caries activity, as compared with the control group. Thus the present experiment confirms the negative findings of previous studies, clinical as well as experimental, on this subject, and indicates that the use of vitamin K active compounds or other quinone derivatives as a control measure against human dental caries should be discouraged. Furthermore, some other implications of these studies have been discussed.

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The Treatment of *Trichomonas vaginalis* Vaginitis with Aureomycin. (17538)

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Since we(1) had been successful in treating amebiasis with aureomycin it was thought possible that this antibiotic might be useful in other parasitic infestations. It was recalled that many amebicidal preparations are effective against *T. vaginalis.* Subsequent *in vitro* studies revealed that aureomycin is likewise trichomonicidal. Therefore, it was decided to investigate the action of the local application of aureomycin into the lower female genital tract in *T. vaginalis* vaginitis. A powder for vaginal insufflation was prepared by adding

1. McVay, L. V., Laird, R. L., and Sprunt, D. H., *Science,* 1949, v109, 590.