No. 17 of early embryonic development. Its transient nature may be the reason for the denial, or questioning, of its existence in the various references mentioned. Nevertheless, it is the contention of this paper, based upon the study of many sectioned embryos during this particular period of development that

a. A neurenteric canal actually does exist in fact as a tubular connection between the neurocoel and the hindgut of the embryo of *Rana pipiens.*

b. The cells of the floor of the neurocoel, of the floor of the neurenteric canal, and of the roof of the archenteron are of the same type and are, in all probability, continuous in early development.

c. The post-anal gut and the neurenteric canal are not synonymous but may be continuous.

d. The neurenteric canal is very small, tubular, and transient. It is formed by the depression of the posterior medullary plate along with the dorsal lip of the blastopore, concurrently with coalescence of the posterior neural folds and the lateral lips of the same blastopore which together form the sides of the temporary neurenteric canal.

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Effect of Quinones on Acid Formation in Saliva.

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Fosdick, Fancher and Calandra[1] noted that a saliva-glucose mixture containing 1 mg per 100 cc of synthetic vitamin K (2-methyl-1,4-naphthoquinone) "forms no acid in a 4-hour incubation period, while the same mixture will produce up to 2 m.eq. of acid under the same conditions in the absence of the vitamin K." These authors emphasized that the effect on bacterial metabolism observed by them to be a property of synthetic vitamin K and discussed the possible uses of this compound in the prevention of dental caries.

The possibility suggested itself to us that the bacterial inhibitory action of synthetic vitamin K in saliva might be due to the fact that this compound is a quinone and that its antibacterial property has no actual relation to its vitamin K activity. It was already known[2] that benzoquinone possesses a substantial bacteriostatic ability. Recently reports[3,4] have appeared of a remarkable bacteriostatic effect exerted *in vitro* by certain dimethoxy quinones related structurally to fumigatin. Fumigatin (3-hydroxy,4-methoxy,2:5-toluquinone), produced by *Aspergillus fumigatus,* in itself possesses a definite ability to inhibit the multiplication of several gram-positive organisms.[5]

All points in Fig. 1 referring to a particular quinone were determined with solutions incubated concurrently using the same lot of pooled saliva to which 10% of its weight of glucose had been added (recommendation of Fosdick *et al.*[1]) Other experiments demonstrated that essentially the same results were obtained when only 2% of glucose was added to the saliva. Those quinones which were not sufficiently soluble in water were made up in alcohol, or chloroform in the case of 2,6-dimethoxy-benzoquinone, so that equal volumes contained equal molecular amounts of the several substances. The required volumes (0.05 to 1.0 cc) of these solutions were added to dry 25 cc volumetric flasks and the solvent evaporated under reduced pressure so that a

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1 Fosdick, L. S., Fancher, O. E., and Calandra, J. C., *Science,* 1942, **96**, 45.

2 Graham-Smith, G. S., *J. Hyg.,* 1919, **18**, 1.

3 Oxford, A. E., *Chemistry and Industry,* 1942, **61**, 189.

4 Oxford, A. E., *J. Chem. Soc.,* 1942, p. 577.

5 Oxford, A. E., and Raistrick, H., *Chemistry and Industry,* 1942, **61**, 128.

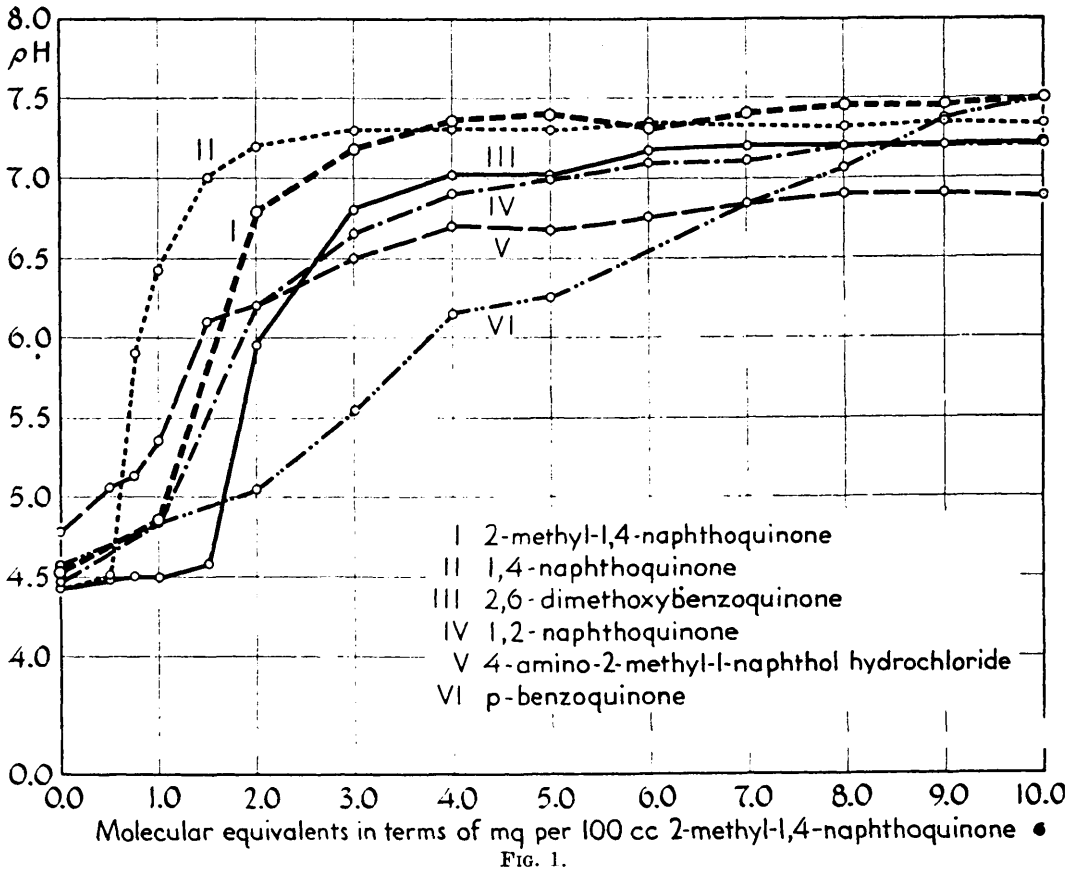

FIG. 1.

Inhibition of acid formation in saliva-glucose mixtures incubated with equal molecular concentrations of quinones.

film of the quinone was deposited over the wall of the flask. The substances soluble in water† were transferred in measured volumes (0.05 to 1.0 cc) to dry 25 cc volumetric flasks as aqueous solutions of the same molecular concentration as the alcoholic solutions referred to above. Sufficient water was added to all flasks to make the total volume of added water 1 cc. Immediately after adding 15 cc of the saliva-glucose mixture to each flask; and after the glass stoppers were tightly inserted, the flasks were incubated with constant shaking at 37° for 4 hours. Although some of the quinones are not directly soluble in water to a degree equivalent to the highest amounts added to the saliva, it was observed, in the case of clear broths and synthetic media, that the technic described above gave clear solutions of the quinones almost at once after the agitation was started. The flasks were allowed to cool to room temperature in a refrigerator and then shaken. The pH of the contents was determined, using a glass-electrode instrument, immediately after opening each flask. The quantities of 2-methyl-1,4-naphthoquinone used were in definite increments in terms of milligrams per 100 cc of the final dilution. It is to be noted throughout this paper that the other substances are compared with 2-methyl-1,4-naphthoquinone on a molecular basis. The data in Table I were obtained in the manner already described using the same lot of pooled saliva-glucose mixture.

† Benzoquinone, hydroquinone, 2-methyl-1,4-naphthoquinone sodium bisulfite addition compound (Hykione), 1-2-naphthoquinone-4-potassium sulfonate, sodium anthroquinone beta sulfonate, sodium-2-methyl-1,4-naphthoquinone disulfate and 2-methyl-4-amino-1-naphthol hydrochloride (Synkamin).

The data in Fig. 1 indicate that while 1 mg of 2-methyl-1,4-naphthoquinone per 100 cc saliva effectively decreases acid formation to a degree below that required for decalcification

TABLE I.

Comparison of Inhibition of Acid Formation in Saliva-Glucose Mixtures by Substances in a Molecular Concentration Equal to 2 mg per 100 cc of 2-methyl-1,4-naphthoquinone.

Compound tested	pH
Unincubated saliva-glucose mixture	7.40
	7.40
Untreated control	4.60
	4.65
1,4-Naphthoquinone	7.35
	7.35
2-Methyl-1,4-naphthoquinone	6.90
	6.90
1,2-Naphthoquinone	6.85
	6.80
2-Methyl-1,4-naphthoquinone sodium bisulfite addition compound*	6.45
	6.45
Benzoquinone	5.60
	5.45
Tolu-*p*-quinone	5.20
	5.20
Quinhydrone	5.20
	5.15
1,2-Naphthoquinone-4-potassium sulfonate	5.15
	5.10
Hydroquinone	5.00
	5.00
2-Hydroxy-1,4-Naphthoquinone	5.00
	4.95
Tolu-*p*-hydroquinone	5.00
	4.90
Sodium-anthroquinone-beta sulfonate	4.75
	4.75
Sodium-2-methyl-1,4-naphthohydroquinone disulfate	4.65
	4.70
Methylene blue	6.95
	6.90
Sodium-2,6-dichlorobenzenone indophenol	5.00
	5.00
Neutral red	5.30
	5.15

* Abbott's "Hykinone."

of tooth structures, a concentration of upwards of 3 mg per 100 cc is required for complete inhibition of acid formation. Consideration of the data presented in this paper demonstrates that the inhibitory effect of synthetic vitamin K on acid formation in saliva, discovered by Fosdick *et al.*,[1] is merely incidental to its properties as a vitamin. It will be noted that 1,4-naphthoquinone, under the conditions of these experiments, has an antibacterial effect somewhat superior to that of 2-methyl-1,4-naphthoquinone; yet the first named substance is reported[6] to exhibit not more than 10% the vitamin K potency of the second. Furthermore, 1,2-naphthoquinone and benzoquinone whose vitamin K activities are negligible[6] are shown to possess in substantial degree an ability to interfere with acid formation from glucose by salivary organisms.

Oxford[3] has recently reported 2,6-dimethoxy benzoquinone to inhibit the growth of a certain strain of staphylococcus in dilutions of 1:400,000 and was inferior in this regard only to 4,6-dimethoxy tolu-quinone. It is to be noted (Fig. 1) that certain naphthoquinones equal or surpass, under the conditions of these experiments, the ability of 2-6-dimethoxy benzoquinone to interfere with the metabolism of salivary organisms. The results obtained (Fig. 1) with 2-methyl-4-amino-1-naphthol hydrochloride (Synkamin of Parke Davis) are to be explained by its ready oxidation to the corresponding 1,4-naphthoquinone, a fact which also accounts for its vitamin K activity.

It might be supposed that there could exist a relationship between the oxidation-reduction potentials of quinones and their relative effectiveness as bacteriostatic agents. While the information at hand will not allow this question to be settled, a consideration of the data in Table I in relation to the E_o values of the several substances makes it appear probable that some character of quinones other than their oxidation-reduction potentials is also determinant of their antibacterial properties. The data referring to the 3 oxidation-reduction indicators appended as the last 3 items of Table I were obtained in an investigation of this point. The marked effect produced on antibacterial ability by substitution in the quinoid ring is seen when one compares the results obtained with 1,4-naphthoquinone and 2-hydroxy-1,4-naphthoquinone and those obtained with 1,2-naphthoquinone and 1,2-naphthoquinone-4-potassium sulfonate.

Fosdick *et al.*[1] indicated that 2-methyl-1,4-naphthoquinone in low concentrations has no effect on bacterial growth. We found that 3 and 5 mg of this quinone per 100 saliva-glucose

[6] Thayer, S. A., Cheney, L. C., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., *J. Am. Chem. Soc.*, 1939, **61**, 1932.

mixture and corresponding amounts of benzoquinone caused a progressive decrease in the *Lactobacillus acidophilus* counts in samples withdrawn at hourly intervals over a 4-hour incubation period. The final count was reduced to about one-third the original number of organisms while that of untreated controls increased by about 30%.

Several of the naphthoquinones mentioned above have been found to inhibit in high dilution the growth of staphylococcus, streptococcus, and pneumococcus in Gladstone's medium and in broth. Quantitative data as to the bacteriostatic and lethal concentrations required against these organisms in several media are now being obtained.

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Growth of *Brucella* in a Simple Chemically Defined Medium.

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Zobell and Meyer[1] studied the metabolism of the *Brucella* in simplified media. In the absence of knowledge of the accessory growth factors required by this group only slight growth was obtained when such media were used. They were able however to show that certain strains of *Brucella* could utilize the ammonium ion as a source of nitrogen and various organic acids as a source of carbon and energy. They did not find that the simple sugars, including glucose, could serve as the sole source of carbon and energy. McNutt and Purwin[2] found that slight growth of some strains occurred with arabinose or xylose as the sole source of carbon. It is worthy of note that in these instances ingredients were used which are now known to carry various accessory growth factor substances as contaminants.

Koser, Breslove and Dorfman[3] were the first workers to obtain luxuriant growth of the *Brucella* in a chemically defined medium by the addition of known accessory growth factor substances. Their medium consisted of 17 amino acids, glucose, inorganic salts, and the appropriate accessory factors. Following the elucidation of at least some of the accessory growth factor requirements of this group it has become possible to obtain abundant growth of some strains of *Brucella* in a very simple chemically defined medium.

Eight strains of *Brucella*, 3 each of *Br. abortus* and *Br. suis*, and 2 of *Br. melitensis*, were used in this preliminary work. Five of these were stock laboratory strains obtained originally from Dr. I. F. Huddleson and from the State Department of Health, Austin, Texas. Two of the strains of *Br. abortus* were recent isolations from Texas dairy cattle, and one strain of *Br. suis* was an isolation from a human case of undulant fever one week prior to its use in this study. All strains grew well in a normal atmosphere.

The inorganic salts used were of C.P. grade. The glucose was the grade usually used in

[1] Zobell, C. E., and Meyer, K. F., *Science*, 1930, **72**, 176; *J. Infect. Dis.*, 1932, **51**, 344.

[2] McNutt, S. H., and Purwin, P., *J. Infect. Dis.*, 1931, **48**, 292.

[3] Koser, S. A., Breslove, B. B., and Dorfman, A., *J. Infect. Dis.*, 1941, **69**, 114.