

first in 0.1 N hydrochloric acid. The reaction of the serum was then adjusted so that the addition of the pepsin solution would bring the reaction to pH 6-7. The process of incubating serum globulin with pepsin was carried out at 37° C., but it was found that the hypertensive substance was produced also at 20° C.

We employed 2 mg pepsin per cubic centimeter of serum globulin. This solution after 30 minutes' incubation was precipitated with three volumes of alcohol, filtered and the filtrate evaporated *in vacuo*. This residue was then taken up in distilled water in such amounts that one cubic centimeter of the solution was equivalent to one cubic centimeter of the original globulin solution. This solution of the filtrate when injected intravenously into dogs under nembutal anesthesia in doses of 0.1 cc per kilo body weight, produced an average elevation in blood pressure of 22 mm Hg. In many of our experiments the pressor activity of this solution equalled that of renin.⁵

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THE EFFECT OF SYNTHETIC VITAMIN K ON THE RATE OF ACID FORMATION IN THE MOUTH¹

IN 1939 evidence was found that the reactions involved in the production of lactic acid in the mouth, with subsequent dental caries, are analogous to the reactions involved in lactic acid production in muscle tissue.² Furthermore, it has been shown that acid formation in the mouth may be very rapid^{3,4} and that a difference between the saliva of caries active and caries immune individuals is the rate of acid formation from sugar in the respective salivas.⁵

On the basis of this it was thought that if some non-toxic substance that would inhibit the chain of reactions involved and thus delay acid formation sufficiently so that the saliva could neutralize them could be found, it could be used to prevent caries.

After an extended search for a product which would fulfil these qualifications, it was found that synthetic

vitamin K (2 methyl 1-4 naphthoquinone) was such a substance.

In vitro experiments indicate that a synthetic vitamin K concentration of 1 mg per 100 cc of saliva, 10 per cent. in glucose, forms no acid in a 4-hour incubation period, while the same mixture will produce up to 2 mil. eq. of acid under the same conditions in the absence of the vitamin K.

Preliminary clinical experiments wherein the pH of carious lesions was measured indicate that synthetic vitamin K in the same concentration as in the *in vitro* experiments effectually inhibits acid formation. In the absence of vitamin K the acidity of the lesion may increase from pH 6.8 to pH 4 in as little as three minutes.

The synthetic vitamin K has no effect on the bacterial growth in the concentrations used, so the inhibition is not caused by any antiseptic properties. It has no effect on the conversion of phosphoglyceric acid to pyruvic acid or on the reduction of the pyruvic acid to lactic acid. However, it prevents the formation of phosphoglyceric acid from the hexose phosphates. This indicates that the synthetic vitamin K prevents the dismutation of the hexose phosphate or the conversion of the dismutation products to phosphoglyceric acid.

On the basis of the above it is quite probable that if synthetic vitamin K were incorporated in sugar candy or gum it would effectively inhibit dental caries.

It is interesting to note that vitamin K is probably one of the substances removed from the sugar-cane juice during the purification of sugar.

Clinical and laboratory studies are being continued, and the physiological effects of the ingestion of synthetic vitamin K continuously for long periods of time is being investigated.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MODIFIED WARBURG REACTION VESSEL

WITHIN recent years, the Haldane-Warburg manometric technique has been widely applied,^{1,2} and

⁵ Helmer and Page (*Proc. Soc. Exp. Biol.*, 49: 389, 1942) have verified the work of Croxatto and Croxatto. Our findings differ from theirs in the optimum pH for the production of the pressor substance by pepsin. This may be due to the longer incubation time that we employ.

¹ This investigation was made possible by a grant from the Good Teeth Council for Children.

² L. S. Fosdick, *Jour. Am. Dental Assn.*, 26: 415, 1939.

³ L. S. Fosdick, H. L. Hansen and Eppler, *Jour. Am. Dental Assn.*, 24: 1275, 1931.

methods based upon the procedure are now commonly used in the routine analytical laboratory.^{3,4,5} Despite the many advances in technique, the design of the

⁴ R. M. Stephan, *Jour. Am. Dental Assn.*, 27: 719, 1940.
⁵ L. S. Fosdick, E. E. Campaigne and O. E. Fancher, *Ill. Dental Jour.*, 10: 85, 1941.

¹ M. Dixon, "Manometric Methods as Applied to the Measurement of Cell Respiration and Other Processes." Cambridge, 1934.

² D. Burke and R. T. Milner, *Ind. Eng. Chem., Anal. Ed.*, 4: 3, 1932.

³ A. S. Schultz, L. Atkin and C. N. Frey, *Jour. Biol. Chem.*, 129: 471, 1939; *ibid.*, 136: 713, 1940.

reaction vessel can still be improved. Thus, in the determination of oxygen consumption, the accidental contamination of the contents of the vessel with alkali, which may occur when vessels of the conventional form are used^{2,6} and which results in loss of the determination, can be prevented entirely by a slight change in design as indicated in the figure.

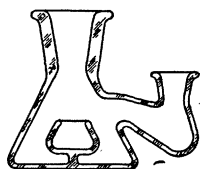


FIG. 1.

A truncated cone-shaped center cup is recommended in place of the usual cylindrical-shaped cup. The center cup in the reaction vessel of Warburg, Kubowitz and Christian⁷ has a similar shape, but it is larger and it is fused to the floor of the vessel. By placing the cup on a pedestal 5 to 7 mm high, the absorbing area of the buffered solution below the cup is considerably increased. The vessel constructed as shown in the figure may be rotated rapidly back and forth through an angle of almost 90° from the vertical without any contamination of the buffered solution with the strong caustic alkali absorbent in the center cup.

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SOME NEW USES FOR THE 2 x 2 PROJECTOR

THE advantages of the small projector now used for 2 x 2 slides are quite obvious. They are small, easily handled and less expensive than the older types designed for the standard 3½ x 4" slides, and a 200 or 300 watt bulb gives good brightness. Besides this, many photographers will welcome the ease of making Kodachrome slides with a miniature camera.

These considerations have led me to experiment with microscopic objects of fairly large size, both in sections and whole mounts. Cross sections of an embryonic dogfish fixed to a 2 x 2 slide and suitably stained serve to show the relationship of such structures as

gills, notochord, neural tube and myotomes. The brook lamprey is another promising subject, and different types of plant stems show up beautifully on the beaded screen.

Whole mounts of a wide variety of subjects may be used, from feathers to chick embryos or insects with spread wings and legs. Small leaves, cleared or skeletonized to show the veining, should offer a promising field to botanists, and I am starting some work along these lines.

In mounting material I have used the ordinary histological techniques for the most part, but prefer thin sheet plastic to glass for cover slips because it can be cut to cover the entire slide, leaving no edges to catch when filing slides. I have mounted a few sections between two sheets of this, putting them in pasteboard "Ready Mounts," but for most purposes a glass slide is preferable.

The limitations of the method are obvious. The usual mask supplied measures 23 x 33 mm, and nothing much larger than this can be used. It must also be plain that histological details seldom show up clearly because of the low magnification, but gross anatomical structures, like xylem and phloem or annual rings, can be seen perfectly. I have not yet tried making a water cell to use with living material like small tadpoles, algae or crustacea, but that should be practical.

In order to try some of the microscopic material in our slide collection I made a slide carrier of wood to take microscopic slides. While this is workable the dimensions of the 1 x 3" slide make it much harder to handle than the 2 x 2. Furthermore, unless this type of slide is used exclusively the frequent changing of carriers is a nuisance. For these reasons I am making a series of 2 x 2 slides for use in the department.

At least one supply house is making 2 x 2 Kodachrome slides from stained histological material, a method that gives beautiful results with a wide variety of subjects and at a reasonable cost.

The arc lamp projector that sends a strong beam through a microscope unquestionably gives much better results for histology, but it is an expensive and cumbersome apparatus and needs complete darkness to give satisfactory results, while the 2 x 2 projector is portable, easily operated and shows any fairly transparent material in enough light for students to take notes.

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⁵ W. R. Johnston and C. N. Frey, *Ind. Eng. Chem., Anal. Ed.*, 13: 479, 1941.

⁶ O. Warburg, E. Negelein and W. Christian, *Biochem. Zt.*, 214: 26, 1929.

⁷ O. Warburg, F. Kubowitz and W. Christian, *Biochem. Zt.*, 242: 170, 1931.

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