The initial high peak of alcohol concentration shown by subject A, seen in Fig. 1, is characteristic of rapid

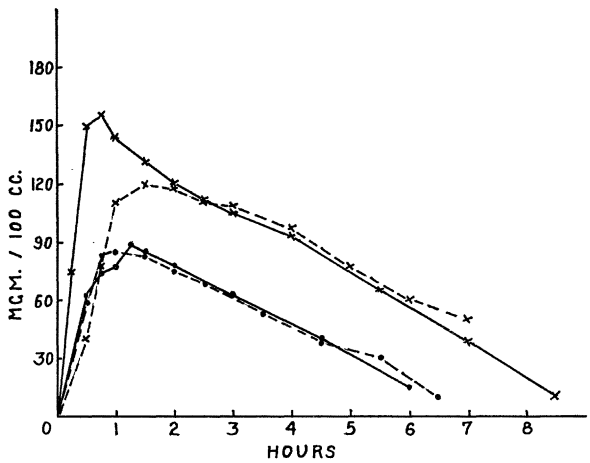

FIG. 1. Blood alcohol concentration following ingestion of diluted alcohol, as shown by the solid lines, and California Burgundy wine, shown by the broken lines. The upper curves are those of subject A, the lower curves those of subject B.

absorption, with failure of equilibration to keep pace. This is notably absent in this subject after the wines.

The same dose of alcohol was again given to subject A, but this time it was buffered to the same pH and buffer capacity as the Port wine. In this case the blood alcohol curve was practically superimposable on that after Port wine, and bore no resemblance to the curve obtained after alcohol alone. Thus there can be no doubt that the slower absorption of wine than distilled liquors is ascribable to its buffer capacity.

CONCLUSIONS

Wines, with their high buffer capacity, are absorbed less rapidly than distilled liquors. This results in the absence of the high peak of blood alcohol concentration seen after ingestion of distilled liquors on an empty stomach. In certain individuals, nausea may so disturb gastric motility as to obscure this difference.

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THE PRODUCTION OF A PRESSOR SUBSTANCE FROM SERUM GLOBULIN BY ACTION OF PEPSIN[1]

THE recent article of H. Croxatto and R. Croxatto[2] has led us to publish some of the work upon which we have been engaged since May, 1941.

The demonstration by Braun-Menendez[3] and others that renin behaves like a ferment led quite naturally to the further study of its ferment-like properties. The discovery by Page[4] that the incubation of blood serum with renin produces a pressor substance, which unlike renin is thermostable, has increased still more the interest in renin and the hypertensive substances which may be formed from or by it.

Our own work followed two lines of investigation. In the first series of experiments, renin was added to various known constituents of serum and the hypertensive effects of the solutions studied on the dog. The substances employed were urea, creatin, creatinin, tyrosin, glycine, histidine, alanine and uric acid in normal saline solution. After incubation for fifteen minutes, the renin solution's activity was destroyed by immersion in boiling water. None of these solutions showed any hypertensive effects. Sheep serum globulin, by contrast, when subjected to the same treatment uniformly gave rise to a pressor substance, called "angiotonin" by Page and "hypertensin" by Braun-Menendez.

In our second series of experiments we studied the effects of various enzymes on serum globulin. We employed trypsin, papain, cathepsin, takadiastase and pepsin. The only positive results were obtained with pepsin, and these results were at first very inconstant. During the past ten months' study of the properties of the pressor substance produced by the action of pepsin on serum globulin, we have learned how to produce this pressor substance with relative ease.

In our experiments we have employed four different commercial preparations of pepsin. The results were identical with each preparation. We have not yet had an opportunity of studying the effect of crystalline pepsin on serum globulin.

The most active pressor substance is produced by incubation between pH 5 and 6 for one-half hour. When more acid reaction such as pH 5, 4 or 3 were employed, the resulting solution was only feebly hypertensive or inactive, or in some instances strongly depressor, possibly because of peptone-like substances, the result of peptic digestion. This observation is in harmony with the fact that pepsin in an acid solution destroys "angiotonin." Incubation for one-half hour produced a much more active preparation than incubation for a shorter length of time.

When the solution of pepsin was immersed in boiling water for ten minutes before addition to serum globulin, no hypertensive substance was formed. Although the hypertensive substance was formed at pH 5–7, it was apparently necessary to dissolve the pepsin

[1] From the Hixon Laboratory for Medical Research, University of Kansas, School of Medicine, Kansas City, Kansas.

[2] H. Croxatto and R. Croxatto, SCIENCE, 95: 101, 1942.

[3] E. Braun-Menendez, J. C. Fasciolo, L. F. Leloir and J. M. Muñoz, *Jour. Physiol.*, 98: 283, 1940.

[4] I. H. Page and O. M. Helmer, *Jour. Exp. Med.*, 71: 29, 1940.

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 MOUTH[1]

IN 1939 evidence was found that the reactions involved in the production of lactic actid 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 nontoxic 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 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

[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.

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[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.