

plete it is impossible to state definitely that the inhibitory effect of these compounds on dental caries results from specific effects on discrete enzyme systems, such as phosphorylation.

*Summary.* Sodium fluoride and iodoacetic acid added to a caries-producing diet fed to albino rats diminished greatly the incidence of carious lesions in the molar teeth.

## 10216

## Phospholipids and Complementary Activity.

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In a previous communication,<sup>1</sup> it was demonstrated that prolonged extraction of active, dehydrated complement with organic solvents, *i. e.*, alcohol, ether, etc., resulted in no reduction of complementary activity. In fact, the recovered lipids often exhibited anticomplementary properties when returned to the extracted residues prior to titrations.

Recently, Bloor and Snyder<sup>2</sup> have noted that a buffered suspension of oxidized phospholipids oxidizes a sensitive methylene blue preparation.

In view of earlier work from this laboratory,<sup>3</sup> which revealed an intimate connection between complementary powers and oxidation-reduction phenomena, it was decided worthwhile to study the effect of oxidized and unoxidized cephalin and lecithin on complementary activity.

Purified preparations of the 2 phospholipids were employed throughout these experiments. The oxidized phospholipids were prepared according to the technic of Bloor and Snider and complement was titrated by the method advocated by Ecker, Pillemer, Wertheimer and Gradis.<sup>4</sup>

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<sup>1</sup> Ecker, E. E., Pillemer, L., and Grabill, F. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 318.

<sup>2</sup> Bloor, W. R., and Snider, R. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 215.

<sup>3</sup> Ecker, E. E., Pillemer, L., Martienssen, E. W., and Wertheimer, D., *J. Biol. Chem.*, 1938, **123**, 351.

<sup>4</sup> Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunol.*, 1938, **34**, 19.

The amounts of reagents employed and general procedures are found in the accompanying protocols. All solutions were prepared in a M/15 phosphate buffer pH 7.2.

The experiments of Bloor and Snider were repeated and confirmed. As reported by these authors, it was also found that phospholipids do not oxidize a sensitive preparation of reduced methylene blue, while the oxidized lipids show a definite oxidizing power.

TABLE I.  
Showing the Relative Inhibiting Actions of Various Phospholipids on Complementary Activity.

Phospholipids	Concentrations of the phospholipids necessary to inactivate 1 cc of a 1:10 dilution of complement
	%
Cephalin	.008
Oxidized cephalin	.05
Lecithin	.10
Oxidized lecithin	.40

Various portions of the phospholipids were then added to fresh guinea pig complement, and their effects noted. Table I shows the relative inhibitory effect of the various phospholipids on complement. Their inhibitory powers were found in the following orders:

Unoxidized cephalin > oxidized cephalin >  
unoxidized lecithin > lecithin

It is of interest, and likewise difficult to explain, that the unoxidized preparations were more inhibitory than the oxidized preparations.

The effect of ascorbic acid and of reduced glutathione were then studied with regard to complements inactivated by these phospholipids. These results are found in Table II.

From the data obtained, it is evident that ascorbic acid and glutathione SH possess the ability to restore the diminished complementary activity of the complements after treatment with lipids. The most marked reactivation was accomplished with serums inactivated by unoxidized cephalin, while the others proceeded in the following order:

Unoxidized lecithin > oxidized cephalin > oxidized lecithin.

In fact, only a slight reactivation was accomplished with the complements treated with the oxidized lecithin. Another point of interest is the observation that ascorbic acid yielded the most marked and consistent reactivations.

Similar findings were obtained when the reductants were incubated first with the phospholipids and then complement added to

TABLE II.  
Reactivation of Complement Inhibited by Various Phospholipids.

Complement 1:10	Initial Hemolysis									
	.01 cc	.02 cc	.03 cc	.04 cc	.05 cc	.06 cc	.07 cc	.08 cc	.09 cc	.10 cc
+ 2 cc M/15 buffer pH 7.2*	O	H	H	H	H	H	H	H	H	H
'' 1 '' cephalin 1:24,000 + 1.0 cc buffer	O	O	O	O	O	O	O	O	O	O
'' '' '' '' '' '' '' .5% ascorbic acid	O	O	H	H	H	H	H	H	H	H
'' '' '' '' '' '' '' .5% GSH	O	O	O	O	O	O	O	O	O	O
'' '' '' '' '' '' '' + 1.0 cc buffer	O	O	O	O	O	O	O	O	O	O
'' '' '' '' '' '' '' .5% ascorbic acid	O	O	O	O	O	O	O	O	O	O
'' '' '' '' '' '' '' .5% GSH	O	O	O	O	O	O	O	O	O	O
'' '' '' '' '' '' '' + 1.0 cc buffer	O	O	O	O	O	O	O	O	O	O
'' '' '' '' '' '' '' .5% ascorbic acid	O	O	O	O	O	O	O	O	O	O
'' '' '' '' '' '' '' .5% GSH	O	O	O	O	O	O	O	O	O	O
'' '' '' '' '' '' '' + 1.0 cc buffer	O	O	O	O	O	O	O	O	O	O
'' '' '' '' '' '' '' .5% ascorbic acid	O	O	O	O	O	O	O	O	O	O
'' '' '' '' '' '' '' .5% GSH	O	O	O	O	O	O	O	O	O	O

H = Hemolysis.

\* = Phosphate buffer.

The phospholipids were incubated for 1 hour with the serums, and then the inactivated complements were incubated for 30 minutes with the various reductants. Ascorbic acid and GSH were prepared in buffer solution 7.2 M/.15.

the mixture. This would indicate that the protective phenomenon observed was due to the interaction of the reductants with the phospholipids, thereby inhibiting their anticomplementary powers.

It has been generally believed that phospholipids play a distinct rôle not only in biological redox phenomena<sup>5, 6</sup> but also in immunological reactions. It is therefore possible that a connection exists between these two assumptions.

Evidence presented here indicates that an excess of cephalin or of lecithin weakens complement and that the effect may be reversed by ascorbic acid or by glutathione SH. These findings, therefore, point to the fact that the phospholipid inactivation of complement may be at least partly oxidative in nature.

The reactivations of the inactivated complements by ascorbic acid and by reduced glutathione may be due either to the reduction capacity of these agents, thereby reducing the oxidized system to a state whereby it can function or that the reductants employed unite directly with the phospholipids and thereby prevent the compounds from exerting an anticomplementary action.

It is at present difficult to explain the differences observed between the oxidized and the unoxidized phospholipids.

## 10217

### Effect of Divinyl Oxide on Intestinal Activity *in vivo*.\*

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The knowledge of the effect of the newer inhalation anesthetic agents on intestinal contraction is far from adequate. In a previous report<sup>1</sup> it was indicated that during the first two planes of surgical anesthesia the effect of cyclopropane on intestinal activity in the intact animal consists of an increase of both intestinal contractions and tone followed by inhibition if narcosis is further deepened. These results agree with the *in vitro* effects observed by Peoples and

<sup>5</sup> Koch, W., *Ztschr. Physiol. Chem.*, 1903, **37**, 181.

<sup>6</sup> Fränkel, S., and Dimitz, L., *Wien. klin. Wchnschr.*, 1909, **22**, 1777.

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<sup>1</sup> Burstein, C. L., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 530.