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QUARTERLY REPORT

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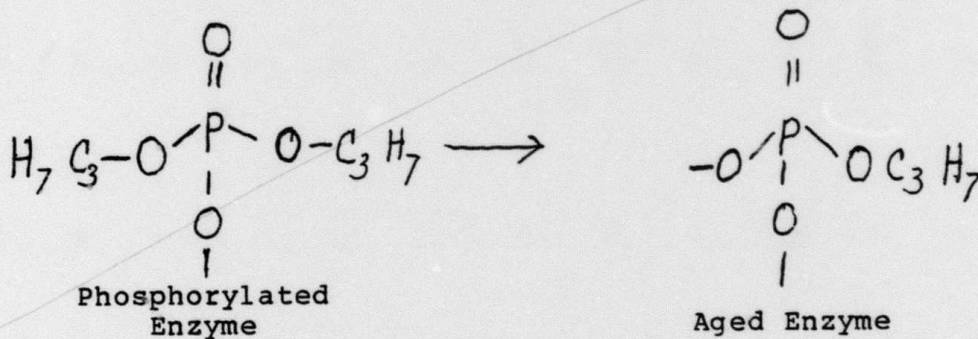
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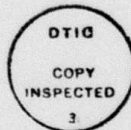
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In this reporting period we have continued to characterize monoclonal antibodies against AChE-DFP. We have focused attention on three monoclonals of interest. These are the V-16 antibody described in our previous report and two additional antibodies designated V-18 and D11B. The V-16 antibody demonstrates higher activity against AChE-DFP conjugate than against AChE alone. The V-18 and D11B antibodies showed higher activity against AChE than against the conjugate. In addition, the D11B antibody blocks AChE enzymatic activity in the Elman assay. Further study showed that these antibodies exhibit a different response to AChE-DFP conjugates on the basis that the AChE ages or loses an isopropoxy residue in the reaction depicted below.



The resulting phosphate diester has a charge, at normal pH, on oxygen. This change could account for the results given below in Table 1.



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TABLE 1

TABLE 1. ANTIBODY ACTIVITY AGAINST AChE/AChE-DFP CONJUGATE

<u>ANTIBODY</u>	<u>AChE</u>	<u>AChE-DFP</u>	
		NEW	AGED
V-18	1.425	1.398	.078
V-16	.221	.230	.491
D11B	.946	.951	.177

Numbers indicate optical density in ELISA assay. Large numbers indicate strong antibody activity.

Clearly the results in Figure 1 indicate that V-18 and D11B bind to both AChE and AChE-DFP conjugate. However, when the aged form of the conjugate is used, binding is lower. This result could be a response to the strong charge on aged conjugate in phosphate ester. It is reasonable to think that such a charge could affect binding of antibody profoundly. This could be the basis of an indirect assay in which antibodies are released from AChE-DFP conjugates as they age. On the other hand, these results may indicate a further conformational change associated with changes taking place in AChE when aging takes place. The V-16 antibody shows higher activity against the aged conjugate. Again this could be a differential affinity for the charge located on the aged phosphodiester or further conformational change in the enzyme.

In conclusion, the V-16 antibody does not react with DFP alone, does not react strongly (as strongly as previous monoclonals against the conjugate) and the conjugate that does react is aged. This

antibody would not, therefore, be appropriate for rapid organophosphate assays as it would require aging of the conjugate. The reverse assay with V-18 and D11B is possible, but would depend on the kinetics of release and would be unacceptably noisy. Since V-16 antibody does not recognize DFP there is reason to suspect a conformational change, but a conformational change that occurs over a long period of time and may not be completely correlated with dealkylation and could be simple enzyme denaturation. This certainly would be of no interest in an ELISA assay. It is therefore fair to conclude that while we will continue to characterize our V-16 antibody, we are not encouraged that this antibody has the characteristics of earlier antibodies. Because of these results, we feel new approaches to the problem may be considered at this time. Of course further immunizations and hybridomas to the conjugate may be attempted in an effort to regenerate monoclonals with the desired specificity to the conjugate antigen. However, we cannot state with confidence that this will work since existing evidence suggest the possibility that the organophosphate may be unavailable for immune processing recognition.

A more standard way to approach the problem is to immunize against organophosphate bound to a common carrier such as BSA. We have conjugated DFP to BSA and will begin immunization shortly. This approach is reasonably reliable in that it will produce antibody to DFP and we can use this antibody in assays for the organophosphate. However, it is also likely that the antibody will have a high dissociation rate from the AChE-DFP conjugate. This will limit the