The results reported above demonstrate a close correlation between the structural organization of glutamate decarboxylase and its specific catalytic activity. The active enzyme macromolecules have the aspect of hexagonal disc-shaped particles. Marked reduction in activity, both of the apoenzyme and of the enzyme exposed to cold in dilute solution, is associated with destruction of the initial shape of the active enzyme, *i.e.* with disorganization of its quaternary structure. Both the activity and structure of glutamate decarboxylase are restored on addition of pyridoxal phosphate. The experimental data thus indicate the role of bound coenzyme in stabilizing the structure of the holoenzyme.

Detailed investigation of the quaternary structure of glutamate decarboxylase by electron-micrographic and other methods is in progress.

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Properties of matrix supported acetylcholinesterase

The use of polymeric matrices to insolubilize proteins with catalytic activity has been described by several authors[1–6]. The interactions between the protein and the matrix is defined in terms of covalent[1–4] or ionic bonds[5] in certain instances while in others the protein is simply entrapped within the lattice of the polymer[6,7]. Those bonding situations involving known linkages hold the greater theoretical interest in the investigation of the relationship between enzyme-membrane binding and enzymatic activity; however, the matrix-entrapped systems may be of significant practical value because they are easily prepared with high activities and may demonstrate some unusual modification of enzyme behavior. In this study, Silastic-entrapped acetylcholinesterase has been prepared and certain of its properties relative to the free enzyme investigated. The Silastic resin employed has the general chemical structure where the

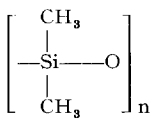


TABLE I

RESULTS OF THERMAL DENATURATION STUDIES

Activities are in terms of % orginal activity.

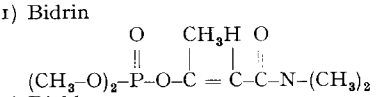
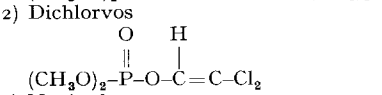
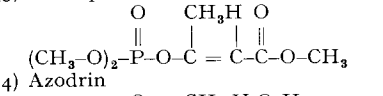
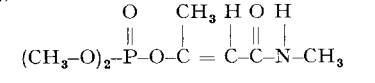
Time (min) at 60°	*Esterase in solution*	*Matrix-supported esterase*
0	100	100
5	8	128
10	0	98
15	0	92
20	0	94
30	0	89
40	0	99
50	0	85
60	0	94
90	0	90
120	0	91

n value is of the order of 10 000. This resin contains additionally, a silica filler and on addition of the proper vulcanizing agents, silicone rubber results.

The entrapped acetycholinesterase was prepared by mixing 10 g of the Silastic resin (a gift from Dr. SILAS BRALY, Dow Corning, Midland, Mich.) with 100 mg of acetylcholinesterase (Sigma Chemical Co., St. Louis, Mo.). This mixture was stirred for 10 min, and 0.5 ml of stannous octoate catalyst was then added. Stirring was continued for 30 min and the mixture allowed to stand overnight (5°). The resulting white slab was cut into sections approx. 0.1 mm × 0.9 mm × 15.0 mm. These prepa-

TABLE II

COMPARISON OF THE EFFECTS OF CERTAIN ORGANOPHOSPHORUS COMPOUNDS ON ACETYLCHOLINESTERASE

Compound (10^{-6} M)	*% Inhibition*		
	Enzyme in solution	*Pretreated with support*	*Entrapped enzyme*
(1) Bidrin 	100	80	51
(2) Dichlorvos 	94	74	83
(3) Mevinphos 	88	84	44
(4) Azodrin 	63	62	31

rations were washed twice with water and assayed for enzymatic activity according to the method of HESTRIN[8]. All activities were corrected for the surface area of the disc exposed to the solution.

The effects of thermal denaturation on the entrapped enzyme were studied by incubating the disc at 60° in 5 ml of water for varying lengths of time and then carrying out the assay procedure described. The results of the thermal denaturation studies are given in Table I. It is apparent that the matrix-entrapped material has achieved remarkable thermal stability. The initial increase in activity on heating has been reported for a variety of solid supported enzymes.

The effects of various organophosphorus inhibitors upon the matrix-supported enzyme as well as the enzyme free in solution were determined. A reference blank was used to determine the effect of interaction between the phosphorus compounds and the support matrix. The results of these experiments are given in Table II. Concentration of the inhibitor was 10^{-6} M in all cases. The second column of data is for the free enzyme in a solution of substrate *plus* inhibitor that had been pretreated with a sample of Silastic matrix. Compounds 3 and 4 appeared to interact only slightly with the matrix material. The prevention of inhibition by the solid support of the enzyme in the other two cases appears to at least be partially due to absorption of the organophosphorus compound by the support material thus reducing its concentration in solution; however, the supported enzyme appears, in all cases, to be less affected by the inhibitor than does the free enzyme in solution.

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