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Distribution of $DF^{32}P$ in mouse organs—III Incorporation in the brain tissue

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DEATH due to an organophosphorus anticholinesterase such as di-isopropyl phosphorofluoridate (DFP) is attributable to failure of respiration brought about, peripherally by paralysis of the respiratory muscles, and centrally, by the depression of the respiratory centre.[1] The mode of action of neither the organophosphate nor the oxime-antidotes on the central nervous system is clearly understood. There is poor correlation between death and the acetylcholinesterase (AChE) content of the brain[2] which differs with different organophosphates.[3] The oxime-antidotes, the most effective of which, such as 2-formyl-1-methyl pyridinium oxime (PAM), 1,1′-trimethylenebis (4-formyl pyridinium) dioxime (TMB-4) and *bis*-(4-hydroxyiminomethyl-pyridinium-1-methyl)ether (Toxogonin) are of the quaternary type have, therefore, very poor[4] or only limited [5, 6, 15] capacity to penetrate the blood–brain barrier. They have a low antidotal effect unless administered together with atropine. The involvement of some selected sites in the brain has also been reported.[7] In such studies the penetration of the organophosphate itself is assumed to be rapid in the brain tissue in the case of compounds like DFP and Sarin which are highly soluble in lipoid media, as there is a good correlation between lipoid solubility and the degree of the effect on the central nervous system.[8] However, the results reported in the present work show that penetration of $DF^{32}P$ and similar compounds in the brain tissue may be slow and probably not uniform.

In the previous paper[9] in which the incorporation of $DF^{32}P$ in mouse organs against atropine and oxime antidotes was reported, the amount of "bound", $DF^{32}P$-derived P was shown to reach a constant value in the liver, kidney and lung even at the level of about 2 LD_{50}s though doses up to 50 LD_{50}s could be administered to mice protected with TMB-4 or Toxogonin with atropine. In the case of brain tissue, however, the "bound" P showed a tendency to increase with the dose of $DF^{32}P$ administered. The bound $DF^{32}P$-derived P was calculated in these cases from the difference between the total radioactivity and the TCA-soluble radioactivity in the tissues. A more direct proof will be to isolate the ^{32}P-labelled phosphoproteins and liberate the labelled organophosphate moieties. This has been done in the present work. The results have been confirmed by the isolation of ^{32}P-labelled serine phosphoric acid in some cases.

MATERIALS AND METHODS

These were the same as in the previous paper.[9] Phosphoproteins containing $DF^{32}P$-derived P were isolated according to Kleinsmith *et al.*[10] Serine phosphoric acid was determined according to Schaffer

TABLE 1. $DF^{32}P$-DERIVED PHOSPHOPROTEIN P IN MOUSE ORGANS

Dose of $DF^{32}P$ + DFP (mg/kg)	$DF^{32}P$-derived phosphoprotein P in mμg/g wet tissue				
	Liver	Kidney	Lung	Brain as protein ^{32}P	Brain as ^{32}P ser-P
3·4–3·8*	921	266	170	27 ± 1·8(5)	6·3
4	871	236	136	30 ± 3·3(7)	6·8
8	891	236	144	47 ± 2·9(6)	7·4
16	831	251	119	54 ± 3·0(4)	9·5
32	868	234	208	66 ± 1·7(6)	10·8
64	769	280	207	80 ± 9·4(4)	
128	809	212	135	90 ± 14·1(3)	

* Control without antidotes. Organs from 6–10 animals were pooled and worked up. The results of a single representative series are given in the case of liver, kidney and lung. In the case of brain the standard deviation and the number of series (in parentheses) are given. Sacrifice was at 4 hr after $DF^{32}P$.

et al.[11] with minor modifications. Only Toxogonin was used in these experiments at a dose level of 100 μmole/kg in combination with atropine at 50 μmole/kg, both given i.p. 15 min before $DF^{32}P$ + DFP subcutaneously. To avoid any free $DF^{32}P$ present in the tissue combining with the esterases during the process of homogenization, sacrifice of the animals was invariably done 4 hr after the administration of $DF^{32}P$.[9]

RESULTS

From results in Table 1 it is seen that the protein-bound ^{32}P remains fairly stationary in the case of liver, kidney and lung irrespective of the dose of $DP^{32}P$ administered. In the lung the deviations are greater due to the indeterminate amounts of blood always present. In the brain the amount of protein-bound ^{32}P increases with the dose. If the values are plotted against the logarithm of the $DF^{32}P$ dose administered, a straight-line graph is obtained (not submitted for publication to avoid duplication). The results are also confirmed by the steady increase in the ^{32}P-labelled serine phosphoric acid content as determined by Dowex-50 column chromatography.

The amount of ^{32}P bound to the protein does not depend upon the interval between the administration of $DF^{32}P$ and sacrifice (Table 2). It seems to attain constancy even at 30 min after which it does not change for a considerable time in the brain. In the case of the other three organs there is a slight decrease with time, due probably to tissue regeneration.

TABLE 2. $DF^{32}P$-DERIVED PHOSPHOPROTEIN P CONTENT OF MOUSE ORGANS AT VARIOUS TIME INTERVALS AFTER $DF^{32}P$ ADMINISTRATION

Interval between $DF^{32}P$ + DFP injection and sacrifice (hr)	Phosphoprotein ^{32}P in mμg/g wet tissue			
	Liver	Kidney	Lung	Brain
0·5	1071	346	216	56
3	1044	377	259	61
6	890	330	209	62
18	853	266	184	56
24	846	265	141	50
48	565	189	173	56

$DF^{32}P$ + DFP was administered subcutaneously at 32 mg/kg to mice protected with atropine and Toxogonin. The results are from one representative series, each value being that of pooled organs from 6–10 animals.

Whatever the amount of protein-bound ^{32}P to begin with, it remains stationary at that level over a length of time. In Table 3 the amounts of protein-bound ^{32}P at two selected intervals, viz. 30 min and 4 hr are given for various doses of $DF^{32}P$.

DISCUSSION

These results indicate that the penetration of $DF^{32}P$ in the brain tissue is slow and dose dependent. At LD_{50} (about 3·8 mg/kg without antidotes) there is a considerable amount of DFP-binding potential (esterase activity)[12] in the brain, as only 27 mμg/g of $DF^{32}P$-derived P is formed as against the possible 90 mμg/g at 128 mg/kg of DFP which is nearly the LD_{50} against Toxogonin and atropine.[9] The distribution of the bound-^{32}P at the sub-maximal doses may be uniform in the brain tissue or may be localized in some selected regions. The latter alternative is probable in view of the findings

reported in an earlier work[13] that $DF^{32}P$ binds itself to the tissue with which it comes into contact first, only the over-flow reaching the rest of the tissues. These findings may help to throw some light on the lack of correlation between death and the AChE levels, as also the mechanism of oxime-induced reactivation of AChE in the brain.

TABLE 3. $DF^{32}P$-DERIVED PHOSPHOPROTEIN P IN MOUSE BRAIN TISSUE AT DIFFERENT DOSE LEVELS AND TIME INTERVALS

Sacrifice (hr)	Phosphoprotein-^{32}P (mμg/g of wet tissue) with $DF^{32}P$ + DFP administered at (mg/kg)				
	3·6*	4	8	16	32
0·5	28	30	47	55	66
4	27	31	44	55	66

* Control without antidotes.

The slow rate of recovery of enzymes in the brain tissue also indicates the possible dangers from cumulative poisoning to those exposed to chronic sub-lethal doses of organophosphorus esters. Though only a small proportion of the enzymes dealt with in this paper may be AChE, the effect of persistent inhibition of other important DFP-susceptible enzymes such as transport-ATPase[14] on general health is still to be assessed.

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