The Kinetics of Reactivation, by Oximes, of Cholinesterase Inhibited by Organophosphorus Compounds

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The inhibition of cholinesterase (ChE) and related enzymes, by organophosphorus compounds, is generally believed to occur by direct phosphorylation of some group at the active centre of the enzyme, together with the liberation of an acid anion (Wilson, 1951; Balls & Jansen, 1952; Hartley & Kilby, 1952; Aldridge, 1954; Kilby & Youatt, 1954).

Inhibition by organophosphorus compounds is generally not reversible by dialysis or dilution, but the inhibited enzyme can be reactivated by treatment with many chemical reagents with a nucleophilic affinity for phosphorus, presumably by direct chemical displacement of the enzyme from the phosphorus atom (Wilson, 1951, 1952). A survey of a wide range of reactivating reagents has been given by Wilson (1955b), who has shown that reactivators are particularly effective if they possess a group capable of forming a complex with the inhibited enzyme. In addition, the rate of reactivation was found to be markedly dependent upon pH, and showed an optimum depending upon the pKₐ of the reactivating agent.

Few detailed studies have been reported upon the kinetics of reactivation. The rate of reactivation by choline of tetraethyl pyrophosphate (TEPP) inhibited ChE was found by Wilson (1952) to approach zero order with increasing concentration of the reactivator, i.e. it shows the phenomenon of saturation as found in normal enzyme-substrate action. This effect was not observed with hydroxylamine, but even so the rate did not show a simple dependence upon hydroxylamine concentration. Cunningham (1954) obtained similar results in the reactivation by hydroxylamine of diethyl p-nitrophenyl phosphate (E800) inhibited chymotrypsin.

Wilson, Ginsberg & Meislich (1955) have reported that the rate of reactivation of inhibited true ChE by hydroxamic acids also shows the saturation effect found with choline, while a similar result was obtained with hydroxamic acids and the pseudo enzyme (Wilson, 1955a).

In a previous paper (Childs, Davies, Green & Rutland, 1955) the reactivating power of a wide range of oximes was reported. The present paper describes the kinetics of reactivation by selected members of this group of compounds and discusses the significance of the results in relation to the structure of the active centre of ChE.

EXPERIMENTAL

Materials

The oximes were prepared by the action of nitrous acid on compounds possessing a suitably activated methylene group. Ditosonitosoacetone was obtained from acetone dicarboxylic acid (Koessler & Hanke, 1918), monosonitosoacetone from ethyl acetocacetate (Freon, 1949), isonitosoacetoephone from acetophenone (Claisen & Manasse, 1897) and isonitosoacetacetylated from acetylaceton (Wollf, 1902). The hydroxamic acids were prepared as described by Hackley, Plapinger, Stolberg & Wagner-Jauregg (1955). They were characterized by melting-point determinations and nitrogen analyses.

Methods

Experiments at pH 7-4. Washed human erythrocytes were suspended in an equal volume of 0-9% NaCl and then allowed to stand in contact with the inhibitor (10⁻⁴ m) for 10 min. at 25°. The cells were washed with ice-cold saline to remove excess of inhibitor, and then stored at 0° in an equal volume of saline until immediately before use. One volume of the inhibited cell suspension was mixed with one volume of the reactivating agent in veronal buffer (sodium diethylbarbiturate, 0-01 M; KH₂PO₄, 0-002 m; KCl, 0-3 m), and stored at the required temperature. At intervals, 1 ml. of this mixture was added to 10 ml. of acetycholine chloride (0-01 M) in the above veronal buffer. ChE determinations were carried out by the electrometric method (Michel, 1949).

Experiments at varying pH. The non-inhibited cell-saline suspension (10 ml.) containing the oxime at twice the required final concentration was treated with 0-1 N NaOH or 0-1 n HCl to bring the pH to the required value and the volume was then made up to 20 ml. with saline. To the inhibited cell-saline suspension (10 ml., prepared as above) was added the same volume of alkali or acid as determined above, followed by the oxime dissolved in the volume of saline required to bring the total volume to 20 ml. Samples (1 ml.) were then taken at intervals and analysed for ChE activity. The pH was checked during each run.

In both types of experiment, controls were run (a) upon the enzyme alone, (b) upon the enzyme in the presence of the reactivator, and (c) upon the inhibited enzyme.

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RESULTS

The kinetics of reactivation

If the reactivation process consists of a direct nucleophilic displacement of the enzyme from the phosphorus atom, it would be expected that the process would obey a second-order rate equation:

\[
\frac{dE}{dt} = k[E_{\infty} - E] \text{[oxime]},
\]

where \([E]\) is the concentration of reactivated enzyme after time \(t\), and \([E_{\infty}]\) is the total inhibited enzyme concentration available for reactivation. This equation will be valid provided that (a) the enzyme does not contain two or more forms which would be reactivated at different rates, (b) the pH and temperature are kept constant, and (c) the oxime does not form a complex with the inhibited enzyme.

This rate law has been demonstrated (Table 1) with mono- and di-isonitrosoacetone at concentrations below 0.01 M and ChE inhibited by TEPP or by isopropyl methylphosphonofluoridate (Sarin). At these concentrations, neither of these oximes inhibits the free enzyme, and hence presumably the oxime does not form a complex with the phosphorylated enzyme. Even with isonitrosoacetophenone, which forms a strong complex with the free enzyme, the observed rate constant does not fall off markedly until the concentration reaches 0.01 M. At concentrations above 0.01 M the rate of reactivation by all three compounds of ChE inhibited with either Sarin or TEPP becomes too fast to measure accurately.

A typical experiment (Fig. 1) shows the first-order dependence of the rate on concentration of inhibited enzyme.

Rate of reactivation by different oximes of inhibited ChE

On the assumption that the second-order kinetics, demonstrated above, are generally valid, irrespective of inhibitor and oxime, the rate constants for a number of the more active compounds described previously (Childs et al. 1955) have been determined for TEPP, diisopropyl phosphoro-

<table>
<thead>
<tr>
<th>Oxime conc. (M)</th>
<th>Sarin</th>
<th>TEPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>23.0</td>
<td>—</td>
</tr>
<tr>
<td>0.002</td>
<td>23.0</td>
<td>21.5</td>
</tr>
<tr>
<td>0.004</td>
<td>24.0</td>
<td>22.2</td>
</tr>
<tr>
<td>0.005</td>
<td>—</td>
<td>6.8</td>
</tr>
<tr>
<td>0.01</td>
<td>—</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Table 1. Second-order rate constants for the reactivation by monoisotrosoacetone, at pH 7.4 and 25°C, of erythrocyte ChE inhibited by TEPP or by Sarin

The values of \(k\) are given as l./mole/min.

Values of \(k\) are given in l./mole/min.; temp., 25°C; pH 7.4 (approx.). The figures in brackets indicate the number of experiments.

<table>
<thead>
<tr>
<th>Compound</th>
<th>TEPP</th>
<th>DFP</th>
<th>Sarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diisotrosoacetone</td>
<td>8.8</td>
<td>0.8</td>
<td>24.3</td>
</tr>
<tr>
<td>Monoisotrosoacetone</td>
<td>6.8</td>
<td>0.7</td>
<td>22.1</td>
</tr>
<tr>
<td>IsoNitrosoacetophenone</td>
<td>10.7</td>
<td>5.1</td>
<td>4.4</td>
</tr>
<tr>
<td>IsoNitrosoacetylecetone</td>
<td>0.7</td>
<td>—</td>
<td>1.1</td>
</tr>
<tr>
<td>Picolinohydroxamic acid</td>
<td>9.2</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Nicotinohydroxamic acid methiodide</td>
<td>0.3</td>
<td>&lt;0.05</td>
<td>0.3</td>
</tr>
</tbody>
</table>
reactivation is due solely to a reaction between the points is
form
shown
are
over
studied
erthrocyte ChE
pH-dependent,
and
identical at different
(A-)
of
anion
carrying
in Table 3. These
determined
in Table 2. In
these values may not give the true
activation energies since the rate constants are
pH-dependent, and this dependence may not be
identical at different temperatures.

Variation in the rate of reactivation with pH

The rate of reactivation of TEPP-inhibited
erthrocyte ChE by monoisonitrosoacetone was
studied over the pH range 6-9. The observed rates
are shown in Fig. 2. Superimposed on the observed
points is a curve derived on the assumption that
reactivation is due solely to a reaction between the
anion (A-) of the oxime (A, pK A 8-3) and a protonated
form of the inhibited enzyme (EI+H, pK A 7-3), i.e. that the reaction is of the form

\[ EI^+H + A^- \rightarrow E + HAI \rightarrow E + \text{products.} \]

Therefore \( v = k_1 [A^-] [EI^+] \]

\[ = k_{\text{observed}} [A]_{\text{total}} [EI]_{\text{total}} \]

i.e.

\[ k_{\text{observed}} = k_1 [A^-] [EI^+] \]

\[ [A]_{\text{total}} [EI]_{\text{total}} \]

The values \( k_1 \) (106 l./mole/min.) and pK A of the
inhibited enzyme (7-3) have been chosen in order to
give the best fit to the observed points.

Effect of storage on ability to be reactivated

It was originally observed that if the enzyme,
freshly inhibited with DFP or Sarin, was stored at
room temperature for about 24 hr., only about
20% of the initial activity could be restored on
treatment with oxime, although the rate at which
reactivation occurred was unchanged. This effect
was not observed, however, with ChE inhibited
with TEPP.

This phenomenon has been studied in greater
detail with ChE inhibited with Sarin. The inhibited
enzyme was stored at 37°, and samples were taken at
approximately hourly intervals and reactivated
with monoisonitrosoacetone (0-01 M) for 30 min.,
i.e. about ten half-lives. Controls on the free
enzyme, the free enzyme in the presence of the
reactor, and on the inhibited enzyme, were run simultaneously to ensure that the enzyme
itself was not denatured under these conditions.
The results of a typical experiment are shown in
Table 4.

A plot of the values of log \((T - I)\) against time,
where \( T \) is reactivated enzyme and \( I \) is inhibited
enzyme, gives a straight line which shows that the
transformation occurs at a first-order rate. As will
be seen from Table 4, the activity of the free enzyme
is not affected by storage at 37°.

The effect of pH on the rate of transformation has
also been studied and the results are shown in
Table 5. The transformation is markedly accelerated
at lower pH and inhibited at higher pH. The free
enzyme itself is also rather less stable at pH values
above and below 7, but this will not seriously in-
fluence the values given for the rates of transform-

At 0°, ChE inhibited by either Sarin or DFP may
be kept for over a week without reduction in its
ability to be reactivated.

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Table 3. Rates of reactivation by diisonitrosoacetone at pH 7-4 and different temperatures of erythrocyte ChE
inhibited with TEPP or Sarin, and the corresponding activation energies

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>10°</th>
<th>25°</th>
<th>37°</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEPP</td>
<td></td>
<td>8-4</td>
<td>17-6</td>
</tr>
<tr>
<td>Sarin</td>
<td>8-9</td>
<td>24-3</td>
<td>53</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Activation energy (Kcal./mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-25°</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>11-2</td>
</tr>
</tbody>
</table>

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Fig. 2. Second-order rate constants for the reactivation
of erythrocyte cholinesterase inhibited with TEPP at 25°
at different pH values.
DISCUSSION

The mechanism of ChE action has been discussed in detail by Wilson, Bergmann & Nachmansohn (1950), who suggested that normal hydrolytic action occurs through the formation of an unstable acylated intermediate, which is spontaneously hydrolysed in water. Inhibition by organophosphorus compounds may then be explained as due to the formation of a corresponding phosphorylated intermediate, which in contrast is relatively stable to hydrolysis (Wilson, 1951; Aldridge, 1954).

From the variation of ChE activity with pH, it has been suggested that the acylated or phosphorylated site in the enzyme may be the basic nitrogen atom of the iminazole ring in histidine (Wilson & Bergmann, 1950). Similar calculations have also indicated histidine as the active nucleophilic molecule in pseudo ChE, chymotrypsin and trypsin (Laidler, 1955; Gutfreund, 1955). Thus if it can be shown that inhibition does occur by the formation of a phosphorylated iminazole ring then this would be strong confirmation of the participation of histidine in the normal process of hydrolysis.

Evidence for the phosphorylation theory of inhibition has been obtained, both from the stoichiometric nature of the inhibition itself (Balls & Jansen, 1952) and from the kinetics of reactivation by water, of ChE inhibited by organophosphorus compounds of the form (RO)₂P(O)X, where the alkyl group R was kept the same and the acid group X was varied (Aldridge, 1953; Aldridge & Davison, 1953).

Although some studies have been reported on the kinetics of reactivation by nucleophilic reagents other than water (Wilson, 1952, 1955a; Wilson et al., 1955; Cunningham, 1954) no simple linear dependence of the rate on the concentration of the reactivator has yet been demonstrated. The saturation effect observed when choline (Wilson, 1952) or hydroxamic acids (Wilson et al., 1955) are used as reactivators suggested that the mechanism of reactivation may be similar to that of normal enzymic hydrolysis, i.e. may be expressed in the form

$$k_1$$

$$\text{EI} + A \rightleftharpoons \text{EIA} \rightarrow \text{E} + \text{IA},$$

$$k_2$$

where A is the actual reactivating species (e.g. for oximes the anion, for hydroxylamine the neutral molecule). If the breakdown of the complex is the rate-controlling step the overall rate may be expressed as

$$v = \frac{dE}{dt} = k_2 \frac{[\text{EI}^*]}{K_A + [\text{A}]}.$$

where $[\text{EI}^*]$ is the total amount of inhibited enzyme remaining, and

$$K_A = \frac{[\text{EI}][\text{A}]}{[\text{EIA}]} = \frac{k_1}{k_2 + k_3}.$$

Wilson et al. (1955) have observed a rate equation for the reactivation by nictinohydroxamic acid of TEPP-inhibited ChE, a value 0.034 being given for $K_A$. If the observed rate, $v$, is proportional to concentration $[A]$, then $K_A$ must be large compared with $[A]$ and the rate $v$ is given by

$$v = \frac{k_1 k_2}{k_2 + k_3} [A][\text{EI}^*].$$

Table 4. Reactivation of ChE inhibited by Sarin after storage at 37°

<table>
<thead>
<tr>
<th>Time of storage (hr.)</th>
<th>0</th>
<th>1.5</th>
<th>2.5</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme alone (E)</td>
<td>110</td>
<td>111</td>
<td>113</td>
<td>106</td>
<td>109</td>
<td>102</td>
</tr>
<tr>
<td>Enzyme + MINA (M)</td>
<td>101</td>
<td>101</td>
<td>107</td>
<td>98</td>
<td>102</td>
<td>100</td>
</tr>
<tr>
<td>Inhibited enzyme (I)</td>
<td>13</td>
<td>10</td>
<td>15</td>
<td>10</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Reactivated enzyme (T)</td>
<td>91</td>
<td>71</td>
<td>62</td>
<td>47</td>
<td>38</td>
<td>33</td>
</tr>
<tr>
<td>$T-I$</td>
<td>86</td>
<td>61</td>
<td>47</td>
<td>37</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Log ($T-I$)</td>
<td>1.935</td>
<td>1.785</td>
<td>1.672</td>
<td>1.568</td>
<td>1.398</td>
<td>1.301</td>
</tr>
</tbody>
</table>

Table 5. Rate of transformation of ChE inhibited by Sarin on storage at 37° and varying pH

<table>
<thead>
<tr>
<th>pH</th>
<th>6.0</th>
<th>7.1</th>
<th>7.2</th>
<th>8.0</th>
<th>8.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of transformation (min.$^{-1}$)</td>
<td>0.010</td>
<td>0.0040</td>
<td>0.0038</td>
<td>0.0030</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

Cholinesterase inhibited by Sarin was stored at 37° at pH 7.2 for varying time intervals; samples were withdrawn and reactivated for 30 min. in the presence of 0.01M monoisonitrosoacetone (MINA) at 25°; enzyme activities are expressed as 100 ΔpH in 45 min.
If \( k_2 \ll k_3 \) then \( v = k_1 [A] [EI^*] \). Under these circumstances, the complex EIA may be regarded as possessing only a transitory existence, in fact it may be looked upon merely as the transition state for the reaction \( EI + A \rightarrow E + IA \). In other words, the reactivation process is a simple chemical displacement reaction.

If \( k_2 \gg k_3 \) then

\[
v = \frac{k_1 k_2}{k_3} [A] [EI^*].
\]

In this case the rate-controlling step is the formation of a complex EIA which can no longer be regarded as the transition state for the reaction.

From the kinetics alone, it was impossible to tell with the oximes which of the above conditions prevailed. Although the rate of reactivation was linear with concentration below 0.01 \( m \), a saturation effect may have occurred at higher concentration, but it was impossible to confirm this owing to the rapidity with which reactivation occurs under these circumstances. However, if reactivation is a normal chemical reaction, it would be expected that the relative rates of reactivation of inhibited ChE (I, where \( E \) represents the whole enzyme phosphorylated as shown at the active site) when \( R \) and \( R' \) are varied would be analogous to the relative rates of reactivation of heteroisosteric reagents with any corresponding set of inhibitors of the form (II).

\[
\begin{align*}
R & \quad \text{(I)} \\
R' & \quad \text{(II)} \\
\end{align*}
\]

The action of nucleophilic reagents (e.g., water, amines and anions) on diethyl- and disopropyl-phosphorochloridates \( (RO)\text{Cl} \) has been studied by Dostrovsky & Halmann (1953), who found that although the relative rate of reaction depended to some extent upon the precise structure of the reagent, the diethyl compound was normally 5–10 times as reactive as the disopropyl compound. Hackley et al. (1955) have reported that hydroxyacids react about 20 times as rapidly with Sarin as with DFP. Thus the ease of reactivation would be expected to be in the order Sarin > TEPP > DFP.

From the data in Table 2, it may be seen that the ease of reactivation by the aliphatic iso-nitroso compounds does fall into this order, whereas with iso-nitrosoacetophenone and the hydroxamic acids considerable discrepancies are found, particularly with Sarin. iso-Nitrosoacetophenone and the hydroxamic acids do themselves inhibit the free ChE, and the formation of a distinct complex EIA may well influence the rate-controlling step.

<table>
<thead>
<tr>
<th>Table 6. Dissociation constant of phosphorylated ChE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Pyridine</td>
</tr>
<tr>
<td>Hydroxylamine</td>
</tr>
<tr>
<td>Nicotinohydroxamic acid</td>
</tr>
<tr>
<td>Methiodide</td>
</tr>
<tr>
<td>Nicotinohydroxamic acid</td>
</tr>
<tr>
<td>Monoisotrosoacetic acid</td>
</tr>
<tr>
<td>Acetohydroxamic acid</td>
</tr>
<tr>
<td>Choline</td>
</tr>
</tbody>
</table>

The approximately equal activation energies (11–12 kcal./mole) found for the reactivation by disisonitrosoacetone of ChE inhibited by TEPP or Sarin are in agreement with the concept of a simple chemical-displacement reaction, the slightly faster rate with Sarin being probably due to lower steric hindrance.

It was observed by Wilson (1955b) that the rate of reactivation of inhibited ChE by a range of reactivators showed a marked \( pH \) dependence. A similar effect has been found with monoisonitrosoacetone. As shown in Fig. 2, the rate of reactivation is approximately proportional to the product of the concentrations of the monoisonitrosoacetone anion and a protonated form of the enzyme. Under these circumstances the optimum \( pH \) equals \( \frac{1}{2} (pK_1 + pK_2) \), where \( K_1 \) is the dissociation constant of the reactivator and \( K_2 \) that of the enzyme. Thus from a knowledge of the optimum \( pH \) and \( pK_1, pK_2 \) can be calculated. Table 6 shows the values of \( pK_2 \) obtained in this way; the data have been taken mainly from Wilson (1955b). The values so obtained for \( pK_2 \) are reasonably consistent with an average of about 7-6.

Wilson et al. (1955) have identified this ionizing group in the inhibited enzyme with the acidic centre in the esteratic site of free ChE, although the \( pK \) value is markedly different from 10-5, obtained from measurements of the variation of ChE activity with \( pH \).

If it be assumed that the initial phase of ChE inhibition consists of phosphorylation of a ring \( N \) atom in histidine, then the \( pK \) value of 7-6 can be attributed to the phosphorylated histidine. The charged form of the phosphorylated iminazole ring will be much more susceptible to attack by nucleophilic reagents than will the uncharged form (see top of p. 534). It might be expected that reactivation of the phosphorylated ChE by water would also be catalysed by acid, but Davison (1955) has reported that although aqueous solvolysis of diethylphosphoryl pseudo ChE shows catalysis by acid, the solvolysis of dimethylphosphoryl true ChE is not catalysed by acid but shows an optimum at \( pH \) 7-8. There seems no obvious explanation of this fact, but it may be connected with the storage effect.
Jandorf, Michel, Schaffer, Egan & Summerson (1955) have observed that ChE inhibited with DFP loses its ability to be reactivated by hydroxamic acids, after storage at 25° for 30 hr., and suggested a transfer of the phosphoryl group from an unstable to a more stable linkage. Wilson (1955b) similarly noted that the diisopropyl- and dimethyl-phosphorylated enzymes underwent this transformation upon storage, but that the diethylphosphorylated enzyme was relatively resistant. This fact has been confirmed in the present work. Although ChE inhibited by TEPP could still be reactivated almost completely after storage at 37° for 5 hr., ChE inhibited by Sarin was found to undergo the transformation at about the same rate as ChE inhibited by DFP. At 37° the transformation obeys a first-order rate law (see Table 4) and shows marked acid catalysis (see Table 5). These results are consistent with the reaction of the protonated form of the phosphorhistidine with another basic group in the enzyme (e.g. the hydroxyl group of tyrosine or serine)

If this group were the hydroxyl of serine then this would explain the isolation of serine phosphate on hydrolysis of ChE or chymotrypsin inhibited with DFP (Schaffer, May & Summerson, 1953, 1954; Cohen, Oosterbaan, Warringa & Jansz, 1955).

Davison (1955) only gives half-lives for the reactivation by aqueous hydrolysis at 37° of dimethylphosphoryl ChE and not the total reactivation obtained. If the dimethylphosphoryl enzyme undergoes the above transformation rapidly (Wilson, 1955b) and the process is catalysed by acid, then the significance of the half-life is uncertain and may not give a true measure of the actual rate of reactivation.

Until this discrepancy is resolved, it cannot be firmly established that histidine is the primary site of phosphorylation in the inhibition of ChE. Nevertheless, the kinetics of reactivation, particularly the pH dependence and storage effect, strongly indicate that such an initial phosphorylation does occur and support the other indirect evidence [e.g. the pH-ChE activity curve (Wilson & Bergmann, 1950) and the photo-oxidation experiments of Weil, James & Buchert (1953)], that this phosphorylation occurs at the ring N of a histidine molecule.

**SUMMARY**

1. The reactivation by α-keto-oximes, at concentrations below 0-01 M, of cholinesterase (ChE) inhibited by tetraethyl pyrophosphate (TEPP) or iodoethyl methylphosphonofluoridate (Sarin) has been shown to obey a second-order rate law, i.e. \( \frac{dE}{dt} = k[E|I|] \) [oxime].

2. The relative ease of reactivation of ChE inhibited by TEPP, diisopropyl phosphorofluoridate (DFP) and Sarin by various oximes and hydroxamic acids has been examined and compared with the reactivity of corresponding organophosphorus inhibitors

   with nucleophilic reagents. Some differences have been interpreted in terms of a preliminary complex formation between the reactivator and inhibited enzyme.

3. Activation energies of 11–12 kcal./mole have been found for the reactivation by diisonitrosoacetone of ChE inhibited by TEPP or Sarin.

4. A bell-shaped pH/activity curve has been found for the reactivation by monooisonitrosoacetone of ChE inhibited by TEPP. This has been interpreted as due to reaction between the oxime anion and a protonated form of the inhibited enzyme.

5. Inhibition by organophosphorus compounds has been shown to be a two-stage chemical process, an initial form of the inhibited enzyme being converted at a first-order rate into a second form which can no longer be reactivated. This reaction is
catalysed by acid and may be due to an initial phosphorylation of histidine followed by migration of the phosphoryl group to the hydroxyl group of serine.

We are indebted to Miss June Petts for her careful and reliable technical assistance.

REFERENCES


A Comparative Study on the Metabolism of 140Ba and 45Ca in Rats

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The tracer technique has made possible measurements of various processes involved in growth and remodelling of the skeleton, and has thus contributed to a better understanding of bone physiology (Bauer, Carlson & Lindquist, 1955a). We have recently described a method with 32P as tracer for calculating the accretion rate of bone salt in humans (Bauer, Carlson & Lindquist, 1955b). Results have been obtained by this method in human cases of rickets and osteoporosis which indicate significant changes from the normal rate of accretion of bone salt (Bauer, Carlson & Lindquist, 1956). It seems probable that isotope methods can be used in humans, not only for basic research on skeletal metabolic disorders but also as diagnostic tests. Part of our research has been to find useful isotopes for this purpose.

Theoretically, skeletal accretion and resorption processes can be studied by means of isotopes of almost any element which forms an appreciable part of the skeletal tissue. For obvious reasons, however, isotopes labelling bone salt are better suited for this purpose than are those which label the organic tissue. Not all the phosphorus outside the bone salt is freely exchangeable, and therefore 32P is not suitable for calculations of the accretion rate in the whole skeleton. All extra skeletal calcium is freely exchangeable, but the commonly available 45Ca has too long a half-life (152 days) to permit a widespread use of this isotope in human experiments. Carrier-free 89Sr is metabolized by the rat skeleton exactly as is 45Ca (Bauer, Carlson & Lindquist, 1955c) but again the half-life (2.5 years) is too long. Other isotopes of strontium, however, may prove useful in humans [e.g. 87Sr (Spencer, 1955), half-life 65 days]. The similarity in the skeletal metabolism of 89Sr and 45Ca has prompted us to investigate isotopes of other elements with even shorter half-lives. The data of Hamilton (1947) suggest that the metabolic behaviour of barium and strontium are similar and 140Ba has a half-life of only 12.8 days.