ALTERATIONS IN SERUM ENZYMES AFTER REPEATED EXPOSURE TO MALATHION

BY

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The alterations in serum pseudo-cholinesterase, transaminases, and aldolase observed in 12 agricultural workers exposed to the organo-phosphorus insecticide, malathion, over a period of six months are recorded. A comparison with a series of unselected controls and a series of manual workers is made. Evidence is produced that the majority of the agricultural workers have been suffering from minor effects of poisoning.

The manifestations of acute intoxication by the more lethal of the organo-phosphorus insecticides have been closely studied and documented. The symptomatology differs only slightly with the compound involved, though the presenting features may be determined by the route by which the compound enters the body (Heath, 1961).

One of the least toxic phosphate ester insecticides in common use is malathion, S-(1:2-dicarboxyethyl) O, O-dimethyl phosphorodithionate. The relative safety of malathion is generally believed to be due to the existence of an enzyme, malathionase, in the liver of man and warm-blooded animals (Johnstone and Seward, 1960). Together with other organo-phosphate insecticides, malathion and/or its metabolites inhibit directly acetylcholinesterase (AChE) by phosphorylation and affect also directly other unrelated enzymes like dehydrogenases, oxidases, and ali-esterases in experimental animals and insects. Besides AChE, other enzymes in man may be indirectly affected, as observed by Fischetti (1957).

Experimental observations (Casula, Cherchi, and Spinazzola, 1959 a, b) have revealed a marked increase in serum aldolase activity in poisoned animals and a less marked rise in serum glutamic-oxaloacetic (GO-T) and glutamic-pyruvic (GP-T) transaminases and of malic and lactic dehydrogenase activity.

In acute poisoning by organo-phosphate insecticides, alterations in serum enzymes other than AChE as well as acute hypoglycaemia and plasma electrolyte changes have been reported by several workers (Casula, Cherchi, and Spinazzola, 1960 a, b; Hruban, Schulman, Warner, Du Bois, Bunnag, and Bunnag, 1963).

The effects of chronic exposure to these insecticides are more scantily recorded in the literature, and then they are concerned mainly with the effects on AChE. The changes in other esterases, transaminases, and aldolase in serum have been studied in a group of workers exposed to risk by Casula, Cherchi, and Spinazzola (1959 a, b).

The present paper reports the alterations in the activity of serum pseudo-cholinesterase (pChE), glutamic-oxalacetic (GO-T) and glutamic-pyruvic (GP-T) transaminases, serum aldolase, and serum albumin concentrations in 12 agricultural workers exposed to malathion over a period of six months. Because of the prevailing heat at the time of the spraying campaign, none of the workers followed the instructions on the wearing of the protective clothing provided. The results are compared with estimations of serum enzyme activities and albumin concentrations in two series of controls studied concurrently.

Materials and Methods

The sera tested were obtained from three classes of subjects. The first was made up of 12 agricultural workers who were to embark on a spraying campaign with malathion. Each subject was tested once before exposure to the insecticide; 10 subjects on three subsequent occasions, one subject on two other occasions, and one subject on one other occasion during the exposure period. The interval between tests was four to 10 weeks.

Thirty blood samples, providing the normal controls, were obtained from unselected healthy blood donors. As an additional control 30 samples were obtained from healthy blood donors engaged in manual labour.

Twenty-three sera from patients in hospital were
included to study the correlation between the two methods used for the estimation of pChE activity.

Serum GO-T, GP-T, aldolase, and albumin estimations were also carried out on the sera derived from agricultural workers and from healthy controls. Serum enzyme assays were in all instances carried out within six hours after venepuncture.

**Serum pseudo-Cholinesterase.**—The colorimetric method of de la Huerca, Yesinick, and Popper (1952) was employed. Acetylcholine bromide substrate is hydrolysed by cholinesterase to choline and acetic acid, and the amount of acetylcholine remaining after incubation is determined by means of the colour reaction with hydroxamic acid.

The unit of activity is that amount of cholinesterase which hydrolyses 1 μmole of acetylcholine per ml. of serum. By this method the normal range is 130 to 310 units per ml. of serum, and 2 units are equivalent to 1 Warburg unit (Smith, Loewenthal, Lehmann, and Ryan, 1959).

The test paper method, 'Acholest’, described by Churchill-Davidson and Griffiths (1961) was applied to 40 sera concurrently with the colorimetric method. The manufacturer’s instructions were followed, and readings were taken by the same person and under constant temperature and lighting conditions.

**Glutamic-oxalacetic and Glutamic-pyruvic Transaminases.**—The serum enzyme activity was assayed by the method of Reitman and Frankel (1957), using reagents supplied by Sigma Chemical Co., St. Louis, U.S.A. The results are expressed in Reitman-Frankel units per ml. serum and are equivalent to Karmen units. Normal values for SGO-T are 8 to 40 units per ml., and 5 to 35 units per ml. for SGP-T.

**Aldolase.**—The colorimetric method of Bruns (1954) was used. The reagents were supplied by C. F. Boehringer & Soehne, Mannheim, W. Germany. The unit of activity is that amount of enzyme which attacks 1/224 μmoles of fructose 1,6-diphosphate in one hour at 37°C. The normal range is 3-0 to 10-0 units per ml. of serum, average 6-0 units (Sibley and Fleisher, 1954).

**Serum Albumin.**—The biuret method for the estimation of serum albumin was used (King and Wootton, 1959).

**Results**

**Serum pseudo-Cholinesterase.**—Table 1 records the values of pChE. Pre-exposure and subsequent exposure values are tabulated against the two groups of controls.

Although the mean values for any period show no significant difference from either series of normal controls, yet the changes in enzyme activity of any single subject vary significantly after exposure (Table 2). A reduction in enzyme activity was observed in 11 of the 12 subjects at some time after exposure, and six showed a sustained fall till the end of the study. Two showed a slight increase over the pre-exposure level at the end.

**Table 2**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pre-exposure</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
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<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>102</td>
<td>105</td>
<td>114</td>
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<tr>
<td>2</td>
<td>100</td>
<td>91</td>
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<td>3</td>
<td>100</td>
<td>76</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>83</td>
<td>88</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>11</td>
<td>100</td>
<td>90</td>
<td>102</td>
<td>109</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
<td>81</td>
<td>77</td>
<td>-</td>
</tr>
</tbody>
</table>

The Figure below records the correlation between the serum pChE activity determined by the chemical method and by the screening 'Acholest' test paper,
expressed as the reciprocal of time in minutes. Forty sera obtained from healthy blood donors, agricultural workers, and patients in hospital were tested.

Serum Glutamic Oxalacetic-Transaminase.—Table 3 records the GO-T values. Pre-exposure and subsequent exposure values are tabulated against the two groups of controls. There is no significant difference between the two groups of controls or between the mean value of the agricultural workers at any period and the controls. However, there is a suggestion that there is some depression of enzyme activity, which is progressive, after exposure to the insecticide.

**TABLE 3**

VALUES OF GO-T IN AGRICULTURAL WORKERS AND CONTROLS

<table>
<thead>
<tr>
<th></th>
<th>Agricultural Workers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-exposure</td>
<td>Period 1</td>
</tr>
<tr>
<td>No. of subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range (units/ml. serum)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Mean value (units/ml. serum)</td>
<td>18-37</td>
<td>16-5-42</td>
</tr>
<tr>
<td>S.D. ±</td>
<td>28-9</td>
<td>24-3</td>
</tr>
<tr>
<td></td>
<td>6-1</td>
<td>6-46</td>
</tr>
</tbody>
</table>

**TABLE 4**

VALUES OF GP-T IN AGRICULTURAL WORKERS AND CONTROLS

<table>
<thead>
<tr>
<th></th>
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<th>Controls</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre-exposure</td>
<td>Period 1</td>
</tr>
<tr>
<td>No. of subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range (units/ml. serum)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Mean value (units/ml. serum)</td>
<td>12-46</td>
<td>14-5-41</td>
</tr>
<tr>
<td>S.D. ±</td>
<td>27-7</td>
<td>23-9</td>
</tr>
<tr>
<td></td>
<td>9-41</td>
<td>6-30</td>
</tr>
</tbody>
</table>

**TABLE 5**

VALUES OF SERUM ALDOLASE IN AGRICULTURAL WORKERS AND CONTROLS

<table>
<thead>
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<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-exposure</td>
<td>Period 1</td>
</tr>
<tr>
<td>No. of subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range (units/ml. serum)</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Mean (units/ml. serum)</td>
<td>6-7-167</td>
<td>3-0-129</td>
</tr>
<tr>
<td>S.D. ±</td>
<td>10-3</td>
<td>9-0</td>
</tr>
<tr>
<td></td>
<td>2-35</td>
<td>2-13</td>
</tr>
</tbody>
</table>

**TABLE 6**

SERUM ALBUMIN CONCENTRATIONS IN AGRICULTURAL WORKERS AND CONTROLS

<table>
<thead>
<tr>
<th></th>
<th>Agricultural Workers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-exposure</td>
<td>Period 1</td>
</tr>
<tr>
<td>No. of subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range (g./100 ml. serum)</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Mean (g./100 ml. serum)</td>
<td>4-20-5-64</td>
<td>4-44-5-06</td>
</tr>
<tr>
<td>S.D. ±</td>
<td>4-76</td>
<td>4-84</td>
</tr>
<tr>
<td></td>
<td>4-98</td>
<td>0-276</td>
</tr>
</tbody>
</table>
Serum Albumin.—Table 6 shows the serum albumin concentrations in agricultural workers and controls.

There is no significant difference between the agricultural workers at any time and the controls.

An additional point borne out by the findings reported above is that there is no correlation between the serum albumin concentration and the pChE activity of the separate groups. Failure of correlation was also observed between these two estimations of individual workers. Some degree of correlation is present, however, between the mean values of the activity of the serum enzymes assayed, but there is a complete lack of correlation between the enzyme activity of single workers tested over the period.

Discussion

This investigation was prompted by the knowledge that these agricultural workers had been using malathion at various times of the year, and that they had reported ill-defined complaints which could possibly be due to intoxication. It was then known that they were not wearing the recommended protective clothing because of climatic conditions. In spite of the acknowledged safety of malathion (acute single dose LD_{50} is more than 4,000 mg./kg. body weight), it was suspected that enough was being absorbed through the skin to provoke these symptoms.

It has been suggested by Holmes and Gaon (1956) that repeated minor exposures may give rise to symptoms at higher pChE levels than in acute poisoning. This is in direct conflict with Heath's (1961) view that no symptoms may develop when the pChE level is lowered slowly by spaced repeated doses. So it is conceivable that these subjects might in fact have been suffering from minor effects of chronic poisoning.

It is generally agreed that the most valuable indication of poisoning by an organo-phosphate insecticide is a reduction in activity of the serum or whole blood cholinesterase. However, poisoning by these compounds is not the only cause of a low pChE. It was therefore thought necessary to exclude associated liver disease. The concurrent assay of serum transaminases and aldolase and an estimation of the serum albumin concentration have been included for this purpose. These would also provide the opportunity of studying the effect of chronic exposure to malathion on these enzymes. Changes in these enzymes have been reported in acute intoxication by parathion by Casula, Cherchi, and Spinazzola (1960 b).

There is ample evidence that the level of various enzymes in the blood, including transaminases (Schlang and Kirkpatrick, 1961) and aldolase (Cantone and Cerretelli, 1960), is elevated after physical exertion. It is unlikely that the initial high concentration of pChE in the agricultural workers in this series was an effect of the physical effort entailed, because all members of the team had been on this type of work for a variable number of years. It was essential that a series of controls engaged in comparable manual labour, but not exposed to the insecticide, should also be studied for better evaluation of the results.

It is generally accepted that a value of pChE level of 50% of the normal mean indicates intoxication by organo-phosphate esters. Greater significance may be attached to the results if the pre-exposure value of the individual is known. A 50% reduction in enzyme activity after exposure provides an alerting signal (Gage, 1955). None of the workers included in this study showed such low values at any time, and the lowest post-exposure value ever recorded was 70% of the pre-exposure level for the individual. However, a detectable depression has been observed in 11 of the 12 workers in the series. Another finding that emerged was that two subjects ended up after six months with a significant increase in pChE when compared with their initial values. Locker and Siedek (1952) have suggested that low levels of inhibitors stimulate the synthesis of pChE, and poisoned animals may recover with pChE concentrations above normal. If such a reaction is a defensive one, and whether it is determined by the amount or mode of entry of the toxic material or whether it is in some way genetically determined, is not yet clear. The only two brothers in this investigation behaved very differently in their reaction, though it is obvious that the amount of organo-phosphate absorbed by each of them could not be determined.

The 'acholest' test paper method is a useful screening test for determining pChE, and any lack of accuracy is outweighed by the practicability of the procedure. Further, a fall in pChE activity beyond the normal limit is definitely accentuated by this method, so that there is absolutely no risk of failure to detect even cases of slight over-exposure with only about 70% of the normal pChE value.

The changes observed in the other serum enzymes, viz., GO-T, GP-T, and aldolase, have been rather surprising. Although there is no very remarkable difference in the mean values for the workers and the normal controls, it is evident that the mean value of all three enzymes is initially, though not significantly, higher. Following exposure to malathion, a progressive fall has been recorded. The present study does not allow the drawing of any definite conclusions, but it does suggest that the depression in these
EXPOSURE TO MALATHION

I am indebted to the Senior Occupational Health Officer, Malta, for the help and facilities he has given me, and to the Principal Government Statistician, Malta, for the statistical analysis of the results.

‘Acholest’ test paper was supplied by the Österreichische Stickstoffwerke Aktiengesellschaft, Linz/Donau, Austria.

REFERENCES

Pergamon, Oxford.

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