said whether or not their level falls outside the normal range, and if it is below normal it is good evidence that they have absorbed some of the inhibitor. Fortunately, the lower normal level of the population is well above the highest level at which symptoms of systemic poisoning are likely to become apparent.

If, however, two successive samples of blood can be taken over a period during which the individual was exposed to these compounds, or over a period immediately following such an exposure, it might be possible to show that the variations of his individual levels varied beyond the range of his personal normal level and indicated absorption of a cholinesterase inhibitor.

The limits have been accurately defined and the degree of significance of the variations has been described.

A more universal application of the technique will await the development of methods less complicated than the one used in this work and the adoption of these newer techniques as a routine method in chemical pathological laboratories.

Summary

The variations in plasma and red-cell cholinesterase have been determined in a large group of 247 healthy adults drawn from the armed Services and civilian population.

Limits of variation have been defined for the plasma enzyme for the whole sample, but separate limits were necessary for the red-cell enzyme from the civilian and Service groups. These limits lie between 57 and 143% of the mean value for the plasma enzyme and 78 and 122% of the mean red-cell enzyme value for Service personnel. The corresponding limits for the red-cell cholinesterase for civilians are 69 and 131% of the mean.

There were no sex, age, or seasonal differences in either enzyme.

It has been shown that the coefficient of variation is a valuable index for defining the limits of variation in both enzymes.

The personal variation has been studied in 10 soldiers by examining blood samples upon eight separate occasions.

It has been possible to make estimates of the fall in enzyme level necessary to indicate departure from the normal. This value varies with the number of initial observations, but is numerically the same (in enzyme units) for both plasma and red-cell enzymes.

A knowledge of the results here presented permits the early recognition of abnormal levels of red-cell and plasma cholinesterase.

We are greatly indebted to Surgeon Commander W. H. McKee, R.N., and to Dr. R. S. Tovey, Director of the South-western Region Blood Transfusion Service, for supplying us with blood samples. We wish also to thank the Service and civilian personnel who co-operated in this inquiry. Finally, we wish to record our appreciation of the careful and accurate assistance we have received from Miss A. M. Eyres and Corporal E. Kerr, R.A.M.C. Acknowledgment is made to the Chief Scientist, Ministry of Supply, for permission to publish this paper. Crown copyright reserved. Reproduced with permission of the Controller of H.M. Stationery Office.

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BLOOD CHOLINESTERASE LEVELS IN WORKERS EXPOSED TO ORGANO-PHOSPHORUS INSECTICIDES

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During the last twelve months several reports have appeared which emphasize the toxic properties of a group of substances commonly referred to as the organo-phosphorus insecticides (Report to Pesticides Committee, 1950; Goldblatt, 1950; Bidstrup, 1950; Grob *et al.*, 1950). At least 30 fatalities have occurred among workers handling these compounds in America (Abrams *et al.*, 1950). No fatal cases of poisoning have occurred in Great Britain, and only a single non-fatal case has been reported (Milles and Salt, 1950). A working party has been set up by the Ministry of Agriculture to consider the various problems that arise in connexion with the use of toxic chemicals in agriculture. Its report and recommendations are awaited.

These organo-phosphorus insecticides are valuable adjuvants to modern agricultural practice. It is obvious that, like any other poisonous compounds, they can be handled only if suitable safe techniques can be devised. One very important safety procedure would be the development of a clinical test that would make it possible to detect the absorption of the poisons before enough had been taken in to produce a dangerous or fatal intoxication.

All the organo-phosphorus compounds used as insecticides have one common property. When they are introduced into the body they inhibit the enzymes that hydrolyse acetylcholine. In man both the "pseudo" and the "true" cholinesterase circulate in the blood; the former is found in the plasma and the latter in the red blood corpuscles. Furthermore, observations by one of us (D. R. D.) on animals have shown that the amount of these enzymes circulating in the blood may be lowered by at least 80% without symptoms of poisoning (due to the accumulation of acetylcholine) appearing.

Since techniques for measuring the level of cholinesterase in the blood are available, a determination of the cholinesterase level at any given time could be used to decide whether the poison was being absorbed even in quantities too small to produce symptoms of poisoning. Garlick (1950) has already emphasized the value of blood cholinesterase determinations to those responsible for the medical care of workers exposed to these insecticides during their manufacture.

Ideally, a series of determinations of the blood cholinesterase should be done on any individual before, during, and after exposure to the insecticides, whether in the factory or in the field. Callaway *et al.* (1951) have determined the range about which the level of a normal individual may vary, and so it would not be possible to say in any individual case whether or not a reduction

in the level of cholinesterase below the level found before exposure was significant and therefore most probably due to absorption of the insecticide. The individual could then be removed from further contact until his cholinesterase level had returned to normal. Grob *et al.* (1947) have shown that the complete restoration of the blood cholinesterase levels in man is comparatively slow.

It is possible to carry out regular cholinesterase determinations on the same individual with little inconvenience to the worker if the modification of Davies *et al.* (1951) to the method of Michel (1949) is employed, using only a drop of blood.

The observations to be described in this paper were made with the technique described in the preceding paper (Callaway *et al.*, 1951) upon plasma and washed red cells. For this purpose a sample of blood had to be obtained by venepuncture. Because of the difficulties of obtaining repeated samples from the workers concerned, only single observations were made in the great majority of cases.

Callaway *et al.* (1951) have determined the range of blood cholinesterase levels for two healthy adult populations and also the range of variation in the level of healthy Service personnel on whom separate determinations were made at different times. It is the existence of these data which has imparted significance to the observations made in this paper.

Materials and Methods

Examination has been made of 130 specimens of blood from 80 men and women who have been exposed to these insecticides in field or factory. These workers were civilians whose ages ranged from 17 to 59, and it is therefore appropriate that the cholinesterase levels observed among this group should be compared with the values for a mixed civilian population rather than the higher levels of the Service personnel group (see Callaway *et al.*, 1951). The specimens were obtained from 30 factory and 50 agricultural workers. The factory workers were employed at three separate factories. The agricultural workers came from orchards in Essex, Sussex, and Worcester, together with a number of men employed on contract spraying in parts of Kent and East Anglia.

The factory workers included both those packing or preparing concentrated materials and also laboratory workers in contact with a number of different active compounds. The majority of the field workers were farm employees doing fruit-spraying as one of their normal activities. All of them had been working for at least two days continuously with the insecticides before their blood was examined. (The normal period of spraying with this type of worker was never more than three to four days continuously.) Only a few of these men come into contact with the concentrated materials, as one man would act as the mixer for a large group applying the spray.

All the field workers and the majority of the factory workers were exposed to insecticides containing either "parathion" (main active principle, 0.0-diethyl-*para*-nitrophenyl-thiophosphate) or "schradan" (active principle, octamethyl-pyrophosphonamide). A few factory workers were exposed to other compounds in the laboratories of the factory concerned.

Blood was collected, either during or at the end of a working day in the field or factory, in a Behring venule containing oxalate. All specimens were sent through the post to a central laboratory, where enzyme analyses were made. There was an inevitable delay of at least 24 hours between taking the blood and the estimation of cholinesterase. This delay was, however, unimportant, since it had previously been shown (Callaway *et al.*, 1951) that the levels in stored blood are unaltered over a period of at least five days, so long as the red cells and plasma are separated before the onset of haemolysis. Under normal conditions of storage even at atmospheric temperatures no significant haemolysis takes place within the first four days in oxalated blood. It is important, therefore, to note whether or not haemolysis has taken place in any specimen of blood where there has been a delay between the time of its collection and the determination of the cholinesterases.

Determinations of plasma and red-cell cholinesterase were made by the method described by Callaway *et al.* (1951). The values of red-cell and serum cholinesterase are expressed in units of enzyme, one unit being the amount of enzyme present in 1/30 ml. of cells or serum which produces one microlitre of carbon dioxide in 30 minutes.

Results

Callaway *et al.* (1951) have found that the red-cell cholinesterase levels of a healthy civilian population will fall between the range of 75 and 142 units. The chances of a healthy individual having a red-cell cholinesterase level below 75 units would be 1 : 40.

Of the 80 individuals examined during this investigation three only were found to have levels below 75 units. All were factory workers and therefore liable to come into contact with strong solutions of the insecticide. Only one exhibited any symptoms, and these were mild. Although the reduction in each case was only just below the normal minimal level in the civilian population, the level of the enzyme rose in two of the subjects after their removal from contact with these materials.

It thus seems reasonable to conclude that in at least two individuals the depression of cholinesterase levels of the red blood corpuscles had resulted from the absorption of small quantities of the insecticides to which they were exposed.

TABLE I.—Red-cell Cholinesterase Levels in Three Male Factory Workers Found to Have Reduced Levels After Exposure to Insecticides

	Cases		
	L.H.	W.F.	R.N.
Red-cell enzyme (units) immediately after exposure	73	69	70
Symptoms	Nil	Nil	Slight tightness of chest
Highest value observed after removal	124	100	—
Estimated rate of regeneration (% per day)	1.0	0.8	—

Normal range, 75–142 units.

Examination of the cholinesterase of the plasma has permitted the recognition of departures from normal levels in five persons, four of whom were laboratory or factory workers, and one a sprayer. The normal lower level of plasma cholinesterase is 51 units. The levels observed in the five cases varied from 44 to 49 units. As in the case of the red cell, the deviation was small but nevertheless quite definite. No symptoms

were observed. This is in keeping with the findings of Mendel, Hawkins, and Nishikawara, who have shown that the plasma enzyme may be inhibited very considerably without the development of symptoms. The results are summarized in Table II.

TABLE II.—*Plasma Enzyme Cholinesterase Levels in Five Insecticide Workers Found to Have Reduced Levels After Exposure*

Case	Enzyme Units	Symptoms	Occupation
F.S.	49	Nil	Sprayer
D.P.	46	"	Research worker
L.H.*	48	"	Factory worker
R.N.*	45	"	"
E.B.	44	"	"

Normal range, 51-127 enzyme units.

* Red-cell enzyme also low.

A third criterion exists for the recognition of disturbances in the normal level of the red-cell or plasma enzymes. It is based upon a knowledge of the limits of the personal variation in the enzyme levels of healthy individuals. The range of these limits and the evidence from which they have been derived are given in the preceding paper (Callaway *et al.*, 1951). In the case of the plasma enzyme a difference of more than 18 units indicates that there has been some abnormal disturbance in the level of this enzyme.

In this inquiry blood specimens were taken on more than one occasion from 14 workers. In eight of these cases the personal variation was greater than normal. The results are presented in Table III. In four cases the level of the enzyme rose in the interval between the determinations. These workers had been removed from contact with the insecticides, and the rise indicated a recovery from the effects of absorption prior to the first determination. In the other four cases contact with the insecticides continued during the interval between the taking of the blood samples. The fall indicated that some absorption of the compounds had taken place though none of the workers developed symptoms.

Thus of the 80 workers examined, 12 showed evidence of absorption of the insecticides with which they had been in contact.

TABLE III

Case	Occupation	Sex	Units of Plasma Enzyme		Difference	
			Initial	Final		
(a) Cases showing recovery from the effects of previous absorption of insecticide						
F.S.*	..	Sprayer	M	49	76	27
R.B.	..	"	M	56	83	27
E.B.*	..	Chemical worker	M	44	82	38
H.H.	..	"	F	51	73	22
(b) Cases showing evidence of absorption of insecticide during interval between the determinations						
E.P.	..	Sprayer	M	104	80	24
M.L.	..	Chemical worker	F	99	74	25
J.W.	..	"	F	79	60	19
C.C.	..	Laboratory worker	F	97	69	28

* Initial values below the normal range.

The estimation of blood cholinesterase was also used as an aid to diagnosis in some instances. The early symptoms of poisoning by these insecticides are mundane in character—headache, dizziness, nausea, abdominal colic, etc. Such symptoms occurring in individuals exposed to these materials would arouse suspicions of poisoning, though similar complaints in unexposed

people might be passed over as of no particular importance. A determination of the blood cholinesterase levels would show whether in fact absorption of the insecticide was responsible for the symptoms. In three individuals complaints such as giddiness, abdominal pain, and nausea were made after exposure to insecticides. A determination of the red-cell enzyme showed values in each case practically at the top of the normal range. Absorption of the insecticide was thus excluded. However, local symptoms such as contraction of the pupil and tightness of the chest may result from contact with airborne particles without there being enough absorbed to produce general symptoms or even a detectable reduction in the cholinesterase levels in the blood stream.

Discussion

Slight but statistically quite significant departures from normality have been observed in the levels of blood cholinesterase in 12 out of a group of 80 workers examined during or after exposure to organo-phosphorus insecticides in the factory or field.

Advanced liver disease and severe malnutrition (McCance, 1950) are the only relatively common causes of a depression of the level of blood cholinesterase, and these can be disregarded in considering the population examined during this inquiry.

The results obtained demonstrate effectively the value of blood cholinesterase determinations as part of the medical supervision of workers exposed to the organo-phosphorus insecticides. In none of the 12 workers showing deviations from normal were there unequivocal symptoms of poisoning, and this can be explained in the light of experiments that have shown that the blood cholinesterase must be depressed to less than 20% of its normal value before symptoms of systemic poisoning appear.

In this inquiry it has been possible to extract information from single determinations of the enzyme levels in individuals. This has been possible only because these results could be compared with data obtained from a large number of determinations from a healthy population.

It is more desirable that a series of determinations should be made on individuals before, during, and after exposure to these materials. Rapid methods requiring only a drop of blood are obviously called for in this kind of work. Such methods do exist, and there is no reason why a sufficiently high standard of accuracy could not readily be reached by any efficient laboratory.

The observations reported here illustrate a number of points. These materials can be handled by field and factory workers without significant absorption taking place. It must, however, be emphasized that the men observed were all told of the poisonous nature of the material they were handling, and, furthermore, that those responsible for the use of these materials recognized that they were in possession of valuable insecticides and that any disaster occurring in the early stages of its introduction might have serious repercussions. All the field workers were observing simple but practical precautions such as the wearing of waterproof gloves, coats, and hats.

The greatest danger lies in the handling of the concentrated solutions of the insecticides. This is indicated by the fact that nine of the 12 workers showing evidence of absorption were factory workers.

Nearly all the reports upon the use of these substances in the field have rightly stressed their extremely toxic nature. In so doing they have tended to throw the issues out of perspective. Garlick (1950) has rightly drawn attention to the fact that many thousands of square miles have been sprayed with parathion without any accidental poisoning. The results of the present investigation offer some information on this issue. Fifty sprayers have been examined during the height of the spraying season. Between them they had sprayed hundreds of acres of fruit trees and thousands of trees. Only three of them exhibited even the slightest indication of absorption. Thirty factory workers or laboratory workers were examined. Only the use of the most stringent criterion of departures of cholinesterase levels from normality permitted the recognition of any abnormality in nine of them. Ten workers who had been engaged continuously in the manufacture of parathion for two years had normal levels of blood cholinesterase.

These facts demonstrate quite clearly that the organo-phosphorus insecticides may be manufactured and applied without risk provided manufacturer, consumer, and their employees co-operate by the rigid adherence to every form of safety device and procedure which has been developed for that end.

The observations here reported provide no grounds for complacency, and those who use or are responsible for encouraging the use of these materials should remember that freedom from accidents in this country during the past year has been the result of the heed given to early warnings rather than to the innocuous nature of the materials handled.

Summary

The cholinesterase levels of 80 men and women exposed to organo-phosphorus insecticides in the field or factory have been determined.

Three criteria of departure from normal variation of enzyme levels have been described. On the basis of these standards abnormal variations which are probably attributable to insecticide absorption have been recognized in 12 individuals.

The departures from normality were very slight. No serious case of poisoning was observed.

These observations emphasize the value of the test, and their significance in relation to the incidence of insecticide poisoning in field and factory workers is discussed.

We wish to acknowledge the generous help and co-operation which we received from those firms and workers, both agricultural and factory, who made this research possible. We are also very appreciative of the careful technical assistance we received from Miss A. M. Eyres. Acknowledgment is also made to the Chief Scientist, Ministry of Supply, for permission to publish this paper. Crown copyright reserved. Reproduced with permission of the Controller of H.M. Stationery Office.

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FAILURE OF ORAL D.D.T. TO INDUCE TOXIC CHANGES IN RATS

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Dichloro-diphenyl-trichloroethane (D.D.T.) remains one of the most widely used of the synthetic insecticides. Appreciable quantities may be found on food, and one report shows that fat taken from ordinary citizens not considered to have been unduly exposed to D.D.T. may contain up to 30 p.p.m. (parts per million) of the insecticide (Laug, Kunze, and Prickett, 1951). At the present time nothing is known about the action of D.D.T. beyond the fact that it interferes in some way with the normal function of the nervous system. A detailed search failed to reveal any evidence of interference with a number of enzyme systems (Judah, 1949). For this reason special importance must still be attached to histological evidence of tissue damage or change occurring in animals exposed to D.D.T.

The pathological changes occurring in animals dying of acute poisoning by D.D.T. have been described, and the most striking lesion is a severe liver necrosis (Cameron and Burgess, 1945). Despite this obvious damage to the liver tissue animals in chronic poisoning die from causes associated with injury to the central nervous system and do not show the characteristic signs of acute liver injury. Fitzhugh and Nelson (1947) claimed that they could see characteristic liver lesions in rats that had received a diet containing 100–800 p.p.m. of D.D.T. for a period of two years. The changes they found were hypertrophy of the centrolobular hepatic cells with increased oxyphilia, increased basophilia, and margination of cytoplasmic granules and a tendency to hyalinization of the remainder of the cytoplasm. Only those animals receiving 400 p.p.m. or more of D.D.T. showed any clinical evidence of poisoning.

Laug, Nelson, Fitzhugh, and Kunze (1950) fed rats for 15–27 weeks on diets containing 1 to 50 p.p.m. D.D.T. and claimed that similar lesions developed in the liver cells of rats receiving as little as 5 p.p.m. D.D.T. These changes were much less prominent in the livers of other rodents and were never found in dogs, cats, monkeys, or the larger experimental animals. The conclusion they drew that as little as 5 p.p.m. of D.D.T. in the diet can produce recognizable changes in the structure of the hepatic cells of the rat has obvious and serious implications bearing on the question of what should be the maximum permitted limit of D.D.T. in foodstuff for human or animal consumption. The implication that a concentration of D.D.T. might be harmful has been accepted in America (Dunbar, 1949).

In 1947 a series of long-term feeding experiments on rats, fowls, sheep, pigs, and cattle were started by the Agricultural Research Council.* A large number of rats

*The Toxicity Subcommittee of the Research and Development Co-ordinating Committee on Insecticides initiated and supervised this work.