CONVERSION OF O,O-DIETHYL-O-*p*-NITROPHENYL THIOPHOSPHATE (PARATHION) INTO AN ACETYLCHOLINESTERASE-INHIBITOR BY THE INSECT FAT BODY

by

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INTRODUCTION

The toxicity of the insecticide O,O-diethyl-O-*p*-nitrophenyl thiophosphate (parathion, E 605) is explained by the inhibition of acetylcholinesterases in the body. Originally it was thought that parathion itself was a strong inhibitor of acetylcholinesterase *in vitro* too, and it was stated that a concentration of $1.2 \cdot 10^{-6}$ *M* produced 50% inhibition of rat brain cholinesterase (DUBOIS *et al.*[1], ALDRIDGE[2]). This statement did not hold true, however, for highly purified samples of parathion. In 1951 DIGGLE AND GAGE[3] demonstrated that parathion purified by chromatography did not show any inhibition of rat brain cholinesterase at all *in vitro*. Normal samples of "pure" or technical parathion show a very wide range in inhibitory activity, owing to the variation in the percentage of O,S-diethyl-O-*p*-nitrophenyl thiophosphate, the S-ethyl isomer of parathion. Using the same material, ALDRIDGE AND BARNES[4] could confirm the results of DIGGLE AND GAGE. Independently of the authors mentioned above, STEGWEE[5] in our laboratory showed that highly pure parathion did not have an *in vitro* inhibitory activity against the acetylcholinesterase of the central nervous system of the American cockroach, *Periplaneta americana* L., whereas cholinesterase activity had been reduced to zero in roaches that died after being poisoned with parathion.

In experiments with rats, MYERS, MENDEL *et al.*[6] studied, both *in vitro* and *in vivo*, the relative inhibitory potencies of parathion, of the S-ethyl isomer and of O,O-diethyl-O-*p*-nitrophenyl phosphate (paraoxon, E600, the oxygen analogue of parathion). From their results the authors surmised that the inhibitory activity of parathion *in vivo* must be due largely to a conversion to paraoxon in the body. DIGGLE AND GAGE[7] showed that incubation with rat liver slices converted parathion into a cholinesterase-inhibiting substance. While it was made clear that the liver cannot be the sole site of this conversion, so far only experiments with liver tissue yielded positive results. In a subsequent communication GAGE AND PAYTON[8] claimed to have demonstrated by chromatographic analysis the occurrence of paraoxon in the liver of rats poisoned with parathion.

In this way the toxicity of parathion in mammals can be explained by an enzymic oxidation of the P=S bond to a P=O bond in the mammalian body, after this conversion the strong acetylcholinesterase-inhibitor paraoxon exerts its action. On the basis of former experiments in our laboratory, as mentioned above, it seemed probable

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that a similar reaction takes place within the insect body, when parathion is applied as an insecticide. After repeating the incubation-experiments of DIGGLE AND GAGE, we have now tried to verify this hypothesis for the insect body and to localise the organ or organs responsible for this conversion.

EXPERIMENTAL

In the incubation experiments with rat liver, blood of a heparinized white rat was incubated with liver slices of the same animal and with parathion. The liver slices were made according to DEUTSCH[9]. In the experiments with insects, *Periplaneta americana* L. was used as test animal. 0.7 ml defibrinated cow blood, 0.1 ml parathion solution (0.00115% in 0.1% ethyl alcohol solution) and 25 mg tissue were incubated for 50 minutes. All incubations were carried out at 37° C. The following tissues were examined: fat body, muscles, cuticle, central nervous system and malpighian tubes. After incubation cholinesterase determinations were carried out in triplicate by means of the Warburg manometric technique similarly as described previously[10]. The medium used was a 0.025 *M* bicarbonate solution, saturated with 5% CO_2 + 95% N_2; the temperature was 37.0° C. The enzyme activity was expressed as b_{30}, representing the amount of CO_2 in μl evolved during 30 minutes by the quantity of enzyme present in 100 mg tissue or blood (when the course of the evolved CO_2-time curve is linear).

The parathion used was supplied and purified by Mr. H. R. GERSMANN of the Laboratory for General and Inorganic Chemistry, to whom we are very indebted. A difficulty arose out of the fact that purified samples of parathion, which did not have an inhibitory activity against cholinesterases, acquired this activity after some weeks or months. In one extreme case, a sample of parathion, which in a concentration of 0.0115% did not possess any inhibitory potency at all, was capable of a complete inhibition of the cholinesterase-activity of cow blood after three weeks. The conversion occurring in stored parathion is unknown so far, but will be discussed later on. In the course of our experiments, the usual successful method of purification by recrystallization failed, presumably due to a different composition of the technical parathion obtained. Now a chromatographic purification was tried; even the sample purified in this way showed a weak inhibitory activity against Periplaneta acetylcholinesterase; in the concentration used however (0.00115% parathion incubated with 0.7 ml blood, see above) the cholinesterase activity of the blood was inhibited only very slightly. In every experiment this inhibition was determined and accounted for.

RESULTS

Experiments with rat liver

In general the results of the short communication of DIGGLE AND GAGE[7] could be confirmed. Fig. 1 shows the results of a typical experiment.

In this experiment two incubations were carried out: *a.* 0.14 ml 0.015% parathion solution was incubated with 3.5 g liver slices and 1 ml heparinized rat blood and *b.* 0.11 ml parathion solution incubated with 3.5 g liver slices and with 0.8 ml plasma of the same rat, both for 15 minutes at 37° C. Afterwards the cholinesterase activity of 0.2 ml blood and of 0.2 ml plasma was determined, together with the activities of the non-incubated blood and plasma. As can be calculated from Fig. 1, the cholinesterase activity of the blood is decreased by the incubation from a $b_{30} = 53$ to a $b_{30} = 0$ (100%), while the plasma-activity decreased from a $b_{30} = 54$ to a $b_{30} = 28.5$ (55%). Incubation of blood with liver slices alone or with parathion alone did not result in a decreased enzyme activity. We observe from the data that the substance into which parathion is converted during the incubation inhibits the red blood cell acetylcholinesterase more strongly than the non-specific rat plasma cholinesterase.

The decrease in cholinesterase activity of the blood was not 100% in all experiments. In a number of determinations the decrease varied between 20 and 50%, while it was 100% in the other ones. Thus it seems that in these types of experiments with rat liver there are still one or more factors involved, which at present are not yet controlled by us.

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At first we thought that the different results which were obtained depended upon the fact whether the incubation was carried out aerobically or anaerobically. Later experiments, however, in which the incubations were carried out at the same time either in a nitrogen atmosphere (constant stream of nitrogen through the incubation tube) or in an oxygen atmosphere, showed no difference between the two procedures. As technical nitrogen was used, the atmosphere was not strictly anaerobical, but in any case variations of the partial oxygen pressure between wide ranges do not influence the results.

In some experiments no liver slices were used, but liver tissue, ground extensively in a mortar. When tissue prepared in this way was used for the incubation, an inhibition of enzymic activity was not observed. So it is possible that intact liver cells are necessary for the conversion of parathion during the incubation. Preliminary experiments, in which ATP was added to the ground liver tissue, did not show an increased conversion.

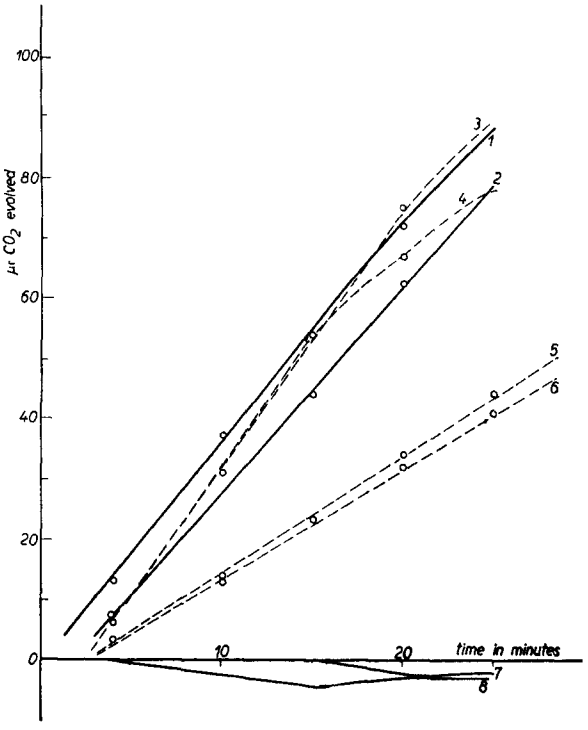

Fig. 1. Two tubes incubated for 15 minutes at 37° C, the contents were: *a*. rat blood + liver slices + parathion and *b*. rat plasma + liver slices + parathion (see text). The cholinesterase-activity of 0.2 ml blood from *a* (curves 7 and 8) and of 0.2 ml plasma from *b* (curves 5 and 6) were determined afterwards and compared with the activities of non-incubated blood (curves 1 and 2) and plasma (3 and 4) as shown.

Experiments with Periplaneta

In the first experiments defibrinated cow blood was incubated at 37° with parathion and slices of one whole roach; determinations of the cholinesterase-activity of the blood afterwards showed a decrease in activity, compared with that of the blood incubated with parathion alone. In later experiments slices of the head did not show any similar capacity of a transformation of parathion into a cholinesterase-inhibitor; so several organs of the abdomen were examined. Of those examined, the intestine showed a considerable decrease of the cholinesterase of the blood by incubation with blood and parathion.

Afterwards this decrease was found to be caused by the intestinal contents (possibly due to the presence of proteinases) as washed intestines did not show a similar decrease. Incubation of blood and parathion with a *Periplaneta* fat body seemed to give a slightly decreased cholinesterase-activity. In order to get incontestable results, more than one fat body had to be used. Nearly all later experiments were carried out with about 25 mg tissue; in order to reach this amount, fat bodies of fifteen animals were necessary. Fig. 2 shows the results of a typical experiment.

It can be calculated from Fig. 2, that incubation of blood together with parathion

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and fat bodies resulted in a 43% decreased cholinesterase-activity (curves 6 and 7, compared with curve 1). As the presence of parathion in itself could not explain this decrease (curves 2 and 3, compared with 1) and the presence of fat bodies alone was without significant effect (compare curves 4 and 5 with 1), it is clear that during the incubation with fat bodies parathion is converted into a more potent cholinesterase-inhibitor.

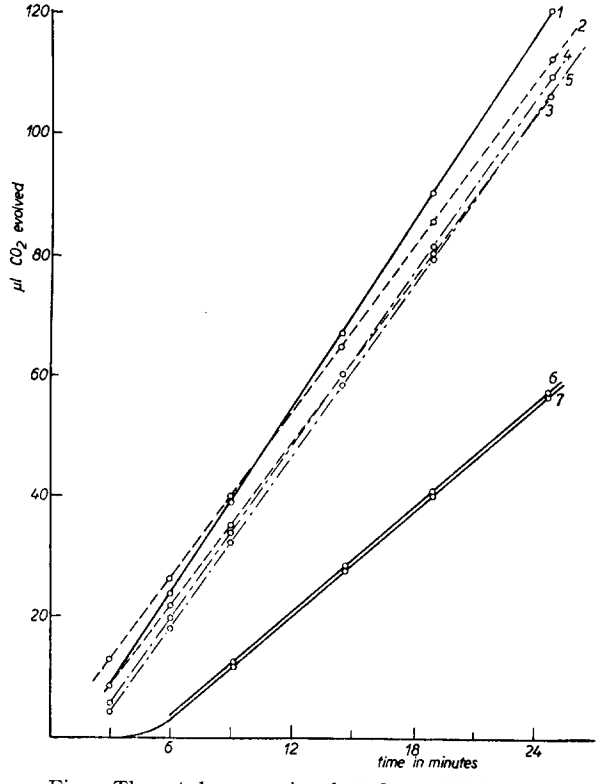

Fig. 2. Three tubes were incubated at 37° C for 15 minutes; the contents were: *a*. 0.7 ml defibrinated cow blood + 0.1 ml 0.00115% parathion, *b*. 0.7 ml cow blood + 25 mg fat bodies, *c*. 0.7 ml cow blood + 0.1 ml 0.00115% parathion + 25 mg fat bodies. Afterwards the cholinesterase-activity of 0.2 ml of the non-incubated blood (curve 1) was compared with that of 0.2 ml blood, withdrawn from tubes a (curves 2 and 3), b (curves 4 and 5) and c (curves 6 and 7). The enzyme activities, expressed in b_{30} are: 1:148, 2 and 3:136 and 134, 4 and 5:141 and 140, 6 and 7:85 and 84. The b_{30} of 1 is highest, because here the blood has not been diluted by 0.1 ml parathion solution.

The experiment from Fig. 2 has been repeated many times. While the results from the experiments with rat liver showed a wide range in the data, in these incubations with fat bodies the results were very constant. The decrease in cholinesterase-activity caused by incubation with fat bodies under the circumstances of Fig. 2, proved to be 44.6% ± 8.3% in 14 incubations.

When the fat bodies were ground in a mortar, their capacity to convert parathion into a cholinesterase-inhibitor was decreased considerably.

Other insect tissues were also investigated. Table I shows the results of the experiments. All the figures were obtained with incubation of 25 mg tissue; in every experiment a successful incubation with fat bodies was carried out simultaneously.

It is evident that incubation with muscles or cuticle has no effect on parathion. There is a rather wide variation in the results of incubation with nervous tissue or malpighian tubes, so that it is difficult to draw any conclusions with regard to these tissues.

TABLE I

Number of experiments	*Tissue*	*% Inhibition*
14	Fat bodies	44.6 ± 8.3
3	Muscles	3.0 ± 3
3	Cuticle	2.9 ± 5.5
4	Nervous system	12.7 ± 6.2
4	Malpighian tubes	14.3 ± 8.7

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DISCUSSION

In a paper, published when our experiments were nearly completed, PAYTON[11] described how parathion solutions develop anti-cholinesterase activity, when exposed to ultraviolet light. This activation occurred too at room temperature in solutions exposed to daylight; a transformation of parathion to paraoxon seemed most likely to the author. As mentioned in the experimental section, we encountered the same difficulties with our purified parathion samples. Our troubles disappeared also when we stored our samples in the dark.

In the experiments with rats most results of DIGGLE AND GAGE[7] could be confirmed. Our quantitative data vary a great deal however, the exact reason of which is not known. ALDRIDGE AND BARNES[4] describe a different sensitivity towards parathion for both male and female rats. It is possible that this difference is coming into play in our results too.

DIGGLE AND GAGE mention that either liver slices or homogenates can be used; our few experiments with ground liver were not successful.

Fig. 1 shows that the parathion after incubation with liver slices is able to inhibit the cholinesterase-activity of rat blood for 100%, that of plasma for only 53%. In a recent paper, DAVISON[12] describes that a paraoxon concentration, which inhibits for 90% rat brain acetylcholinesterase, gives only 50–60% inhibition of the nonspecific cholinesterase. These data fit nicely with ours, making it more probable that the parathion during the incubation has been converted in paraoxon.

The experiments with *Periplaneta* demonstrate in the first place that in insects also parathion is converted into a cholinesterase-inhibitor. No attempts were made to identify this inhibitor; direct identification would be rather difficult, owing to the small weights of the fat bodies involved. The hypothesis that in insects also parathion is oxidized to paraoxon, is obvious however.

As shown in Fig. 2 the *Periplaneta* fat body is the most active tissue in this conversion of parathion. There may be such a conversion in malpighian tubes or nervous tissue. With muscles and cuticle, however, this conversion could not be demonstrated.

The insect fat body may be considered to possess certain of the functions of the vertebrate liver. The fat body is the principal site for glycogen storage; furthermore there are indications that in the fat body glycogen is synthesized from other dietary carbohydrates and it is probable that this organ is the major site for interconversions among fats, proteins and carbohydrates[13]. Now we found that the conversion of parathion into a cholinesterase-inhibitor is another property which *Periplaneta* fat body and the mammalian liver have in common. In mammals the liver is the most active organ for this conversion, while in roaches it is the tissue of the fat body.

SUMMARY

1. Pure parathion (O,O-diethyl-O-*p*-nitrophenyl thiophosphate), which does not possess an *in vitro* inhibitory activity against cholinesterases, acquires this inhibitory capacity after incubation with insect tissue.

2. Among the tissues of *Periplaneta americana* L. investigated, the fat body was the most active in this conversion of parathion.

3. The capacity for this conversion of parathion (probably into O,O-diethyl O-*p*-nitrophenyl phosphate, paraoxon) is another property, which the fat body of the insect shares with the mammalian liver.

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RÉSUMÉ

1. Le parathion pur (O,O-diéthyl-O-*p*-nitrophényl thiophosphate) qui n'exerce pas, *in vitro*, d'action inhibitrice sur la cholinestérase, acquiert des propriétés inhibitrices lorsqu'il est incubé avec un tissu d'insecte.

2. De tous les tissus de *Periplaneta americana* L. essayés, c'est le corps gras qui est le plus actif dans cette transformation du parathion.

3. La capacité de transformer le parathion (probablement en O,O-diéthyl-O-*p*-nitrophényl phosphate, paraoxon) est une des propriétés communes au corps gras des insectes et au foie des mammifères.

ZUSAMMENFASSUNG

1. Reines Parathion (O,O-Diäthyl-O-*p*-nitrophenylthiophosphat), das *in vitro* keine hemmende Wirkung auf Cholinesterasen ausübt, erhält diese hemmende Fähigkeit nach Inkubation mit Insektengeweben.

2. Unter den untersuchten Geweben von *Periplaneta americana* L. besass der Fettkörper die grösste Aktivität bei dieser Umwandlung des Parathions.

3. Die Fähigkeit zur Umwandlung des Parathions (wahrscheinlich in O,O-Diäthyl-O-*p*-nitrophenylphosphat, Paraoxon) ist eine Eigenschaft, die der Fettkörper der Insekten mit der Leber der Säugetiere teilt.

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