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THE INFLUENCE OF OXIMES ON THE DISTRIBUTION OF ^{32}P IN THE BODY OF THE RAT AFTER INJECTION OF ^{32}P-SARIN

R. L. POLAK and E. M. COHEN

Medical Biological Laboratory of the National Defence Research Organization TNO, 139 Lange Kleiweg, Rijswijk(ZH), The Netherlands

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Abstract—One hr after the i.v. injection of 50 μg/kg of ^{32}P-sarin into rats considerable concentrations of ^{32}P containing material were found in the blood, the kidneys and the lungs and relatively small concentrations in the brain and the m.gastrocnemius. In rats which had received an i.p. injection of DAM or MINA 1·5 min after the ^{32}P-sarin decreased amounts of ^{32}P in the blood plasma and increased amounts in the liver were found. P-2-AM did not influence the distribution of ^{32}P in the body of the rat after injection of ^{32}P-sarin.

In anaesthetized rats injected with ^{32}P-sarin about 30 per cent of the injected amount of ^{32}P was excreted 45 min after the injection and about 40 per cent 90 min after the injection.

In ^{32}P-sarin injected anaesthetized rats in which the radioactivity of the blood circulating through an extracorporal cannula was measured continuously, DAM and MINA administered at different intervals after the nerve gas, produced sudden decreases in the ^{32}P-concentration of the blood. It could be demonstrated that a simultaneous rise in the liver radioactivity occurred.

The influence of the i.v. injection of 50 μg/kg of sarin into rats and the effect of the subsequent i.p. injection of therapeutically effective amounts of P-2-AM, DAM and MINA on the ChE- and AE-activities of the brain, the blood plasma and the liver were examined. One hr after the injection of the nerve gas the ChE-activities of the plasma and the brain and the AE-activity of the plasma were partly inhibited. DAM and MINA reactivated the plasma AE, MINA partially reactivated the ChE-activities of the plasma and the brain.

It was concluded that the ^{32}P-concentration of the plasma presumably represented phosphorylated AE and that the radioactivity found in the brain and the liver was not due to ^{32}P attached to either AE or ChE.

PRE-TREATMENT of rats with TOCP, an irreversible inactivator of plasma AE,* strongly enhances the toxicity of the ChE-inhibitor sarin and profoundly changes the distribution of ^{32}P in the body of the rat after injection of ^{32}P-sarin. It reduces the amounts of ^{32}P in the plasma and increases the ^{32}P-activity of the brain.[1, 2] From these observations the importance of the plasma AE for the distribution of sarin in the body of the rat became apparent.

Several oximes are therapeutically effective against nerve gas poisoning. Some of

* The following abbreviations are used: ChE (cholinesterase), AE (aliesterase = tributyrinase), sarin (isopropyl methylphosphonofluoridate), P-2-AM (pyridine-2-aldoxime methyl iodide = 1-methyl-2-hydroxyiminomethylpyridinium iodide), MINA (monoisonitrosoacetone=2-oxopropanal-1-oxime), DAM (diacetylmonoxime = 2,3-butanedione-oxime), TOCP (triorthocresylphosphate), ACh. (acetylcholine chloride).

these agents such as P-2-AM and MINA quickly reactivate phosphorylated ChE. DAM, on the other hand, is only a relatively weak reactivator of sarin inhibited ChE in rats[3] although it has a strong antidotal action against sarin poisoning in this animal. It has been postulated that DAM owes this antidotal effect to its ability to produce a rapid reactivation of sarin-inhibited plasma AE.[1] The availability of ^{32}P-sarin enabled us to study the distribution of sarin in the body of the rat after injection of a sublethal dose and the way this distribution is influenced by oximes. P-2-AM, DAM and MINA were used.

A short communication of some of the present results has been published earlier.[4]

MATERIALS

The ^{32}P-sarin was synthetized by the Chemistry Section of the Defence Research Establishment, Suffield, Ralston, Alberta, Canada.* The specific activity at the time of arrival at Rijswijk varied between 50 and 120 mc/g. Immediately after arrival a stock solution of ^{32}P-sarin in a concentration of approximately 10^{-2} M in anhydrous isopropyl alcohol was prepared. This stock solution was stored at −20°. The purity of the compound, determined a few days after arrival in this laboratory, varied between 90 and 97 per cent.

P-2-AM, DAM and MINA were synthetized by the Chemical Laboratory RVO-TNO, Rijswijk (ZH), The Netherlands. Atropine sulphate and the emulsifier α-Lubrol® (ICI) were obtained commercially. All solutions were freshly prepared on the day of the experiment. Male and female albino rats from one of the strains of this laboratory were used.

METHODS

The distribution of ^{32}P in the body of the rat

The ^{32}P-sarin was dissolved in a 0·9 per cent NaCl solution and injected in a dose of 50 μg/2·5 ml/kg in an exposed saphenous vein of female rats of 140–210 g under ether anaesthesia. Except when stated otherwise an aqueous solution of atropine sulphate (36 mg/kg) was administered i.p. 1·5 min after the nerve gas in order to keep the animals alive. When oximes were injected, they were dissolved in the atropine solution and injected in the therapeutically effective doses, conventionally used in this laboratory. When MINA was administered in the dose of 130 or 150 mg/kg an i.p. injection of 1 ml/kg of a solution of 1,000 mg sodium thiosulphate + 22·5 mg sodium nitrite in 1 ml water was given 10 min prior to the oxime in order to prevent the toxic effects of CN-ions released by this dose of MINA.[5]

At different intervals after the ^{32}P-sarin injection the animals were killed by bleeding from a carotid artery. This haemorrhage was preceded by an i.v. injection under ether anaesthesia of 0·1 ml of a 5 per cent heparine solution. The blood was collected in graduated test tubes and in some experiments it was centrifuged at 3000 rpm during 10 min in order to separate the red cells from the plasma. The other organs were weighed and wet-ashed with equal parts of concentrated nitric acid and sulphuric acid and the clear fluids thus obtained were made up to 10 or 20 ml with water. The radioactivity of the blood and of the wet-ashed organs was measured in a Geiger–Müller liquid counter and compared on the same day with that of a standard solution prepared from the ^{32}P-compound used.

* Thanks are due to the Defence Research Board of Canada for providing us with this material.

The measured amounts of ^{32}P were usually expressed as mμg ^{32}P-sarin from which the ^{32}P was derived per gram wet organ weight or per ml blood. In the case in which the amounts of ^{32}P in the different organs were expressed as percentages of the injected amount of radioactivity, these values were based on the measured radioactivity of the whole organs as far as the brain (always without cerebellum, pons and medulla), the lungs, the liver and the kidneys are concerned, whereas the values of the radioactivity in the total blood and in all the muscles together were calculated from the measured concentration on the assumption that the blood represents 6·5 per cent of the body weight[6] and the voluntary muscles 30 per cent. The latter assumption is somewhat arbitrary as to the knowledge of the authors no data concerning the total muscle volume are available in the literature. Moreover, it was arbitrarily assumed that the radioactivity of the m.gastrocnemius is representative for that of all voluntary muscles.

In eight rats the total recovery of the injected ^{32}P was measured after 1 hr. The blood and the organs were treated as described above. The carcass and pieces of filter paper containing urine and excrements were wet-ashed by boiling with concentrated nitric acid. The turbid fluid and the fat layer thus obtained were emulsified separately with water and α-Lübrol® and the ^{32}P content of each of these emulsions was measured. The fat contained only very small amounts of ^{32}P. In these experiments 86–103 per cent of the injected ^{32}P was recovered.

The excretion of ^{32}P in the urine

Female rats of 185–200 g were anaesthetized by an i.p. injection of urethane (1·2 g/12 ml water/kg) or of barbital sodium (215 mg/6 ml water/kg) and the bladder was cannulated via the urethra. The bladder was washed twice with 0·2 ml of a 0·9 per cent NaCl solution 45 and 90 min after the i.v. injection of 50 μg ^{32}P-sarin/2·5 ml 0·9 per cent NaCl/kg. The washings were added to the small amounts of urine collected during the preceding 45 min. All animals received 1·5 min after the ^{32}P-sarin an i.p. injection of 1 ml/kg of an aqueous solution of 36 mg atropine sulphate/ml, either or not containing 35 mg MINA/ml.

The continuous measurement of the ^{32}P concentration of the blood

Male albino rats (220–250 g) were anaesthetized with urethane (1·2 g/12 ml/kg i.p.) and a carotid artery was interrupted by a cannula (Sterivac no. 1). Part of this cannula was coiled into the shape of a watch spring and was glued to a little plate of perspex that could be attached under an end-window Geiger–Müller counter. The volume of the cannula was approximately 0·4 ml. Before being connected to the animal the cannula was filled with 0·5 per cent heparine dissolved in saline. The blood flow through the cannula was examined at regular intervals by observation of the red cells moving under a microscope. This could be realized by squeezing the cannula in a perspex press during the examination. Moreover, at certain times a very small air bubble was introduced into the blood stream. This allowed macroscopic estimation of its flow rate. The circulation time through the cannula was 1–2 min; after 1–2 hr 0·2–0·4 ml 0·5 per cent heparine was injected again in order to prevent clotting. After the cannula had been connected an i.v. injection of 50 or 25 μg/kg ^{32}P-sarin, 1·5 min later followed by the i.p. administration of 36 mg/kg of atropine sulphate, was given. After different intervals in different experiments one of the oximes was injected i.p.

At the end of each experiment the system was calibrated by filling the cannula with a standard solution of ^{32}P-sarin.

In some experiments the kidneys were removed before the ^{32}P-sarin injection and in other experiments the intestine between the stomach and the rectum was extirpated in addition. In one experiment the stomach and the spleen were also removed and the hepatic artery was ligated. The atropine and the oximes were injected i.v. into the eviscerated animals as i.p. application could not be expected to be effective under the conditions of these experiments. In the eviscerated animals after 10–60 min the blood stream through the cannula became very slow. A second heparine administration was ineffective. The experiment was terminated when the movement of the red cells had nearly stopped.

The ^{32}P activity of the liver in vivo

Male albino rats (220–225 g) were anaesthetized with barbital sodium (215 mg/2 ml/kg i.p.) and the abdomen was opened. An end-window counter was placed on the liver. Next atropine sulphate (36 mg/kg) was administered i.p. or i.v. ^{32}P-sarin (50 μg/kg) was injected i.v. 2 min after the atropine sulphate. MINA (150 mg/kg i.p. or i.v.) or DAM (150 mg/kg i.p. or 75 mg/kg i.v.) was injected 7–50 min after the ^{32}P-sarin. Ten min before the MINA 22·5 mg/kg of $NaNO_2$ and 1000 mg/kg of $Na_2S_2O_3$ were administered i.p.

Enzyme activity determinations

The ChE- and AE-activities of the plasma and of brain and liver homogenates from female rats (153–203 g) were measured by means of the Warburg method at 37° in a Krebs–Ringer-bicarbonate medium, equilibrated with a mixture of 95 per cent N_2 and 5 per cent CO_2 (pH = 7·4).

For the ChE determinations 0·75 ml plasma and 0·75 ml 10 per cent brain and 10 per cent liver homogenate and for the AE-determinations 0·25 ml plasma and 0·75 ml 10 per cent brain and 0·5 per cent liver homogenate was pipetted into the main compartment of the manometric vessel and made up to 2·75 ml with Krebs–Ringer solution. The side arm contained 0·25 ml of one of the following solutions: 200 mg acetylcholine chloride in 9 ml Krebs–Ringer solution for the determination of the ChE-activity and 0·48 ml glycerol tributyrate plus 240 mg gum arabic in 10 ml Krebs–Ringer solution for the AE determination. The final concentration of ACh was 10^{-2} M, that of glycerol tributyrate $1{\cdot}37 \times 10^{-2}$ M. The usual control determinations (thermo-barometer and spontaneous ACh hydrolysis) were included.

The statistical evaluations of the results

The statistical significance at the 5 per cent level of the difference between the ^{32}P-contents of the organs of oxime treated and untreated animals injected with ^{32}P-sarin was determined by the rank sum test.[7] For the oxime effects on the continuously monitored ^{32}P-concentration of the extracorporally circulating blood of anaesthetized rats Poisson distributed observations were compared.[8] For the enzyme determinations Welch's[9] t-test at the 5 per cent level of significance was used.

RESULTS

The distribution of ^{32}P in the body of the rat 1 hr after the i.v. injection of ^{32}P-sarin

One hour after the i.v. injection of 50 μg/kg of ^{32}P-sarin considerable concentrations

of ^{32}P were found in the blood plasma, the kidneys and the lungs, whereas the concentrations in the brain and the m.gastrocnemius were relatively small. The liver occupied an intermediate position. This is shown in the first vertical column of Table 1. The values of this table represent mμg ^{32}P-sarin equivalents per ml blood and per gram wet organ. When the amounts of ^{32}P in the different organs were expressed as percentages of the injected amount of radioactivity per total organ, the following percentages were calculated on the assumptions mentioned sub methods: about 26 per cent of the injected radioactivity was present in the blood, 10 per cent in the muscles, 3·6 per cent in the liver, 2·2 per cent in the kidneys, 1·5 per cent in the lungs and 0·3 per cent in the brain.

TABLE 1. THE DISTRIBUTION OF ^{32}P IN THE BODY OF THE RAT 1 hr AFTER THE i.v. INJECTION OF 50 μg/kg OF ^{32}P-SARIN

	Control	P-2-AM	DAM	MINA
Blood	201 ± 13 (6)	197 ± 10 (5)	**65** ± 4 (5)	**76** ± 4 (6)
Erythrocytes	58 ± 7 (6)	63 ± 8 (5)	43 ± 2·3 (5)	43 ± 2·1 (6)
Plasma	144 ± 12 (6)	134 ± 11 (5)	**22** ± 2 (5)	**33** ± 3 (6)
M. gastrocnemius	17 ± 2·5 (6)	12 ± 2·3 (5)	14 ± 1·2 (5)	18 ± 2·2 (6)
Brain	27 ± 3·2 (7)	26 ± 2·2 (6)	29 ± 5·4 (6)	28 ± 3·3 (7)
Lungs	147 ± 10 (7)	146 ± 5 (6)	114 ± 14 (6)	133 ± 8 (7)
Liver	49 ± 3 (7)	46 ± 3 (6)	**86** ± 5 (6)	**103** ± 5 (7)
Kidneys	164 ± 15 (5)	190 ± 41 (4)	160 ± 19 (4)	184 ± 7 (5)

The figures represent mμg ^{32}P-sarin equivalents per ml blood and per gram organ. Means ± S.E.M. followed by the numbers of experiments between brackets are given. Results from oxime-treated animals which are significantly different ($P_2 < 0{\cdot}05$) from those from control animals are in bold type. All animals received 1·5 min after the ^{32}P-sarin injection an i.p. injection of atropine (36 mg/kg) with or without either P-2-AM (53 mg/kg), DAM (150 mg/kg) or MINA (130 mg/kg). Ten minutes before the injection of MINA, $NaNO_2$ (22·5 mg/kg) and $Na_2S_2O_3$ (1000 mg/kg) were administered i.p.

The injection of the oximes DAM and MINA produced a strong decrease of the amounts of ^{32}P in the blood plasma and an increase in the radioactivity of the liver. The distribution of ^{32}P in the body of the rat was not influenced by P-2-AM.

The ^{32}P-concentration of some organs at different times after the i.v. injection of ^{32}P-sarin

In a small series of experiments the ^{32}P-concentration of the blood and the liver was determined at different intervals after the i.v. injection of 50 μg/kg of ^{32}P-sarin. The results (Fig. 1) show that the ^{32}P-concentration of these organs decreased during the 4 hr of observation. MINA produced a decrease of the radioactivity of the blood and an increase of that of the liver.

The excretion of ^{32}P in the urine from rats after injection of ^{32}P-sarin

In order to assess a possible influence of oximes on the excretion of ^{32}P in the urine, rats under urethane anaesthesia, in which the bladder had been cannulated, received an injection of ^{32}P-sarin and the amounts of ^{32}P appearing in the urine were measured. In 5 rats 33 per cent (range 27–38 per cent) of the injected amount of radioactive material had been excreted 45 min after the i.v. injection of 50 μg ^{32}P-sarin/kg and 47 per cent (range 37–53 per cent) 90 min after the injection. In four rats which had received an i.p. injection of 35 mg MINA/kg 1·5 min after the ^{32}P-sarin 25 per cent

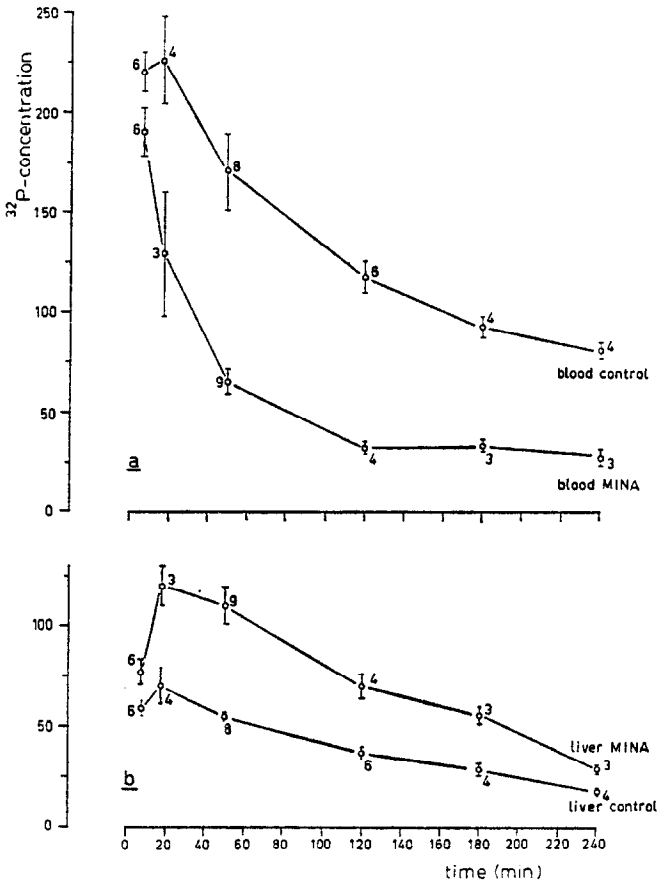


FIG. 1. The ^{32}P-concentrations of the blood and the liver at different intervals after the i.v. injection of ^{32}P-sarin into rats. Atropine (36 mg/kg) with or without MINA (35 mg/kg) was injected i.p. 1·5 min after the ^{32}P-sarin. Results are expressed as mμg ^{32}P-sarin equivalents per ml blood and per gram tissue. Means ± S.E.M. Numbers of observations indicated by the numbers in the figure.

(range 5–53 per cent) of the injected amount of ^{32}P was found after 45 min and 42 per cent (range 30–56 per cent) after 90 min. Thus no significant effect of MINA was seen. This, however, does not exclude a possible influence of MINA on excretion, since the absolute amounts of ^{32}P disappearing from the blood under the influence of this oxime were smaller than the variation in the amounts of ^{32}P excreted by the kidneys.

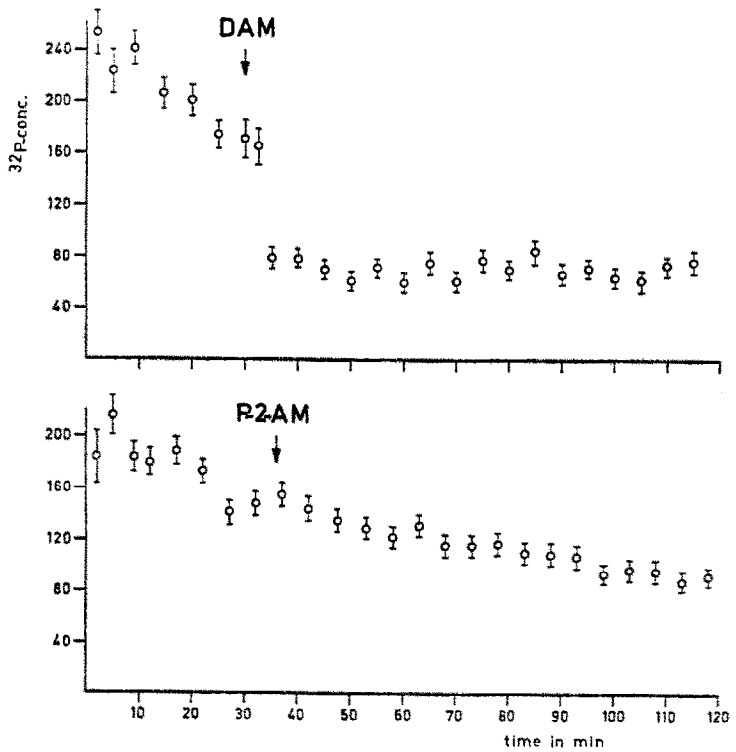
In most animals of both groups there was a striking oliguria. This may have contributed to the variation in the results. In a few similar experiments on animals anaesthetized with barbital sodium, the excretion of ^{32}P varied also greatly from animal to animal and oliguria also occurred.

Continuous determination of the ^{32}P-concentration of the blood in anaesthetized rats after injection of ^{32}P-sarin

In a number of anaesthetized rats the ^{32}P-concentration of the blood circulating through a cannula which interrupted a carotid artery and which passed under an

end-window Geiger–Müller counter, was measured continuously during some time after the i.v. injection of ^{32}P-sarin. The acute effects of the administration of oximes on the radioactivity of the blood and the influence of nephrectomy and evisceration on the oxime effects were studied.

Immediately after the i.v. injection of 50 μg/kg of ^{32}P-sarin the ^{32}P-concentration of the blood rose to about 200 mμg ^{32}P-sarin equivalents/ml. In the subsequent hours the ^{32}P-concentration gradually decreased in about the same way as shown in Fig. 1.



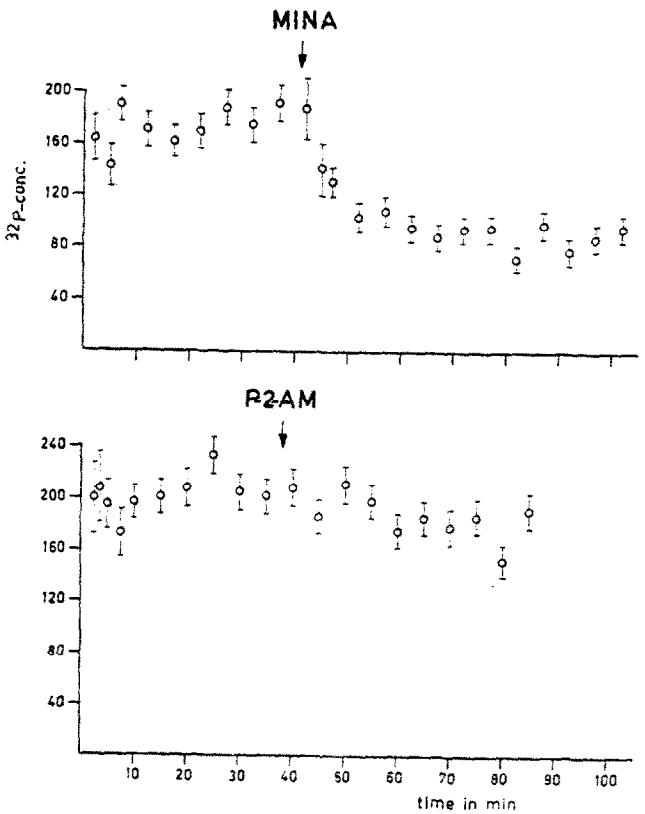
FIGS. 2 and 3. Two experiments in which the radioactivity of the circulating blood in a rat was measured after the i.v. injection of 50 μg/kg of ^{32}P-sarin followed by an i.p. injection of either DAM (150 mg/kg) or P-2-AM (53 mg/kg). Sarin was injected at time = 0 and the administration of the oxime is indicated by an arrow. The ^{32}P-concentration of the blood is expressed as mμg ^{32}P-sarin equivalents per ml. The vertical bars indicate standard deviations calculated from the square root of the number of counts from which each value was derived.

In three rats either DAM (150 mg/kg), MINA (35 mg/kg) or P-2-AM (53 mg/kg) was administered i.p. about 30 min after the i.v. injection of 50 μg/kg of ^{32}P-sarin. The ^{32}P-concentration, which had risen to about 200 mμg ^{32}P-sarin equivalents/ml immediately after the injection of the nerve gas, sharply decreased after the injection of DAM (Fig. 2) or MINA, but not after that of P-2-AM (Fig. 3). After P-2-AM it decreased only gradually with time.

In two nephrectomized rats i.p. injections of DAM (150 mg/kg) and MINA (35 mg/kg) 30 and 22 min after the i.v. injection of 25 μg/kg of ^{32}P-sarin immediately

lowered the ^{32}P-concentration of the blood from about 140 mμg to approximately 70 and 50 mμg ^{32}P-sarin equivalents/ml, respectively. In these experiments the nerve gas was administered in the dose of 25 μg/kg instead of 50 μg/kg, because the nephrectomized animals were found not to survive the higher dose.

In a nephrectomized rat from which the intestine between stomach and rectum had been extirpated (Fig. 4) an i.v. injection of MINA (35 mg/kg) administered 38 min after the i.v. injection of 25 μg ^{32}P-sarin/kg, effectively lowered the ^{32}P-concentration of the blood. A similarly eviscerated and nephrectomized rat, treated with P-2-AM (53 mg/kg) could serve as a control since this oxime had no effect(Fig. 5). In both



FIGS. 4 and 5. Two experiments in which the ^{32}P-concentration of the circulating blood was measured after the i.v. injection of ^{32}P-sarin (25 μg/kg) into a rat after nephrectomy and extirpation of the intestine between stomach and rectum. The influence of an i.v. injection of MINA (35 mg/kg) and of P-2-AM (53 mg/kg).

experiments the ^{32}P-content of the blood was monitored for more than 80 min after the ^{32}P-sarin injection, although the animals were in a bad condition. In a rat from which the kidneys, the stomach, the spleen and the intestine had been removed and the hepatic artery ligated, MINA (35 mg/kg i.v.), given 41 min after the i.v. injection of ^{32}P-sarin (25 μg/kg), produced a distinct decrease of the ^{32}P-content of the

blood. In this case the ^{32}P-content of the blood could be monitored during 100 min following the ^{32}P-sarin injection.

From these observations it may be concluded that the lowering of the concentration of ^{32}P in the blood, produced by DAM or MINA, was not caused by an effect on the organs of excretion.

The radioactivity of the liver of the anaesthetized rat after the injection of ^{32}P-sarin and MINA or DAM

When the distribution of ^{32}P in the body of the rat 1 hr after the injection of ^{32}P-sarin was studied, it was found that the radioactivity of the liver from MINA- or DAM-treated animals was higher than that of control animals (Table 1). In order to investigate whether MINA or DAM caused an actual increase in the ^{32}P-concentration of the liver or merely impeded its gradual decrease with time, the effects of these oximes on the radioactivity of this organ *in vivo* were studied in anaesthetized rats, in which an end-window Geiger–Müller counter was placed on top of the liver in the opened abdomen.

After the i.v. injection of 50 μg/kg of ^{32}P-sarin the liver became radioactive. In three experiments the effect of an i.p. or i.v. injection of MINA (150 mg/kg) 30–50 min after the ^{32}P-sarin and in two experiments the effect of DAM (150 mg/kg i.p. and 75 mg/kg i.v. given 20 and 7 min respectively after the ^{32}P-sarin) was studied. In all

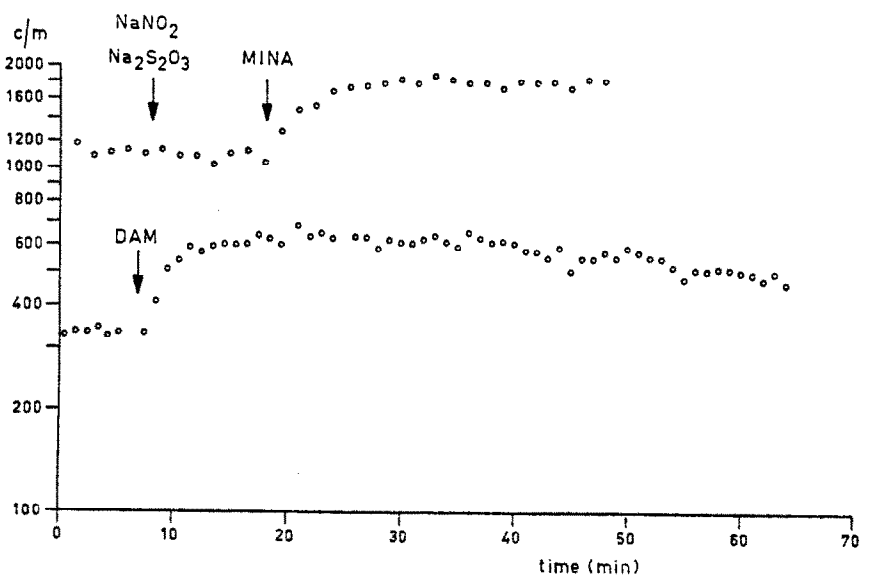


FIG. 6. Two experiments in which the effects of DAM (75 mg/kg i.v.) and MINA (150 mg/kg i.p.) on the radioactivity of the liver of a rat after i.v. injection of ^{32}P-sarin (50 μg/kg) were measured. Injection of ^{32}P-sarin at $t = 0$. Two min earlier atropine (36 mg/kg) was administered i.p. Ten min before MINA $NaNO_2$ (22·5 mg/kg) and $Na_2S_2O_3$ (1,000 mg/kg) were injected i.p. Abscissa: time in min, ordinate: counts/min. Since there was a considerable time interval between the two illustrated experiments the specific activities of the injected ^{32}P-sarin were different. For the sake of comparison the observed values therefore have been plotted on a logarithmic scale. The standard deviations of the measured radioactivities have not been indicated. They are the square roots of the presented numbers of counts.

experiments the radioactivity of the liver increased after the injection of the oxime. The beginning of this increase was observed approx. 3 min after the oxime injection. Within 10–15 min the radioactivity reached a maximum of 130–160 per cent of the pre-injection value. During the following hours it decreased only gradually. Two representative experiments are illustrated in Fig. 6.

Enzyme activity determinations

One hr after the i.v. injection of 50 μg/kg of sarin into rats the ChE-activities of the plasma and the brain and the AE-activity of the plasma were partly inhibited (Table 2). DAM and MINA reactivated the plasma AE to a large extent and MINA reduced the inhibition of the ChE-activity of the brain and the plasma significantly. P-2-AM had no significant reactivating effect.

The ChE- and AE-activities of liver homogenates from rats 1 hr after the injection of sarin with or without oxime therapy were not different from those obtained from not injected rats.

TABLE 2. THE INFLUENCE OF OXIMES ON THE ChE- AND AE-ACTIVITIES OF THE PLASMA, THE BRAIN AND THE LIVER IN RATS AFTER i.v. INJECTION OF SARIN (50 μg/kg)

	Sarin atropine (a)	Sarin atropine P-2-AM (b)	Sarin atropine DAM (c)	Sarin atropine MINA (d)	No drugs (e)
ChE					
Plasma	404 ± 67 (9)	550 ± 88 (8)	490 ± 48 (8)	**622** ± 43 (8)	**908** ± 37 (29)
%	44	61	54	69	
Brain	550 ± 121 (9)	641 ± 85 (8)	601 ± 127 (8)	**928** ± 79 (8)	**1126** ± 15 (29)
%	49	57	53	82	
Liver	230 ± 21 (7)	212 ± 21 (6)	228 ± 28 (6)	218 ± 15 (6)	213 ± 10 (25)
%	108	100	107	102	
AE					
Plasma	2492 ± 246 (9)	3089 ± 219 (8)	**4715** ± 254 (8)	**4883** ± 360 (8)	**5420** ± 183 (29)
%	46	57	87	90	
Brain	887 ± 30 (7)	943 ± 39 (6)	910 ± 37 (6)	925 ± 66 (6)	920 ± 16 (25)
%	96	103	99	101	
Liver	22,433 ± 1194 (7)	23,183 ± 563 (6)	23,373 ± 1496 (6)	22,707 ± 1438 (6)	23,060 ± 579 (25)
%	97	101	101	98	

Enzyme activities expressed as μl CO_2 per ml plasma and per ml 10 per cent homogenate of brain and liver per hour and as percentages of the control values. The control values of column (e) were obtained with organs from rats not treated with sarin or other drugs. Means ± S.E.M. followed by the number of observations between brackets. Each observation is the result of a duplicate manometric determination. Significant (Welch's t-test at the 5% level) differences from values sub (a) are in bold type. All animals received 1·5 min after the sarin injection an i.p. injection of atropine (36 mg/kg) with or without either P-2-AM (53 mg/kg), DAM (150 mg/kg) or MINA (130 mg/kg). The injection of MINA was preceded by the i.p. administration of $NaNO_2$ (22·5 mg/kg) and $Na_2S_2O_3$ (1000 mg/kg).

DISCUSSION

One hr after the injection of ^{32}P-sarin into the rat approx. 25 per cent of the injected ^{32}P was present in the blood and about 18 per cent in the plasma. In anaesthetized animals more than 30 per cent of the injected ^{32}P was excreted in the urine. The remaining ^{32}P could be recovered from different tissues.

The concentrations of radioactive material were relatively high not only in the

plasma, but also in the lungs and the kidneys. In the brain and the m.gastrocnemius they were relatively low.

As demonstrated earlier[2] the ^{32}P in the plasma probably represented sarin attached to AE. This is supported by the present observation that the oxime DAM, which is known to be a very effective reactivator of phosphorylated rat plasma AE,[1, 10] produced a striking decrease of the ^{32}P-concentration of the blood.

One hr after the injection of sarin the ChE- and AE-activities of the blood plasma and the ChE-activity of the brain were partly inhibited. DAM and MINA reactivated the plasma AE. MINA in addition had a reactivating effect on the ChE of the plasma and the brain. P-2-AM, on the other hand, had no significant action on the enzyme activities. The latter observation was surprising, as far as the plasma ChE is concerned, because P-2-AM is an effective reactivator of ChE phosphorylated by sarin,[11, 12] although it does not pass the blood–brain barrier. The lack of significance in the present experiments may be explained by the rather large variation in the results.

Although the ChE-activity of the red cells was not determined in the present experiments their ^{32}P-content probably was not due to ^{32}P attached to ChE as it was not altered by P-2-AM and MINA.

The radioactivity of the brain did not represent phosphorylated AE or ChE, as demonstrated by the absence of an analogy between the ^{32}P-measurements and the results of the Warburg experiments: the AE-activity of the brain was not altered after the injection of sarin and its ChE-activity, which was inhibited, was reactivated by MINA whereas this oxime produced no decrease of its ^{32}P-content in rats after injection of ^{32}P-sarin.

The ^{32}P-content of the liver from rats after the injection of ^{32}P-sarin could also not be caused by phosphorylation of AE or ChE as the activities of these enzymes were not affected.

The fact that large amounts of ^{32}P are excreted into the urine within 45 min after the injection of ^{32}P-sarin could indicate that the kidneys take part in its detoxification. This notion is supported by the observation that the acute toxicity of sarin for rats was increased after nephrectomy. The relatively large amounts of ^{32}P excreted in the urine (more than 40 per cent of the injected amount of ^{32}P within 90 min) contrast with the much smaller excretion of ^{32}P found by Heilbronn *et al.*[13] after the i.v. injection of 80 μg/kg of ^{32}P-tabun into rats (about 15 per cent of the injected radioactivity in 24 hr).

The role of excretion in the mechanism of the effects of DAM and MINA on the distribution of ^{32}P in the body of the ^{32}P-sarin-injected rat could not be elucidated by directly measuring the excretion of ^{32}P in the urine since this excretion varied greatly from animal to animal. This variation may have been due to oliguria, possibly caused by vasopressin release as a result of the anaesthesia, the insertion of a cannula in the urethra and/or the injection of sarin.[14]

The experiments in which the ^{32}P-concentration of the circulating blood was measured by means of an end-window Geiger–Müller counter above a cannula interrupting a carotid artery demonstrated that the oximes DAM and MINA, in contrast to P-2-AM, caused a sudden decrease of the amounts of ^{32}P in the blood. This effect also occurred after nephrectomy and evisceration and therefore could not be dependent on the organs of excretion. Apparently the reactivation of AE by oximes was accompanied by a release of ^{32}P which then leaved the vessels, presumably by simple diffusion. As demonstrated by the rise in the radioactivity of the liver occurring

at the same time, part of the released radioactive materials was stapled in this organ.

In this connection the difference between TOCP and DAM should be stressed. In TOCP-pretreated rats the toxicity of injected sarin is enhanced.[1, 2] This was explained on the assumption that the occupation by TOCP of certain non-vital sarin binding sites, such as the plasma AE, allows a larger than normal part of the injected nerve gas to penetrate as such into the vital centres. DAM, in contrast to TOCP, releases the non-toxic hydrolyzed product of sarin (isopropyl hydrogen methylphosphonate) from plasma AE.[15]

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