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DISTRIBUTION OF DF^{32}P IN MOUSE ORGANS—I.
THE EFFECT OF ROUTE OF ADMINISTRATION ON INCORPORATION AND TOXICITY

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Abstract—The LD_{50} of di-isopropyl phosphorofluoridate (DFP) for mice by the intraperitoneal route is found to be 6·8 mg/kg while that by the subcutaneous or intravenous route is found to be much lower (3·8 and 3·4 mg/kg respectively). When radioactive di-isopropyl phosphorofluoridate (DF^{32}P) is injected to mice or rats by the intraperitoneal route there is a higher uptake of radioactivity in the liver with a corresponding reduced incorporation in other organs than when the administration is by the subcutaneous or the intravenous routes. Death occurs when the concentration of DF^{32}P reaches a certain critical value in some organs including the brain whatever the route employed. A large part of the detoxication of DFP which is known to take place in the liver is thus due to its absorption by the liver esterases whose inhibition obviously does not contribute materially to the overall toxicity.

THOUGH it is well known that organophosphorus esters owe their toxicity to their ability to combine irreversibly with the active site of acetylcholinesterase it is difficult to establish any predictable pattern correlating the chemical structure of an organophosphate with its lethal effect.[1] Even within a given species the LD_{50} is dependent upon the route of administration. These differences are sought to be explained by many factors the most important being the extent of detoxication taking place in the animal system.

In a previous paper[2] it was shown that the enzyme DFPase which hydrolyzes di-isopropyl phosphorofluoridate (DFP) is present only in small amounts in rat and rabbit tissues. It was also reported[3, 4] that liver microsomes contain large amounts of non-specific esterases which incorporate DFP. In the present work data are presented to show that the detoxication occurring in the liver is mainly due to the absorption of DFP by microsomal esterases whose inhibition obviously does not contribute to the overall lethal effect. The LD_{50} of DFP by the intraperitoneal (i.p.) route is found to be much higher than those by the subcutaneous (s.c.) and the intravenous (i.v.) routes. This excess dose is correlated to the higher uptake of DFP in the liver when the injection is given by the i.p. route. There is a correspondingly reduced uptake in the other organs which are probably the foci of lethal action since death occurs when the DFP concentration reaches a certain critical value in these organs whatever is the route of administration. Radioactive di-isopropyl phosphorofluoridate (DF^{32}P) admixed with DFP is used in these studies.

MATERIALS AND METHODS

The samples of DFP used in this work were obtained from the Research Institute of National Defence, Department 1, Sundbyberg 4, Sweden. Stock solutions were

prepared which contained 25 mg/g in dry propylene glycol. $DF^{32}P$ with a specific activity of 300 mc/g was obtained from the Radiochemical Centre, Amersham. Stock solutions of this contained 1·85 mg (10 μmoles)/g in propylene glycol. The solutions were preserved at $-16°$ and quantities were accurately weighed out and dissolved in 0·9% sodium chloride solution just before injections. When mixtures of DFP and $DF^{32}P$ were to be used 20 per cent of the organophosphate was derived from the $DF^{32}P$ stock solution. It was found that $DF^{32}P$ usually contained 8–15 per cent of a radioactive impurity which was not extractable by ether.

Male albino mice weighing 22–25 g were used throughout. Animals of uniform weight ($\pm$ 1 g) were chosen for any series of experiments and a constant volume of solution (0·08–0·1 ml) was injected to each mouse. The s.c. injections were given under the loose skin in the back and the i.v. injections were in the tail vein. Since the rate of i.v. injection is known to influence toxicity[5] these were administered at a controlled rate of 0·1 ml/20 sec. The LD_{50} values were calculated by Thompson's method[6] using the tables compiled by Weil.[7] Four groups of 6 animals each were usually employed for each assay, the dosages being so arranged in a geometric progression that their logarithms differed by 0·025 or 0·03. A 24-hr observation period was allowed for death or survival.

For the determination of radioactive DFP incorporated in various organs, groups of 5 or more animals were injected with the necessary amount of DFP + $DF^{32}P$ mixture at suitably arranged time intervals and each animal was killed individually exactly 30 min after the injection. Sacrifice was by stunning followed by exsanguination to remove as much of the blood as possible. The liver, kidneys and lungs were quickly removed, cut into small bits and rinsed with physiological saline and finally homogenized in a Potter–Elvehjem type of homogenizer. The volume was made up to 25 ml with saline and 1 ml aliquots of the uniform suspensions were pipetted into glass cups and dried after the addition of a drop of 4 N NaOH. The brain tissue was prepared by dissolving in 6 ml of hot 1 N NaOH. Each animal was worked up separately and radioactivity measurements were taken in triplicate in a Robot Equipment (LKB-Produkter, Stockholm) fitted with a Tracerlab Compumatic Scaler. The lung tissue was often contaminated with blood especially at dose levels approaching the LD_{50}.

Studies on rats were performed with male animals weighing about 300 g. The injection volume in these cases was 1 ml per rat.

RESULTS

LD_{50} *of* DFP *by various routes*

This was determined using DFP unadmixed with $DF^{32}P$. Two different batches of the substance were used. The results are given in Table 1. Most of the deaths occurred in the first few hours, late deaths being more frequent in i.v. studies.

Incorporation of $DF^{32}P$ *administered s.c. and i.p.*

Quantities of DFP and $DF^{32}P$ were weighed out and dissolved in the necessary volume of saline so that 0·1 ml contained the required amount of the organophosphate to give dosages of 0·5, 1·0, 2·0 and 4·0 mg/kg body weight. In the last dose level there were a few deaths by the s.c. route but only the survivors at 30 min were analysed. Since the LD_{50} by the i.p. route has been found to be 6·8 mg/kg groups of mice were

TABLE 1. LD_{50} OF DFP FOR MICE BY DIFFERENT ROUTES OF ADMINISTRATION

Route	LD_{50} (mg/kg)	Range (95% confidence limits) (mg/kg)
i.p.	6·7	6·0–7·4
	6·8	6·4–7·1
s.c.	3·6	3·5–3·7
	3·9*	3·7–4·0
i.v.	3·2	3·0–3·4
	3·5	3·3–3·8

LD_{50} was determined using 4 groups of 6 mice each (5 mice in the case of series marked *). DFP solution was freshly prepared by weighing accurate amounts of the stock solution in propylene glycol and dissolving in the necessary volume of 0·9 per cent saline. The dosages were in geometric progression, their logarithms differing by 0·025 or 0·03. The LD_{50}s were calculated according to the method of Thompson[6] using the tables compiled by Weil.[7] A 24-hr observation period was allowed for death or survival.

also injected with the DFP + $DF^{32}P$ mixture at dose levels of 6·0, 6·5 and 7·0 mg/kg by this route. There were a few deaths at the last dose level. The results are given in Table 2. The numbers in brackets in the following text refer to the order numbers of experiments in the table.

It is seen that at any dose level the uptake of $DF^{32}P$ in the liver is much higher by the i.p. than by the s.c. route (1–8). All the differences are statistically significant. It is also observed that at 4·0 mg/kg (s.c.) which is the approximate LD_{50} by the s.c. route there is less $DF^{32}P$ in the liver than when the same amount is injected i.p. (4). This indicates that the $DF^{32}P$ content of the liver does not determine death. On the other hand, there is actually an un-utilized detoxicating potential in the liver at the time of death of the animal by 4·0 mg/kg s.c.

The uptake of $DF^{32}P$ in the kidney follows a reverse order, the amounts incorporated being less by the i.p. route than by the s.c. route (9–12) at sublethal doses. However, when the dose level approaches the LD_{50} the differences are hardly significant (13–16). In the case of the lung the incorporation is, in general, less in the case of i.p. injections (17–21). However, in the course of the experiments it was observed that the lung tissue was often contaminated with large amounts of blood which was difficult to be rinsed out by cutting the tissue in small bits in saline. Erratic values for radioactivity were obtained in such cases and these were more frequent as the DFP dose level approached the LD_{50}.

The incorporation of $DF^{32}P$ in the brain follows the same pattern as in the kidneys but is more consistent. The differences between the s.c. and i.p. values are highly significant up to 4·0 mg/kg which is the approximate LD_{50} (s.c.) after making allowance for the slight impurity in the $DF^{32}P$ (25–28). At this level by the i.p. route it is seen that the incorporation in the brain is considerably less. When the i.p. dose is progressively increased from 6·0 mg/kg the amount of $DF^{32}P$ in the brain approaches a value which is very near that of the 4·0 mg (s.c.) value (29–32). This indicates that at the time of death the DFP content of the brain is the same whatever the route of administration.

TABLE 2. INCORPORATION OF $DF^{32}P$ IN MOUSE ORGANS WHEN ADMINISTERED BY THE S.C. AND I.P. ROUTES

No.	Organ	Dose DFP + $DF^{32}P$ mg/kg	Activity injected	Activity incorporated in the organs, s.e.m. and number of animals — s.c. route	i.p. route	s.c./i.p. ratio	P
1	Liver	0·5	190	32± 0·9 (5)	69± 1·1 (5)	0·46	<0·001
2		1·0	380	101± 2·6 (5)	147± 8·2 (4)	0·69	<0·001
3		2·0	760	186± 6·4 (5)	217±10·2 (5)	0·86	<0·001
4		4·0	1520	259±15·7 (5)	319±10·2 (5)	0·81	<0·001
5		4·0	294	60± 3·6 (5)			
6		6·0	441		75± 6·4 (3)	0·80	<0·01
7		6·5	520		76± 3·5 (5)	0·79	<0·001
8		7·0	553		83± 3·4 (4)	0·72	<0·001
9	Kidney	0·5	190	15± 1·2 (5)	8± 1·2 (5)	1·88	<0·001
10		1·0	380	24± 1·9 (5)	18± 1·5 (5)	1·33	<0·005
11		2·0	760	35± 1·7 (5)	27± 3·2 (3)	1·30	<0·005
12		4·0	1520	78± 7·8 (5)	66± 2·4 (5)	1·18	<0·02
13		4·0	294	17± 1·6 (7)	10± 0·7 (5)	1·70	<0·001
14		6·0	441		20± 3·5 (5)	0·85	>0·1
15		6·5	520		16± 1·8 (4)	1·06	>0·4
16		7·0	553		18± 1·9 (3)	0·94	>0·4
17	Lung	0·5	190	4·4 ± 0·2 (4)	4·3 ± 0·3 (4)	1·02	>0·5
18		1·0	380	5·5 ± 0·8 (5)	5·8 ± 0·5 (5)	0·95	>0·5
19		2·0	760	7·7 ± 0·7 (5)	5·9 ± 0·3 (5)	1·31	<0·005
20		4·0	1520	9·3 ± 1·0 (3)	7·9 ± 0·2 (5)	1·18	<0·01
21		4·0	294	2·6 ± 0·3 (7)	1·8 ± 0·2 (5)	1·44	<0·005
22		6·0	441		1·8 ± 0·3 (6)	1·44	<0·005
23		6·5	520		2·3 ± 0·3 (5)	1·13	>0·2
24		7·0	553		2·4 ± 0·5 (4)	1·08	>0·4
25	Brain	0·5	190	0·62± 0·03 (5)	0·30± 0·02 (5)	2·07	<0·001
26		1·0	380	1·04± 0·04 (5)	0·54± 0·05 (5)	1·93	<0·001
27		2·0	760	1·92± 0·09 (5)	0·93± 0·12 (5)	2·06	<0·001
28		4·0	1520	3·82± 0·06 (5)	1·89± 0·10 (5)	2·02	<0·001
29		4·0	294	1·10± 0·07 (7)	0·69± 0·07 (4)	1·59	<0·001
30		6·0	441		0·71± 0·16 (6)	1·55	<0·001
31		6·5	520		1·04± 0·09 (4)	1·06	>0·3
32		7·0	553		1·28± 0·24 (4)	0·86	>0·05

Values are expressed in count/min $\times 10^{-3}$ in whole organs. Albino mice of 22–25 g were used in these experiments but in any one series animals of uniform weight (±1 g) were chosen and a constant volume of DFP + $DF^{32}P$ solution (0·08 to 0·1 ml) was injected. This solution was prepared fresh by weighing out quantities of a stock solution of DFP (25 mg/g) and $DF^{32}P$ (1·85 mg/g) in propylene glycol and dissolving in the required volume of saline to give the necessary dosage. 20 per cent of the total DFP was derived from the $DF^{32}P$ stock solution. Five or more animals were used for each determination but each animal was worked up separately on a previously worked out time schedule for injection and sacrifice. To minimise errors due to spontaneous hydrolysis of DFP in aqueous medium fresh quantities of the stock solutions were weighed and diluted for every 20 animals (about 100 min). The interval between injection and sacrifice was 30 min in all cases. Other details are given under Materials and Methods.

Incorporation of $DF^{32}P$ *in organs when administered s.c. and i.v.*

The amounts of $DF^{32}P$ incorporated in the organs when graded doses of DFP + $DF^{32}P$ are injected by the s.c. and i.v. routes are given in Table 3. As in the case of the s.c. and i.p. experiments three i.v. dose levels near the LD_{50} were also included in the series, viz., 3·2, 3·4 and 3·6 mg/kg, but all the mice administered 3·6 mg/kg i.v. died within 30 min and these values are therefore not presented.

In general, the differences between the s.c. and i.v. values are less significant than those in the s.c. and i.p. series. It is also noteworthy that the LD_{50}s by these routes lie close together viz., 3·8 and 3·4 mg/kg respectively (Table 1).

TABLE 3. INCORPORATION OF DF^{32}P IN MOUSE ORGANS BY THE S.C. AND I.V. ROUTES

No.	Organ	Dose DFP + DF^{32}P (mg/kg)	Activity injected	Activity incorporated in the organs, s.e.m. and number of animals — s.c. route	Activity incorporated in the organs, s.e.m. and number of animals — i.v. route	s.c./i.v. ratio	P
33	Liver	0·5	37	6·8 ±0·71 (6)	9·5 ±1·12 (6)	0·72	<0·005
34		1·0	74	18·6 ±1·33 (6)	22·9 ±1·15 (6)	0·81	<0·001
35		2·0	148	27·6 ±3·40 (6)	36·1 ±2·39 (6)	0·76	<0·005
36		4·0	296	46·9 ±3·49 (5)			
37		3·2	237		50·9 ±2·43 (5)	0·92	>0·05
38		3·4	252		48·9 ±2·33 (7)	0·96	>0·1
39	Kidney	0·5	37	2·4 ±0·15 (6)	2·7 ±0·42 (6)	0·89	>0·2
40		1·0	74	3·7 ±0·27 (6)	2·8 ±0·13 (6)	1·32	<0·001
41		2·0	148	5·2 ±0·80 (6)	4·5 ±0·27 (6)	1·16	>0·1
42		4·0	296	14·8 ±3·29 (5)			
43		3·2	237		16·0 ±2·71 (5)	0·93	>0·7
44		3·4	252		11·1 ±0·96 (7)	1·33	<0·05
45	Lung	0·5	37	0·75±0·06 (6)	0·83±0·10 (6)	0·90	>0·1
46		1·0	74	0·89±0·06 (6)	0·75±0·05 (6)	1·19	<0·005
47		2·0	148	0·92±0·15 (6)	1·19±0·12 (6)	0·77	<0·02
48		4·0	296	2·72±0·28 (5)			
49		3·2	237		1·47±0·15 (5)	1·85	<0·001
50		3·4	252		1·67±0·12 (7)	1·63	<0·001
51	Brain	0·5	37	0·13±0·006 (6)	0·14±0·005 (6)	0·93	<0·05
52		1·0	74	0·25±0·018 (6)	0·28±0·006 (6)	0·89	<0·02
53		2·0	148	0·33±0·030 (6)	0·41±0·030 (6)	0·80	<0·005
54		4·0	296	0·80±0·160 (5)			
55		3·2	237		0·80±0·070 (5)	1·00	>0·9
56		3·4	252		0·79±0·040 (7)	1·01	>0·8

Values are expressed in count/min × 10^{-3}. The details are the same as given under Table 2 and Materials and Methods. The i.v. injections were given in the tail vein keeping an approximate rate of 0·1 ml/20 sec.

There are significant differences in the DF^{32}P uptake in the liver at sublethal doses (33–35) the incorporation being higher by the i.v. route. As the dosages approach the LD_{50} (36–38) the differences are no longer significant. The values for the kidneys and lungs do not show consistency, the s.c./i.v. ratio being found to lie on either side of unity. As pointed out earlier the lung tissue gives erratic values at near LD_{50} levels. As in the case of the s.c.–i.p. comparison experiments the DF^{32}P uptake in the brain shows a remarkable consistency. There are statistically significant differences between the s.c. and i.v. values at sublethal dose levels (51–53), the i.v. values being higher. At doses near the LD_{50} the DF^{32}P content of the brain reaches an almost identical value by either mode of administration (54–56).

Dose-uptake curves

If the uptake of DF^{32}P (Tables 2 and 3) is plotted against the logarithm of the doses from 0·5 to 4·0 mg/kg, it is possible to obtain a straight line curve for the liver up to 4·0 mg/kg. However, in the case of the other organs the curves are linear only up to 2·0 mg/kg after which there is a sudden steep rise in the slope. For economy of space these curves are not given but attention is drawn to the high values of item numbers 12, 28, 42, 48 and 54 in Tables 2 and 3. A possible explanation can be that the detoxication capacity of the liver is getting rapidly exhausted in the region 2·0 to 4·0 mg/kg and this results in an abrupt overflow into the other organs.

Excretion of $DF^{32}P$ *after administration by the s.c. and i.p. routes*

An organophosphate has to pass through the liver first if it is injected by the i.p. route. It can therefore be expected that its enzymic degradation will be much higher if administered by this route. Adie[8] found that there is a relationship between the sarinase level of the rabbit liver and the dose of sarin needed to kill the animal. To determine if the higher LD_{50} of DFP by the i.p. route is due to its higher enzymic degradation by the liver DFPase, excretion studies were conducted on 2 sets of rats injected $DF^{32}P$ by the s.c. and i.p. routes respectively. A higher excretion of radioactive material should

TABLE 4. INCORPORATION OF EXCRETION OF $DF^{32}P$ IN RATS

Organ	Interval between injection and sacrifice (hr)	Radioactivity* when injected (s.c. route)	(i.p. route)	s.c./i.p.
Liver	1	277	371	0·75
	24	188	303	0·62
Kidney	1	162	81	2·00
	24	58	41	1·41
Lung	1	27	28	0·96
	24	17	18	0·94
Brain	1	4·1	2·3	1·78
	24	2·3	1·1	2·09
Excreta (urine + faeces)	24	2190	2212	0·99

* The values are expressed in count/min $\times 10^{-3}$. Each figure respresents the average of 2 rats of weight 290–310 g. The rats were injected 1 ml each of a $DF^{32}P$ solution (0·8 mg of DFP/kg) which contained $3·53 \times 10^6$ count/min of radioactivity. The animals used in the excretion studies were allowed free access to food and water. The 24-hr values for the organs pertain to these animals.

result in the set of rats injected i.p. as the $DF^{32}P$, once it is hydrolyzed to di-isopropyl phosphate by the DFPase, will not bind to tissues[9] and will be excreted. As results in Table 4 show this does not seem to take place. The excretion of radioactive phosphorus is almost identical in both the sets and it can therefore be concluded that the higher uptake of $DF^{32}P$ in the liver by the i.p. mode of administration is compensated by a lower uptake in the other organs and that there is no significant overall increase in the degradation of $DF^{32}P$ if it is injected intraperitoneally.

DISCUSSION

From the foregoing results it is apparent that the wide gap between the LD_{50}s of DFP by the s.c. and i.p. routes of administration can be explained by the differences in the distribution ratio of the organophosphate in the organs when administered by these routes. It is found that more $DF^{32}P$ is incorporated in the liver when the injection is by the i.p. route with a correspondingly reduced uptake in the other organs which are probably the vital targets of lethal action (Table 2). The higher uptake of $DF^{32}P$ in the liver by the i.p. route is predictable since the liver has a high content of DFP-susceptible esterases[3, 4] and the injected substance has to pass through this organ. As stable phosphorylated enzymes are formed[3] the initial quantities of the injected $DF^{32}P$ will be used up by the microsomal esterases, only the overflow reaching the other organs. It is clear that the liver itself is not a vital organ involved in the lethal effects of DFP as it is possible to have a high concentration of DFP in the liver without

causing death (see item 4 in Table 2). On the other hand, the liver seems to act as a buffer absorbing the organophosphate in the esterases, whose inhibition, whatever may be its long-term effect, does not apparently contribute to the acute toxicity. The detoxication effected by the liver is thus mainly by the absorption of DFP by the microsomal esterases and enzymic degradation seems to play only a minor role since there is no increased excretion of radioactivity when $DF^{32}P$ is injected i.p. (Table 4). This confirms our earlier finding that the enzyme DFPase has only a low activity in the animal system.[2] An abrupt rise in the uptake of $DF^{32}P$ by the kidney, lung and the brain in the region of the LD_{50} also points to a buffering effect by the liver at the initial stages.

It is likely that the liver acts in a similar way in modifying the effective doses of many other active substances. Especially of interest will be a re-assessment of the action of oxime antidotes in the light of the above findings as these are most often administered by the i.p. route in experimental work. Studies along these lines are in progress.

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