Die Retention der Metalle bei Mäusen ausgedrückt in Prozenten aus der gesamten applizierten Dosis (mit 95% Vertrauensintervall in Klammern)

| Versuch (i.v. Injektion) | Zeit nach der Applikation | | |
|---|---|---|---|
| | 1. Tag | 7. Tag | 28. Tag |
| $ZnCl_2$ | 87,1 (85,8–88,4) | 56,8 (54,3–59,3) | 29,2 (24,8–33,6) |
| $ZnCl_2 + Na_2SO_3$ s.c. | 92,1 (85,8–98,3) | 57,7 (55,7–59,7) | 30,0 (26,8–33,2) |
| $ZnCl_2 + Na_2TeO_3$ s.c. | 89,6 (85,8–93,4) | 56,5 (54,6–58,4) | 31,0 (28,3–33,7) |
| $ZnCl_2 + Na_2SeO_3$ s.c. | 90,1 (88,8–91,4) | 57,7 (55,4–60,0) | 28,1 (26,0–30,2) |
| $CdCl_2$ | 92,6 (90,1–95,1) | 86,9 (83,2–90,6) | 75,2 (71,1–79,3) |
| $CdCl_2 + Na_2SO_3$ s.c. | 92,5 (90,6–94,4) | 87,3 (83,9–90,7) | 77,0 (67,3–86,7) |
| $CdCl_2 + Na_2TeO_3$ s.c. | 95,1 (90,0–100,2) | 89,0 (83,6–94,4) | 70,6 (64,8–76,4) |
| $CdCl_2 + Na_2SeO_3$ s.c. | 97,6 (94,7–100,5) | 91,4 (88,1–94,7) | 83,3 (77,0–89,6) |
| $HgCl_2$ | 75,3 (73,3–77,3) | 24,9 (22,9–26,9) | 1,6 (0,8–2,4) |
| $HgCl_2 + Na_2SO_3$ s.c. | 69,5 (65,2–73,8) | 22,6 (19,9–25,3) | 1,7 (1,1–2,3) |
| $HgCl_2 + Na_2TeO_3$ s.c. | 93,3 (90,5–96,1) | 51,8 (41,8–61,8) | 25,5 (22,9–28,1) |
| $HgCl_2 + Na_2SeO_3$ s.c. | 99,8 (98,3–101,3) | 66,2 (62,8–69,6) | 31,9 (28,2–35,6) |

Anionen steigt in Richtung $SO_3^{2-} \rightarrow TeO_3^{2-} \rightarrow SeO_3^{2-}$[4]. In der gleichen Reihenfolge erhöht sich der Einfluss dieser Anionen auf die Retention der verwendeten Metalle. Mit den Redoxpotentialen mancher biologischer Systeme verglichen[5], hat von den benützten Anionen $SeO_3^{2-}$ das positivste Redoxpotential. Das reduzierte Selen reagiert mit Quecksilber und Kadmium. Es bilden sich so Verbindungen (wahrscheinlich Selenide), die relativ unlöslich und wenig toxisch sind und im Organismus gespeichert werden. So lässt sich der Mechanismus der Detoxikationswirkung des Selenits bei der Quecksilber- und Kadmiumvergiftung[6–8] erklären. Die Reduktion des Tellurits im Organismus ist geringer und deshalb auch seine Wirkung bei unserem Versuch schwächer. Das Redoxpotential des Natriumsulfits kommt den Redoxpotentialen der biologischen Systeme am nächsten, weshalb sich die Wirkung des Natriumsulfits nicht zeigt. Aus unseren Ergebnissen ist im weiteren die unterschiedliche Affinität der verwendeten Metalle zu Schwefel, Tellur und Selen ersichtlich.

*Summary.* The influence of sodium sulfite, sodium tellurite and sodium selenite on the retention of zinc, cadmium and mercury in mice was studied. The retention of mercury was increased by sodium selenite and by sodium tellurite. The retention of cadmium was increased only by sodium selenite. Sodium sulfite did not influence the retention of metals studied. The retention of zinc was not influenced by any compounds used.

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## Changes in Acetylcholine Content of Rat Brain by Toxic Doses of Di-Isopropyl Phosphorofluoridate

It is well established that acute poisoning by organophosphorous compounds like di-isopropyl phosphorofluoridate (DFP), tetraethyl pyrophosphate (TEPP) and isopropyl methylphosphonofluoridate (Sarin) can be attributed to their inhibition of acetylcholinesterase (AChE) in nervous tissues[1]. While the inhibition of AChE by these compounds has been the subject of numerous studies, relatively few investigations have been carried out on the increased levels of ACh in brain-tissues[2–5]. Further there are no reports on the extra- and intracellular levels of ACh after injection of toxic doses of DFP.

By subcellular fractionation techniques, it has been established that about 70% of the intracellular ACh in rat[6] and guinea-pig brain[7] may be isolated with the detached nerve terminal particle (synaptosomes). In the present report, possible changes in the ACh content of the synaptosomes following toxic doses of DFP were investigated. The degree of inhibition of AChE and the

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time course of the ACh level in brain under these conditions have also been studied.

*Methods.* Male rats (200 g) were given 1.8 mg DFP/kg s.c. All animals showed severe symptoms of poisoning and 30% of the animals died. Control animals were injected with a similar volume of saline. The animals were killed by decapitation and the heads dropped into liquid nitrogen. The heads were removed after 20 sec. At this time the temperature of their brains had fallen to just above 0 °C[8]. The cerebra were quickly dissected out by discarding the parts caudal to the superior colliculi. The cerebra was homogenized in icecold 10% trichloroacetic acid to determine the total ACh or in 0.32 *M* sucrose under conditions which preserve the synaptosomes, to obtain the particulate ACh[6,7,9]. In order to obtain a quantitative yield of synaptosomes, they were not isolated by density-gradient centrifugation but by centrifuging at 100,000 *g* for 60 min. The high speed pellet was resuspended in 10% trichloroacetic acid. The latter, which was easily removed by ether extraction, destroyed all AChE activity and released all the particulate ACh in brain into solution[10].

ACh was determined by its effect on the ventricular frequency of the heart of the mollusc *Mya arenaria*. The sensitivity of the method was high enough to permit the assay of 5–50 pmoles. ACh per sample with an accuracy of 10%[11]. AChE was assayed manometrically[12].

*Results.* The total ACh concentration in rat cerebra was found to increase 100% above normal level 1 h after injection of DFP. This level was maintained for more than 4 h (Table). After 16 h the ACh content had returned to normal value. The AChE concentration in the brain was reduced to 10% 15 min after the injection and the activity remained at 5–10% of the normal value for the first 4 h. After 16 h, when the level of ACh had reached normal values, the AChE activity corresponded to only 10–20% of that of the control animals.

The particulate ACh was obtained from rats (4 DFP-treated and 4 controls) decapitated 1 h after injection of DFP or saline. The amounts of ACh recovered in the high speed pellet (expressed as mean value ± S.D.) was 13 ± 2 nmoles ACh/g original tissue for the DFP-treated animals and 11 ± 2 nmoles ACh/g original tissue for the control animals. In contrast to the large increase in the total ACh content of brain on exposure to DFP, the amount of particulate ACh, which is identical to that present in the synaptosomes, increased therefore only 15%. Part of this increase was due to supernatant trapped in the high speed pellets.

In control animals, the particulate ACh was 67% of the total ACh. This value agrees with previous reports on the percentage of ACh in synaptosomes from guinea-pig[7] and rat[6] brain. In these control animals, the difference between total and particulate ACh represents for the greater part the ACh present in neuronal cell bodies ruptured by homogenization[6,7].

Levels of ACh and AChE in rat cerebra at various times after s.c. injection of 1.8 mg DFP/kg rat. The results are expressed as mean value ± S.D. Number of animals in brackets

| | ACh (nmoles/g) | AChE (µl $CO_2$/h/g) |
|---|---|---|
| Control (h) | 16.5 ± 3 (9) | 12,500 ± 1,400 (5) |
| 1 | 29 ± 4 (4) | 900 ± 450 (3) |
| $2^1/_2$ | 34 ± 7 (6) | 600 ± 200 (2) |
| 4 | 27 ± 7 (4) | – |
| 16 | 16 ± 1 (5) | 1,760 ± 1,000 (3) |

*Discussion.* Rats treated with toxic doses of DFP showed a marked inhibition of brain AChE and subsequently a large increase in the ACh content of their cerebra. The increase in ACh agrees with that obtained after treatment with other organophosphorous compounds such as TEPP[2–4]. The high level of ACh was maintained for several hours. Similar results were obtained from rabbit cortex after treatment with DFP[5]. Since the ACh content of the synaptosomes increased far less than the total ACh of the brain, the increase in the latter was almost entirely due to a rise in extracellular ACh.

The low increase in ACh content of the synaptosomes was unexpected for two reasons. Firstly, although the localization of AChE within the synaptosome is still an open question[13], there have been reports on the presence of AChE inside the neuron[14,15]. Secondly an uptake of ACh into synaptosomes have been demonstrated[16,17]. As the AChE activity in the brain was considerably reduced by DFP, and since also the uptake of ACh into synaptosomes should have been favoured by the high level of extracellular ACh surrounding the nerve terminals, it was contrary to anticipation that the ACh content of the synaptosomes was only moderately changed.

Only a small increase in particulate ACh was also found in mice brain after treatment with physostigmine[18]. The effects of these two compounds cannot be directly compared since physostigmine, but not organophosphorous anticholinesterases, inhibits the uptake of ACh[19,20]. The results on distribution of ACh after treatment with an acetylcholinesterase inhibitor differ from those of 5-hydroxytryptamine after treatment with the monoamino oxidase inhibitor iproniazid. The latter increased both total and particulate 5-hydroxytryptamine to the same extent[21].

*Zusammenfassung.* Die Behandlung mit Phosphorsäure-Di-Isopropylester-Fluorid ergibt im Rattengehirn eine 100 prozentige Erhöhung des totalen ACh-Gehaltes. Der ACh-Anstieg in den Synaptosomen war nur gering.

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