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SERUM LEVEL AND EXCRETION OF DIISOPROPYLFLUOROPHOSPHATE (DFP) IN CATS

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Abstract—During the first 30 min after the i.v. injection of ^{3}H-diisopropylfluorophosphate in cats the arterial serum content of the drug lies well above the venous serum level. The arterial serum level declines in two exponential phases with half times of about 7 resp. 200 min. The first one reflects the fast accumulation of DFP and its metabolite by the tissue, the second one the elimination. DFP is rapidly metabolized to diisopropyl-phosphate and excreted mainly via the kidneys. Less than 1 per cent are eliminated via the bile and the lungs during two hours after injection.

DISTRIBUTION and excretion of DFP have been investigated under various conditions. In mice,[1, 2] the DFP content in organs after injection of different doses has been determined. Similarly the distribution of DFP and additionally the degree of cholinesterase inhibition have also been estimated[3]. In human subjects Cohen and Warringa[4] investigated the excretion of metabolites of DFP in urine and faeces, using DF^{32}P. The serum level, the distribution and the metabolic degradation of tritium labelled DFP in guinea pigs has recently been reported by Hansen, Schaum and Wassermann.[5] This paper deals with the excretion of DFP and its metabolites in cats after i.v. injection of ^{3}H-DFP.

METHODS

Adult cats of either sex were anaesthesized with chloralose (60 mg/kg). The ureters and bile ducts were exposed and cannulated with appropriate polyethylene tubes. The abdominal wall was closed immediately after the operation. Bile and urine were collected 5, 10, 30, 60 and 120 min after the injection of ^{3}H-DFP into the jugular vein. Arterial blood samples were taken from a carotid artery, venous blood via a polyethylene catheter in the jugular vein, in the peripheral direction. Artificial respiration was applied throughout the experiments. The exhaled air was bubbled through absolute ethanol at −5° in order to collect possibly expired DFP or its metabolites.

The DFP used for the investigation had been labelled with tritium. Its sp. act. was 1·02 Ci/m-mole (Buchler, Braunschweig). The stock solution was diluted to 40 μCi/mg DFP/kg in order to yield an appropriate amount for the injection. The purity of the substance has been checked prior to the experiments by means of radiochromatography. The radioactivity was determined in the following solution: 0·02 ml serum, bile or urine, plus 13 ml scintillation fluid containing 4·0 g PPO and 0·1 g POPOP in 1000 ml of toluene, plus 2 ml of pure ethanol, plus 0·7 g thixotropic gel powder (Packard). The samples were counted in a Packard liquid scintillation spectrometer (Mod. 3002). All counts were corrected by means of an internal tritium standard.

DFP and its metabolites were separated by TLC and determined upon radiochromatographic scanning.[5] Each value given represents the mean ± S.E.M. of at least 7 determinations.

RESULTS

(a) *Serum level*

The decline of the arterial and venous serum levels after i.v. injection of ^{3}H-DFP is demonstrated in Fig. 1. The arterial curve consists of two components. During the first 20 min a rapid decline occurs. Subsequently, the slope becomes flatter. The venous level lies significantly below the arterial level during the first phase and does not show the steep phase. In a semilogarithmic plot the arterial curve may be separated into two different exponentials (Fig. 2). Their half times amount to 7 resp. 200 min. The

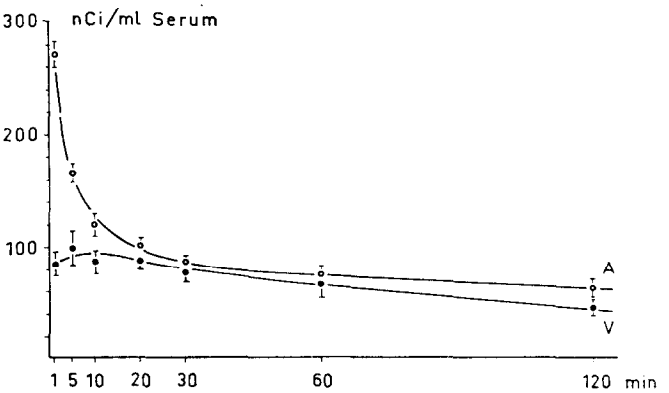

FIG. 1. Serum radioactivity (carotid ○, jugular vein ●) after the i.v. administration of 40 μC$_i$ ^{3}H-DFP/kg.
Abscisse: time, elapsed after drug administration (min) Ordinate: radioactivity (nCi/ml serum)

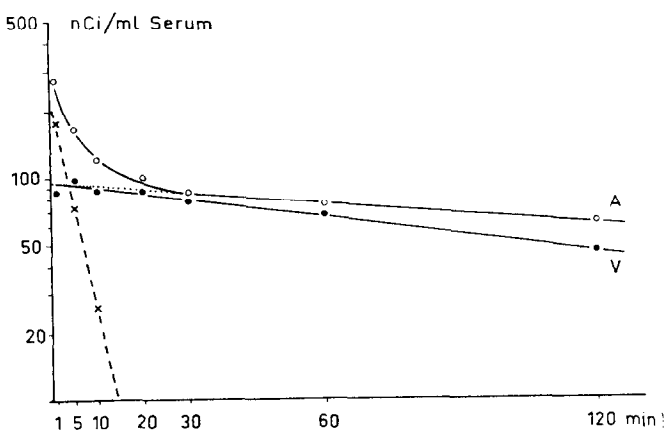

FIG. 2. Radioactivity in arterial (A, ○) and venous serum (V, ●) (drawn curves). Graphical separation of the curve for arterial serum radioactivity into two different components.
Fast component ($t_{\frac{1}{2}}$ = 7 min) — — — —
Slow component ($t_{\frac{1}{2}}$ = 200 min) . . . . . .
Abscisse: time, elapsed after drug administration. Ordinate: radioactivity (nCi/ml serum), logarithmic scale

decline in the venous serum is characterized by a similar, long half time as the slow phase of the arterial curve.

((b) Excretion with the bile, the urine and via the lungs

The total excretion of radioactivity with the bile is demonstrated in Fig. 3. During the first 10 minutes the rate of excretion amounts to about 0·8 nCi/10 min and later on increases until about 2·4 nCi/10 min. The cumulative plot yields an almost straight line. After 2 hr 0·13% of the injected radioactivity was excreted with the bile.

The rate of excretion of labelled material with the urine is depicted in Fig. 4. It increases until the 20th minute after injection and reaches the value of about 4 μCi/10

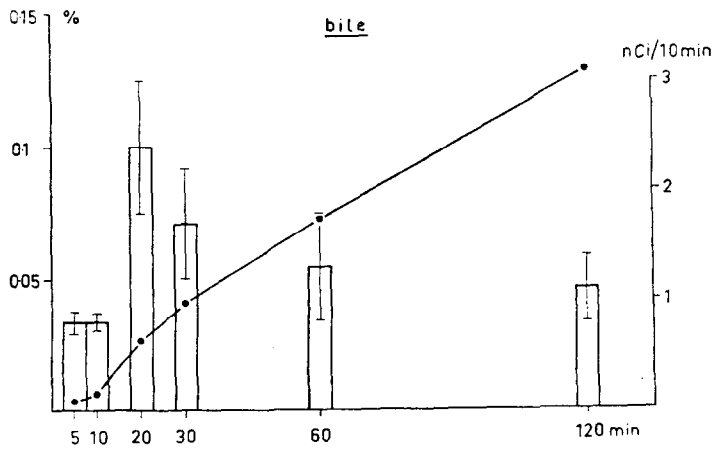

FIG. 3. Excretion of radioactivity with urine and bile.
Drawn curve: cumulative representation of the percentage excreted of radioactivity (left ordinate)
Columns: rate of excretion (right ordinate), nCi/10 min (bile).
Abscisse: time elapsed after drug administration.

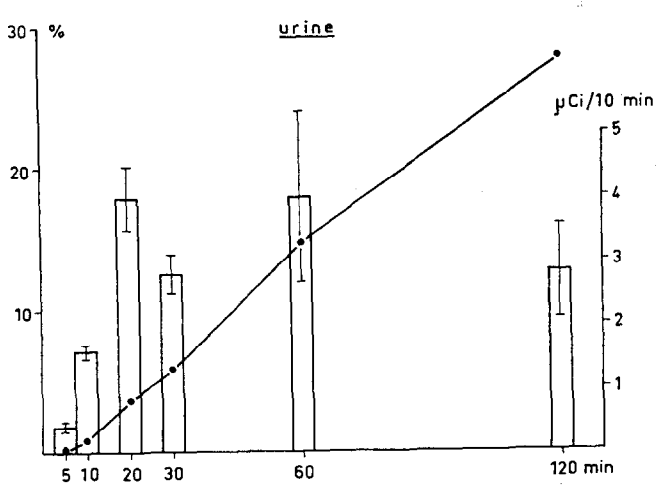

FIG. 4. Excretion of radioactivity with urine and bile.
Drawn curve: cumulative representation of the percentage excreted of radioactivity (left ordinate).
Columns: rate of excretion (right ordinate), μCi/10 min (urine).
Abscisse: time elapsed after drug administration.

min. Again the cumulative plot yields a straight line. 30 per cent of the injected tritium has been excreted via the kidneys during 2 hr.

The expired air contains radioactivity, which amounts to 615 ± 60 nCi in 2 hr. This corresponds to about 0·5 per cent of the injected doses.

(*c*) *Metabolites of* ^{3}*H-DFP*

One minute after the injection of the organophosphate only 15% of the labelled material in serum samples consists of non-metabolized DFP. This portion slowly decreases and completely disappears after about 1 hr. Corresponding to the disappearance of DFP, one single metabolite occurs in the appropriate amount to compensate for the radioactivity, namely diisopropyl-phosphate (DP). While the expired air contains only non-metabolized DFP, the radioactive material in the urine and the bile consists of about 5–10% DFP during the first 30 min, thereafter DP alone proved responsible for the activity.

DISCUSSION

In experiments on cats the injected ^{3}H-DFP leaves the blood so rapidly that a remarkable difference between the concentration of radioactivity in the arterial and venous serum occurs. According to results with guinea pigs[5] the lungs at once accumulate DFP to a great extent and may act as a buffer. The fast component of the arterial serum curve reflects the rapid accumulation of DFP and its metabolite by different tissues. Thirty minutes after the injection, the decline curves in the arterial and venous serum approximate each other. The slow component (half time about 200 min) probably represents the elimination process. The main excretion occurs via the kidneys (30% in 2 hr), while the elimination via the liver (about 0·1%) and via the lungs (0·5% in 2 hr) may be neglected. The results are in good agreement with those reported by Cohen and Warringa[4] in human subjects.

The metabolic degradation of DFP to DP occurs rapidly. DFP can be traced in the serum only for the first 30 min after injection. The main product in the urine and bile is also DP.

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