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# Translobular Uptake Patterns of Environmental Toxicants in the Rat Liver

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Recent autologous blood perfusion and autoradiographic studies in this laboratory have shown that uptake of the organophosphate insecticide, parathion and its metabolite, paraoxon, by the rat liver is extremely rapid. As a result, essentially all the molecules entering the liver are first taken up by a narrow band of periportal hepatocytes and undergo "chromatographic" migration downstream repeating reversible uptake and release (Nakatsugawa et al. 1980; Bradford and Nakatsugawa 1982; Tsuda et al. 1987). A small fraction of influent parathion (1 to 2%) never enters hepatocytes due to tight binding to blood components and is eluted in a "void volume" spike. Except for this small portion, however, essentially all the dose is subjected to biotransformation by hepatocytes in a sequential manner along the sinusoidal flow (because of the repeated uptake and release). Low doses can be totally degraded precluding systemic exposure. It is important to realize that the efficient metabolism of these organophosphorus esters during the first hepatic passage results from a favorable combination of two independent factors, i.e., the titer of biodegradation enzymes within the lobule and the mode of translobular uptake. If this scenario also applies to other chemicals, we may be able to define their threshold doses for systemic exposure. Such a possibility has far-reaching toxicological implications, and prompted this study to explore the less defined of the two underlying factors, i.e., translobular uptake pattern of xenobiotics using the recently developed autologous blood recirculating liver perfusion technique (Tsuda et al. 1987).

We have limited ourselves to non-ionic chemicals to avoid complications due to active transport. Because water solubility/lipophilicity is likely to be a critical factor in the binding of xenobiotics to the blood and hepatocytes and thus in their translobular behavior, xenobiotics of varied lipophilicity were pulse-infused and their elution pattern examined in the recirculating autologous blood perfusion system (Tsuda et al. 1987). Three chemicals, i.e., 1,2- and 1,3-dichlorobenzene and 4-nitroanisole (reported water solubilities are $9.9 \times 10^{-4}$ M at $25^{\circ}$ C, $8.4 \times 10^{-4}$ M at $25^{\circ}$ C and $3.8 \times 10^{-3}$ M at $30^{\circ}$ C, respectively; Verschueren 1983), were chosen as

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examples of relatively water-soluble xenobiotics compared with parathion ($8.2 \times 10^{-5}$ M at $20^{o}$ C; Verschueren 1983). Benzo(a)pyrene (water solubility: $2.0 \times 10^{-9}$ M at $20^{o}$ C; Eisenbrand and Baumann 1969) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, $6.2 \times 10^{-10}$ M; Crummett and Stehl 1973) were studied as highly lipophilic toxicants. Elution of the chemical from the liver in situ at the "void volume" (about 10 sec after input) served as an indicator of hepatic uptake efficiency as discussed before (Tsuda et al. 1987).

## MATERIALS AND METHODS

The following chemicals were purchased from Amersham/Searle Corp. (Arlington, IL) and were 97-99% radiochemically pure: ($^{3}$H) hexadecane (2.0 $\mu$Ci/g), (U-$^{14}$C) sucrose (477 mCi/mmol), (G-$^{3}$H) benzo(a)pyrene (40 Ci/mmol), (phenyl-1-$^{14}$C) 4-nitrophenol (22.5 mCi/mmol). The labeled 4-nitrophenol was used to synthesize (phenyl-1-$^{14}$C) 4-nitroanisole (22.5 Ci/mmol, prepared by methylation with diazomethane and used after dilution to 0.815 mCi/mmol). (1-$^{14}$C) Hexadecane (1.10 $\mu$Ci/g) was obtained from Packard Instrument Co., Inc., Downers Grove, IL. (U-$^{14}$C) 1,3-Dichlorobenzene (0.63 mCi/mmol) and (U-$^{14}$C) 1,2-dichlorobenzene (2.06 mCi/mmol) (both 99% pure, New England Nuclear, Boston, MA) were gifts of Drs. H. Sikka and S. Banerjee of Syracuse Research Corp., Syracuse, NY. ($^{3}$H)TCDD (50 Ci/mmol) was purchased from KOR Isotopes (Cambridge, MA). Although the manufacturer indicated the purity of the tritiated TCDD to be 90%, thin-layer chromatograms (silica gel with hexane, hexane:dioxane (5:1), or hexane:ethyl acetate (5:1) as solvent) revealed radioactive impurities only at the origin in amounts which were 1% when the chromatogram was developed immediately after spotting, but increased to over 10% as the time between spotting and developing increased. Similar results were obtained with the tritiated benzo(a)pyrene, indicating the instability of the tritium labels on silica gel. Since high-performance liquid chromatography of these tritiated chemicals revealed only single radioactive materials, they were generally used without further purification. Bovine serum albumin (fraction V) and heparin (sodium salt, grade II) were obtained from Sigma Chemical Co., St. Louis, MO and Waymouth MB 752/1 powder was purchased from Grand Island Biological Co., Grand Island, NY.

Experimental protocol and apparatus for the recirculating autologous blood liver perfusion procedure have recently been described in detail (Tsuda et al. 1987). Male Sprague-Dawley rats (Tac:(SD)fBR) (300-315 g at the time of experiments) were purchased from Taconic Farms, Germantown, NY. Rats were prepared for in situ perfusion, about 10 ml of blood was collected and reintroduced to begin a recirculating perfusion at a flow rate of 20 ml/min. Packed cell volume (PCV), normally about 42% in undiluted blood, was decreased to 28 to 33% due to some dilution with Waymouth's medium during collection. Following the infusion, 0.1 ml effluent samples were collected at intervals and each aliquot was mixed with 0.4 ml of distilled water in a test tube for extraction and analysis. When very low doses of benzo(a)pyrene or TCDD were infused in the single-pass perfusion mode, the first 20-second eluate was collected in a single tube and the remainder in a second tube. Following the perfusion, the liver was removed, weighed, and homogenized with 40 ml of 5% (w/v) trichloroacetic acid in a Sorvall Omnimixer.

To separate the original compound (and any lipophilic products) from polar metabolites, 2 ml of hexane was shaken with 0.5 ml of an aqueous sample containing eluate or liver homogenate. Also, a 20-$\mu$l aliquot of the original infusion solution was mixed with 0.4 ml of water and similarly processed. One milliliter of the hexane phase, which contained essentially all the parent compound, was removed for radiometry in a Packard Tri-Carb Scintillation Spectrometer. The hexane extraction was repeated to remove any residual parent compound, and 0.1 ml of the aqueous phase was counted. When the total radioactivity was determined, 0.1 ml aqueous sample was directly counted. The counting efficiency was determined with radiolabeled hexadecane as the internal standard.

## RESULTS AND DISCUSSION

Fig. 1 (A and B) shows typical elution profiles obtained by pulse-infusions of $^{14}$C-labeled 1,2- and 1,3-dichlorobenzene during the recirculating autologous blood perfusion. The hexane fraction profiles had no peak at the void volume position (ca. 10 sec.) indicating complete absorption of these compounds during the first passage through the liver. Subsequent elution clearly reflected chromatographic migration of these chemicals due to rapid reversible uptake and release, with average hepatic transit times of 2.3±0.5 (n=4) and 1.3±0.0 (n=2) min for 1,3- and 1,2-dichlorobenzene, respectively. Gas chromatographic analysis of eluate samples near these peaks showed that the radioactivity in the hexane extracts indeed represented the original dichlorobenzenes. Radioactivity in aqueous fractions only slowly increased with time, indicating slow metabolism.

Fig. 1 (C) also presents the behavior of 4-nitroanisole during the recirculating autologous blood perfusion. The lack of the void-volume peak again showed the total absorption during the first passage and the chemical was eluted rapidly with the first peak at 0.92±0.12 (n=2) min and secondary peaks thereafter. Gas chromatographic analysis of the hexane fractions of the first-peak samples revealed that up to one-third of the radioactivity was due to metabolites. The true transit time of the parent compound, therefore, may be slightly different. Radioactivity in the aqueous phase increased rapidly, reflecting the biodegradability of this compound.

The behavior of two highly water-insoluble chemicals, benzo(a)pyrene and TCDD, was examined, generally with a very low infusion concentration to ensure that the chemicals were truly dissolved, albeit in a protein-bound form. Fig. 2 (A and B) illustrates elution profiles of these chemicals infused in autologous blood or other media. A normalized unit was used on the ordinate to permit a composite plot of different experiments and a void-volume elution of sucrose, which is not taken up by hepatocytes. In the autologous blood perfusion, elution of these chemicals was markedly different from that of more water-soluble chemicals. A substantial portion of the dose flowed through the liver without entering hepatocytes, and was eluted as a large void-volume peak regardless of the concentration (Table 1). Infusion of these chemicals dissolved in plasma during the autologous blood perfusion also produced a sizable void-volume peak.

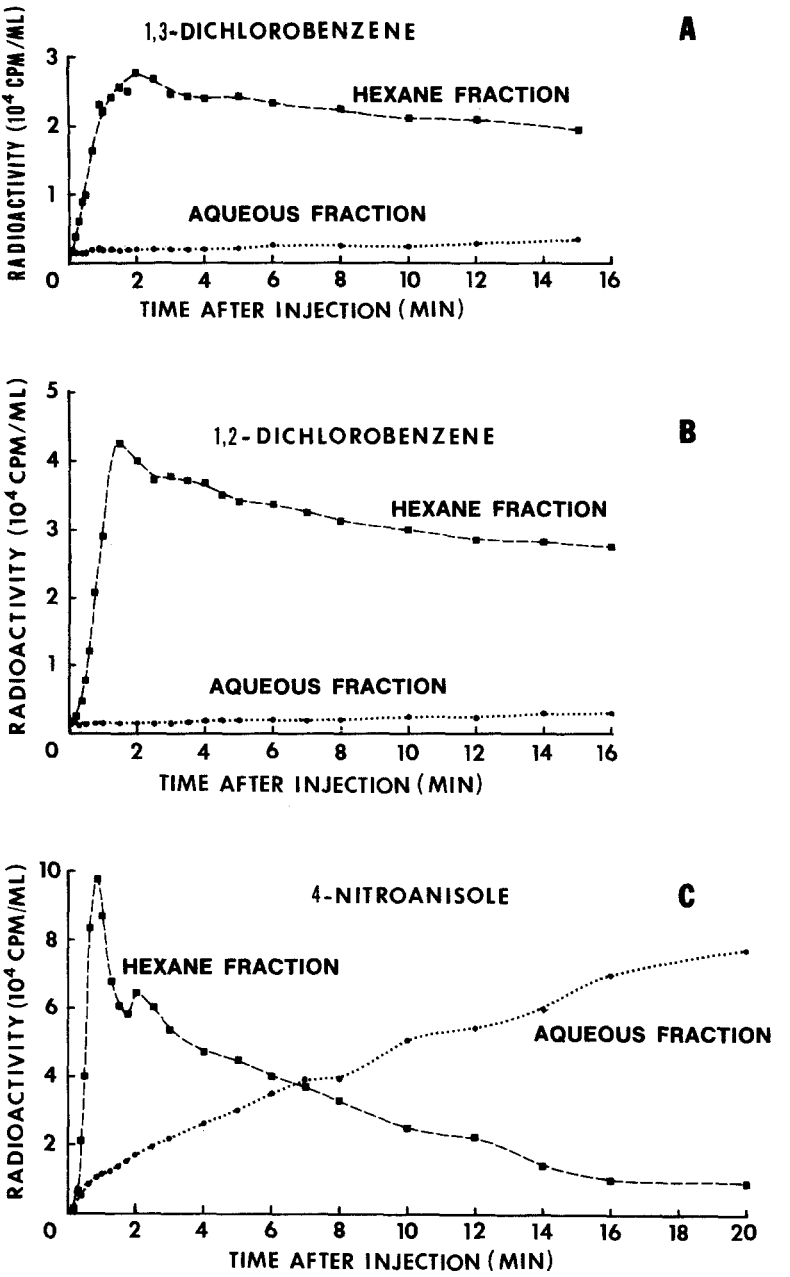


Fig. 1. Elution patterns of $^{14}C$-labeled chemicals. (1A): 1,3-Dichlorobenzene ($1.2 \times 10^{-6}$ mol) was infused in the recirculating perfusion with autologous blood (PCV 30.4%). (1B): 1,2-Dichlorobenzene ($6.5 \times 10^{-7}$ mol) was infused in autologous blood (PCV 35.6%). (1C): 4-nitroanisole ($1.9 \times 10^{-6}$ mol) was infused in autologous blood (PCV 29.1%).

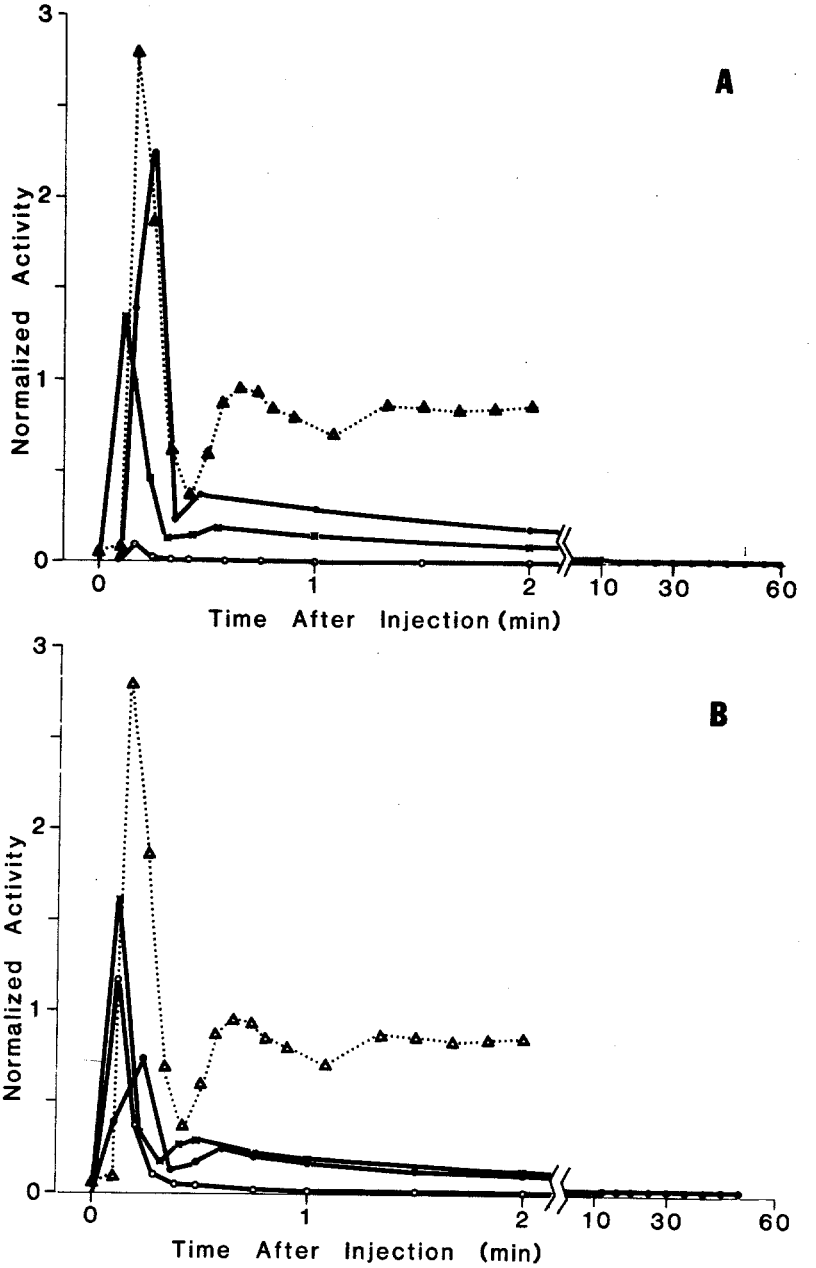


Fig. 2. Typical elution patterns of highly lipophilic chemicals. Also see Table 1. Benzo(a)pyrene (2A) or TCDD (2B) was infused in autologous blood (PCV 29%) [●], autologous plasma [■] (recirculating perfusion with autologous blood) or Waymouth's medium containing 3% bovine serum albumin [○] (single-pass perfusion with the same medium). For comparison, sucrose elution profile (Fig. 2A) is superimposed [▲] and all values are expressed normalized as % of infused amount in 0.1 ml effluent sample.

Table 1. The size of the void-volume peaks of benzo(a)pyrene and TCDD under various conditions. The number of replicates is given in parentheses.

| | benzo(a)pyrene | | TCDD | |
|---|---|---|---|---|
| Perfusate[a] | dose ($10^{-9}$ M) | peak[b] (%) | dose ($10^{-9}$ M) | peak[b] (%) |
| blood | 356±0 | 58±8 (2) | 1275±0 | 30±6 (2) |
| blood | 119±31 | 42±23 (3) | 70±10 | 34±17 (3) |
| blood[c] | 0.10±0.01 | 60±10 (2) | 0.15±0.02 | 31±8 (2) |
| 3% BSA in Waymouth's | 37±1 | 3.6±2.3 (2) | 60±0 | 24.5±4.9 (2) |
| Waymouth's[c] | 0.59 | 3.0 (1) | 0.46 | 2.5 (1) |
| Waymouth's[c] | 0.08±0.01 | 4.7±0.8 (2) | 0.14±0.02 | 2.9±0.9 (3) |

a/ Autologous blood containing about 30% Waymouth's medium, plain Waymouth's medium or Waymouth's medium containing 3% bovine serum albumin was used as the perfusate.
b/ Percentages of 0.4-ml infused dose recovered in the void volume are given.
c/ Unlike other experiments listed, perfusion was carried out in the single-pass mode and the first 20-sec eluate was collected as a single void-volume sample for these very low-dose infusions.

These results suggest that the hepatic uptake of benzo(a)pyrene and TCDD is limited by the slow reversal of macromolecular binding during passage through the lobule. In fact, at concentrations below water solubility (thus, presumably in true solution), these chemicals in non-proteinaceous Waymouth's medium were much more efficiently taken up by hepatocytes, eluting a void-volume peak corresponding to only 3-5% of the dose (Table 1). Binding, however, appears to depend upon the combination of a chemical and binding macromolecules. Benzo(a)pyrene infused in the single-pass perfusion with 3% bovine serum albumin in Waymouth's medium, for example, yielded a much smaller void volume peak (4%) whereas the peak of similarly infused TCDD was like those observed in the autologous blood perfusion. At least in the case of benzo(a)pyrene, sequestration by chylomicron is not the cause of the void-volume peak since the peak was essentially unaltered with a rat starved for 36 hours before experiments.

With benzo(a)pyrene and TCDD, the void-volume peak in the autologous blood perfusion was followed by another smaller peak at about 30 sec. The latter peak is probably not due to the elution of the chemical from hepatocytes, but due to recirculation of the initial void-volume peak. In fact, the peak position coincided with that of the second peak of sucrose

elution in separate experiments (data not shown) in which sucrose was co-infused with benzo(a)pyrene or TCDD. No further peaks could be discerned and the concentration decreased steadily towards zero. The radioactivity in the aqueous phase of the eluate increased only slightly over a period of 60 min. About 80% of the total radioactivity of benzo(a)pyrene and 90% of that of TCDD were recovered from the liver at the end of the 60-min recirculating perfusion experiments. Thus, once taken up by the liver, these highly lipophilic chemicals seem to be released back to circulation extremely slowly, if at all.

All the chemicals so far examined seem to have high intrinsic rates of uptake by the hepatocyte as evidenced by the absence or small size of void-volume peak following their pulse infusion in a non-proteinaceous medium (Table 1; Nakatsugawa et al. 1980; Bradford and Nakatsugawa 1982; Tsuda et al. 1987). Translobular uptake of xenobiotics in vivo, however, appears to be affected by lipophilicity via macromolecular binding in the blood so that two types of behavior are apparent. Relatively water-soluble, non-ionic xenobiotics are likely bound to blood macromolecules only weakly and thus are liable to rapid and total uptake by periportal hepatocytes and subsequent translobular migration with concomitant hepatic action. Tight binding to blood, on the other hand, seems to deliver highly lipophilic xenobiotics directly to the entire lobule and beyond.

Lipophilicity also seems to control the fate of chemicals already taken up by hepatocytes by deterring the release from cellular macromolecules. For instance, the apparent absence of elution of benzo(a)pyrene and TCDD from hepatocytes probably reflects extremely slow reversal of hepatocellular binding of these chemicals. In addition, cellular binding probably underlies the excellent correlation recently observed between log(elution volume) and log P (P = octanol:water partitioning coefficient) of a series of dialkyl dithiolanylidenemalonates perfused at 5°C in protein-free or low protein media (Murakami et al. 1987). For chemicals undergoing chromatographic translobular migration in vivo, the transit time (or elution volume) will likely depend upon the ratio of the blood and hepatocyte binding, i.e., partitioning between the blood and the hepatocyte. There appears to be a degree of lipophilicity above which translobular uptake is rate-limited by blood binding, yielding a sizable void-volume peak. These generalizations require further experimental verification and form the subject of our ongoing research involving a wider range of xenobiotics.

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