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The release of plasticizer from polyvinyl chloride haemodialysis tubing

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The extent of migration of plasticizer from haemodialysis lines into saline and plasma has been examined and a number of different types of tubing have been compared. A model system designed to simulate *in vivo* haemodialysis was evolved. The method uses a continuous cyclohexane extraction in order to mimic uptake of plasticizer by body tissues. Although not an ideal system, the results obtained are indicative of the extent to which the plasticizers are leached.

The migration of plasticizers from flexible polyvinylchloride medical devices and storage bags into biological fluids has been reported (Jaeger & Rubin, 1970, 1972; Marcel & Noel, 1970; Fishbein & Albro, 1972; Janimet & Dive, 1974; Vessman & Rietz, 1974) and special attention has been given to the plasticizer content of blood and plasma stored in plastic bags. The transfer of plasticizers from haemodialysis tubing to the blood of patients undergoing regular haemodialysis has also caused concern (Neergaard, Nielsen & others, 1971, 1975; Ono, Tatsukawa & Wakimoto, 1975). We have examined a number of currently available haemodialysis lines for the type of plasticizer present and the extent to which it may be leached into the patient's bloodstream.

MATERIALS AND METHODS

Complete haemodialysis lines as supplied to hospitals were used. The type of plasticizer in the different types of line was determined by Soxhlet extraction of a portion of the tubing with hot ethanol and examination of the residual oil after evaporation of the solvent. Only two plasticizers were found, di-2-ethylhexyl phthalate (DEHP) and di-2-ethylhexyl adipate (DEHA) normally amounting to 20–30% of the weight of tubing. Reference samples of DEHA and DEHP were obtained from Koch Light Laboratories.

Solvents were redistilled until satisfactory blank values were obtained. All other materials were checked for DEHA and DEHP and if necessary were continually extracted in a Soxhlet apparatus until free from contamination.

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Column chromatography was on silica gel (Kieselgel 60, 70–230 mesh. Merck) using a column of 8 mm i.d., dry packed with 4 g of adsorbent.

G.l.c. conditions. Analyses for DEHA and DEHP were on a Pye 104 Gas Chromatograph using a flame ionization detector at 300°, nitrogen carrier gas, 60 ml min^{-1}. A 5 ft glass column containing 5% OVI on Chromasorb WHP was used. Oven temperatures were 245° and 255° for DEHA and DEHP respectively and retention times of 2·9 min and 4·0 min were obtained. External standards and peak height measurements were used for quantitation.

Plasticizer extraction and determination in saline, plasma and whole blood. The sample, saline, plasma or blood, (1–5 ml) was shaken with acetonitrile (0·5–2·5 ml) and 30% ether in light petroleum (40–60° b.p. range), (50 ml) for 1 h. For whole blood and some plasma samples, prior absorption on cotton wool (0·05–0·25 g) reduced the extent of emulsification. A suitable aliquot of the solvent layer was evaporated to dryness at 40° under reduced pressure. (Extracts from saline or water were determined directly at this stage without the chromatographic clean-up). The residue was dissolved in 10% ether in light petroleum (5 ml) and transferred to a chromatography column which was eluted with light petroleum and the yellow band which first separated was discarded. Subsequent elution with 100 ml of 5% ether in light petroleum gave a solution of plasticizer which was evaporated to dryness as before, redissolved in acetone (0·2–1·0 ml) and injected into the gas chromatograph. From a 5 ml sample, consistent recoveries of DEHA of 90–110% were achieved at

plasma concentrations down to 0·2 ppm. Comparable recoveries of DEHP could only be obtained at plasma concentrations greater than 1 ppm.

Perfusion procedures. Closed perfusion was by circulating 250 ml of bovine plasma through dialysis lines, one arterial and one venous connected in series, for 5 h. A Watson Marlowe external roller pump was used at a flow rate of 300 ml min^{-1} and the contents were maintained at 37° throughout.

Perfusion with continuous extraction. The procedure was essentially the same as above except for the inclusion of the assembly shown in Fig. 1. The apparatus was assembled and the reservoir filled to capacity with perfusing fluid. Cyclohexane (350 ml)

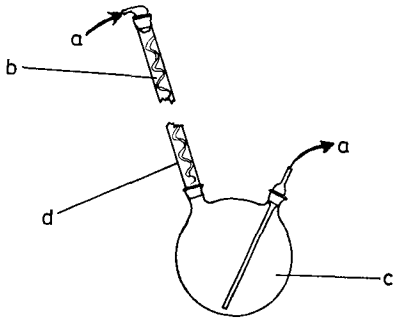

FIG. 1. Apparatus for continuous extraction. a—Perfusate, b—cyclohexane, c—perfusate reservoir, d—glass column (2 m × 20 mm) with spiral glass insert.

was then added to the top of the column and then more perfusate until the liquid level was raised to the top of the column. The system was arranged so that complete separation of the solvent and perfusate occurred high up in the reservoir. At the end of the perfusion, aliquots of solvent and perfusate were taken and the plasticizer content determined.

RESULTS AND DISCUSSION

Previous workers (Neergaard & others, 1975) have developed test procedures which involve the continuous circulation of saline, plasma or whole blood through haemodialysis lines in a closed system for a specified time. The concentration of plasticizer in the perfusate is then determined and the different types of lines are compared on this basis.

The migration of plasticizers into the perfusing fluid under these conditions may be considered to be dependent on the following factors:

1. The amount of 'free' surface plasticizer on the inner walls of the dialysis tubing.
2. The rate at which the 'free' surface plasticizer is renewed by diffusion and displacement from the bulk of the PVC to the inner wall of the tubing.
3. The solubility of the plasticizer in the perfusate and the partition coefficient of the plasticizer between the perfusate and the PVC tubing wall.

This can be represented diagramatically as in Scheme 1.

$$\text{PVC} \overset{K_1}{\rightleftharpoons} \text{'Free' surface plasticizer} \overset{K_2}{\rightleftharpoons} \text{Perfusate}$$

Scheme 1

It follows that the evaluation of haemodialysis lines using the systems described above will reflect K_1, K_2 and the total amount of 'free' surface plasticizer. When saline is used as perfusate, the very low aqueous solubilities of DEHA and DEHP result in low concentrations of them in the perfusate (Table 1).

Table 1. *Closed perfusion (one arterial, one venous line in series).*

Tubing type	Plasticizer	Perfusate	Concn of plasticizer in perfusate (ppm)
I*	DEHA	Saline	< 0·05
II	DEHP	Saline	< 0·05
I	DEHA	Bovine plasma	80–90
II	DEHP	Bovine plasma	25–30
III	DEHP	Bovine plasma	25–30
IV	DEHA/DEHP	Bovine plasma	< 0·1/25

* Now withdrawn.

This indicates the limiting effect of K_2. With plasma as perfusate, much higher concentrations of both DEHA and DEHP are found due to their much greater solubility in plasma (Table 1). However in any system of this type, unless extemely large volumes of perfusing fluid are used, equilibrium concentrations may be approached thereby limiting the amount of plasticizer removed from the tubing wall. For these reasons, we consider these systems are unsatisfactory since they provide little information about the total amount of plasticizer available to the patient during dialysis.

Reports by Schulz & Rubin (1973) and our own work with animals indicate that both DEHA and DEHP are rapidly removed from the blood and are concentrated at various points in the body. This being so, the perfusate saturation effects mentioned above are no longer significant *in vivo* and the limiting effect of K_2 may be ignored. Thus the *in vivo* situation is better represented by Scheme 2.

$$\text{PVC bulk} \overset{K_1}{\rightleftharpoons} \text{'Free' surface plasticizer} \overset{K_2}{\rightleftharpoons} \text{Blood} \overset{K_3}{\rightleftharpoons} \text{Body tissues}$$

Scheme 2

Here the effect of K_3 is to rapidly remove the plasticizer from the blood to the tissues and it follows that appreciable transfer of plasticizer from the tubing into the patient can occur if the amount of 'free' surface plasticizer is substantial or if K_1 is significant. It also follows that substantial transfer of plasticizer can occur without significant elevation of the concentration in the blood and consequently only limited information can be obtained by monitoring the blood concentration of plasticizer.

The situation is further complicated by the fact that invariably we have observed background concentrations of plasticizer (0·1–0·2 ppm) particularly DEHP in all subjects examined, even those not undergoing haemodialysis or transfusion procedures. Rubin & Nair (1973) have reported similarly. The source of plasticizer in these cases is assumed to be dietary.

In an attempt to overcome these problems, a test system which more closely resembles the *in vivo* situation has been developed. To simulate the uptake of plasticizer by body tissues, the perfusate was extracted with an organic solvent before being recirculated through the dialysis lines. At the end of the perfusion, the total weight of plasticizer in the organic phase was determined and in addition, the concentration of plasticizer in the perfusate (Table 2).

Cyclohexane was found to be the most satisfactory solvent phase. Other more powerful solvents caused extensive emulsification with plasma or showed much greater solubility in the perfusate. Both of these factors must be avoided since incomplete separation of the two phases allows solvent to come into direct contact with the tubing wall, whilst high concentrations of dissolved solvent affect the solubility and partition coefficient of the plasticizer in the perfusate. (K_2 Scheme 2).

Using the system described, appreciable amounts of plasticizer remain in the plasma perfusate (Table 2). This indicates that the cyclohexane column is significantly less effective than the patient in extracting plasticizer from the blood since *in vivo* plasma concentrations do not exceed 2 ppm even during dialysis with Type I tubing. We have also observed that the patient's blood concentrations of plasticizer rapidly return to pre-dialysis values after disconnection of the lines.

Table 2. *Perfusion with continuous extraction.*

Tubing type	Plasticizer	Perfusate	Concn plasticizer in perfusate (ppm)	Wt of plasticizer in cyclohexane (mg)
I	DEHA	Plasma	39–51	350–450
II	DEHP	Plasma	11–14	1·2–3·5
III	DEHP	Plasma	17	7·5–9·5
IV	DEHA/DEHP	Plasma	*	0·5–1·0/8–11
V	DEHA	Plasma	28–35	100–127
I	DEHA	Saline	< 0·1	70–80
II	DEHP	Saline	< 0·1	0·8–1·5

* Determination not carried out.

While the model system falls short of the clinical situation, it is possible to draw definite conclusions about the different types of haemodialysis lines. This is particularly true for Type I tubing which has a performance only slightly below average in the closed perfusion system (Table 1) whereas in the continuous extraction system, a dramatic increase in plasticizer leaching is observed. This is in agreement with clinical effects which have been reported in the use of this type of tubing (Gibbins & Martin, 1976).

On the basis of these findings we suggest that all perfusion tubing should be evaluated under similar conditions which more closely reflect the *in vivo* situation. We also suggest that whilst the extent of plasticizer migration can be significantly reduced by minimising the amount of 'free' surface plasticizer and the value of K_1, some migration is inevitable. Consequently the problem may best be resolved by the use of plasticizers which are rapidly metabolized and are much less toxic than those now in use (Singh, Lawrence & Autian, 1972, 1975; Milkov, Aldyreva & others, 1973; Lake, Gangolli & others, 1975; Schulz, Rubin & Hutchins, 1975). This is of particular importance for patients with renal impairment since in such cases the normal method of excretion of these compounds may be highly compromised.

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