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Plasma Extraction of Plasticizers from "Medical Grade" Polyvinylchloride Tubing (38389)

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(Introduced by John M. Weller)

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Up to 2 mg of di-2-ethylhexyl phthalate (DEHP) have been extracted in 5 hr from polyvinylchloride (PVC) tubing by a plasma perfusate (1). This highly lipid soluble compound has been shown to cause chemical peritonitis (1) and to be toxic in human tissue cultures (2). Little information is available regarding the extraction of DEHP and other phthalates from PVC tubings available commercially. In this study, we have demonstrated *in vitro*, that DEHP and di-2-ethylhexyl adipate (DEHA) are extracted in milligram quantities from tubings used for hemodialysis.

Materials and Methods. Tubings supplied to conduct blood to and from artificial kidneys were obtained from the following sources: Travenol Laboratories, Inc., Morton Grove, IL (Sample A); Cobe Laboratories, Inc., Lakewood, CO (Sample B); Extracorporeal Medical Specialties Company, King of Prussia, PA (Sample C); Sweden Artificial Kidney Supply Company, Seattle, WA (Sample D), and Life Med Corporation, Compton, CA (Sample E). In each case a specimen of tubing designed to deliver blood to the hemodialyzer was attached to the tubing designed for returning blood from the hemodialyzer using the hard plastic fitting provided by the manufacturer. This system was then perfused with 500–700 ml of human plasma from a glass reservoir by a roller pump at 200 ml/min. The perfusate was maintained at 37° by a water bath. Samples for phthalate determinations were taken from the reservoir.

Human plasma was obtained from blood collected in plastic bags in a blood bank. Blood specimens were immediately centrifuged and the plasma transferred to glass containers. The plasma was in contact with the plastic collection container for less than 30 min.

Plasma samples were extracted by the procedure of Folch *et al.* (3) with chloroform and methanol. The plasticizers were subsequently quantitated by a computerized gas liquid chromatographic-mass spectrometer (4). This assay measures as little as 0.5 μg/ml of the compounds examined and distinguishes changes of one μg/ml.

Results. DEHP was found in all samples of plasma before recirculation was begun (Table I). This phthalate probably originated from the plastic collection bags used to separate plasma from red blood cells. However, the presence of phthalates was not determined in the blood of these normal donors.

The DEHP level in the plasma perfusing the blood tubing progressively increased over the 5–6 hr of perfusion reaching levels as high as 35.9 μg/ml (Table I). Table II lists the amount of DEHP extracted in the perfusate. The extraction rate for tubing sample A was markedly reduced during the second hour of recirculation when the pump was turned off. From 8.9–13.2 mg of DEHP were extracted from the four sets of tubing within 6 hr.

DEHA was found only the perfusate for sample A. No DEHA was identified in the plasma before the perfusion began. Thereafter, hourly concentrations were 2.7, 3.7, 7.3, 8.5, and 9.7 μg/ml. Like DEHP, the rate of extraction of DEHA into the plasma was markedly reduced during the hour without recirculation. The total

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TABLE I. DEHP Concentration in Plasma Perfusing PVC Tubing.[a]

Tubing	Hours of Perfusion 0	1	2	3	4	5	6
A	3.0	5.6	5.7[b]	10.7	13.6	15.5	—
B	1.1	6.2	10.8	16.5	18.5	28.9	27.5
C	2.0	5.6	8.7	12.9	16.7	24.0	28.7
D	1.4	3.9	6.3	9.2	12.8	—	21.0
E	0.5	5.4	10.4	18.4	—	27.0	35.9

[a] μg/ml.
[b] Recirculation stopped during second hour.

amount of DEHA extracted after 5 hr was 4.2 mg.

Di-tert-butyl cresol (DTBC), an antioxidant, was also found in tubing D. None was identified in the plasma used for perfusion and only trace amounts were found after 1 and 2 hr of perfusion. Thereafter, the perfusate concentration of DTBC (μg/ml) was 0.3 after 3 hr, 0.5 after 4 hr and 0.9 after 6 hr.

Discussion. Since extraction of DEHP was nearly linear in the tubing examined, even higher levels probably would be achieved with longer perfusion at 37°. The observation that DEHA was also found in one specimen and DTBC was also found in another specimen, in addition to DEHP, suggests that at least three formulations of PVC tubings were involved. Accordingly, when the toxicology of such materials is in question, observations should be made for each source of the PVC tubing.

These results do not shed light on the biological significance of the phthalates extracted from the PVC tubings. However, the concentration of DEHP achieved in these experiments has been shown to be toxic for cells in tissue culture (2). There is also evidence suggesting toxicity of DEHP for an isolated liver preparation (1). These reports indicate the need for controlled studies with other conduit materials (such as tetrafluorethylene polymer or silicone rubber) when PVC tubing is used to perfuse isolated organs.

TABLE II. DEHP Extracted by Plasma Perfusing PVC Tubing.[a]

Tubing source	Hours of Perfusion 1	2	3	4	5	6
A	1.15	1.16	3.1	4.15	4.45	—
B	2.0	3.8	5.9	6.6	9.9	9.6
C	1.6	2.65	4.15	5.35	7.6	8.9
D	1.4	2.7	4.3	6.2	—	10.15
E	2.15	4.25	7.45	—	10.2	13.2

[a] Cumulative amounts of DEHP (mg) extracted by 500–700 ml of human plasma.

The significance for the intact organism of phthalate extracted into protein containing solutions is less clear. While the rat metabolizes DEHP slowly (1, 5) there is evidence suggesting that DEHP is rapidly metabolized in man (1, 6). However, the potential toxicity of such metabolites is unknown.

Amounts of phthalate comparable to those recovered in the studies reported here must also be extracted from PVC tubings during hemodialysis for patients with renal failure. The loss of extracted phthalate through the cellulosic membrane of the hemodialyzer is probably minimal because of the limited water solubility of these compounds (7). Although the total amounts of phthalate which are extrated *in vitro* and which could be given intravenously during hemodialysis are considerably below oral toxic doses (8), the ratio of urine to fecal ^{14}C is higher after intravenous than after oral administration of ^{14}C-labeled DEHP (5), suggesting that in anephric patients more DEHP and its metabolites accumulate after intravenous infusion than after oral ingestion. Furthermore, hemodialysis patients may be exposed to the PVC tubing of the artificial kidney three times a week for many years. Since patients undergoing chronic dialysis do not have a normal life span (9) and chronic phthalate toxicity cannot be excluded in man, further study to clarify these points *in vivo* is warranted.

Summary. Up to 13 mg of DEHP and 4 mg of DEHA were extracted from "medical grade" polyvinylchloride tubing by human plasma recirculated for 6 hr at 37°. The demonstrated toxicity of DEHP for *in vitro* systems suggests that this type of tubing should be evaluated for toxic effects on *in vitro* organ perfusion systems. The significance of these observations for medical applications has not been determined.

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