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GAS CHROMATOGRAPHIC—MASS SPECTROMETRIC DETECTION OF CIRCULATING PLASTICIZERS IN SURGICAL PATIENTS

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SUMMARY

Gas chromatographic and gas chromatographic—mass spectrometric analytical techniques were employed to quantitate and confirm levels of circulating organic plasticizers in critically ill surgical patients. Two plasticizers, dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP), have been identified. DEHP can be found in many plastic medical devices. The DEHP levels were significant soon after transfusion or in the presence of renal dysfunction. The source of DBP is not clear at present and requires further study. The prevention of this contamination and the toxicity of these plasticizers should be investigated to ensure the safe use of plastic medical devices.

INTRODUCTION

We have employed gas chromatography (GC) and gas chromatographic—mass spectrometric (GC—MS) analytical techniques to confirm and quantify the levels of circulating plasticizers in selected surgical patients requiring intravenous therapy. This study was prompted by the identification of dibutyl phthalate (DBP) in the lipid fraction of the serum of one of our surgical patients maintained on continuous therapy for 3.5 months and the subsequent

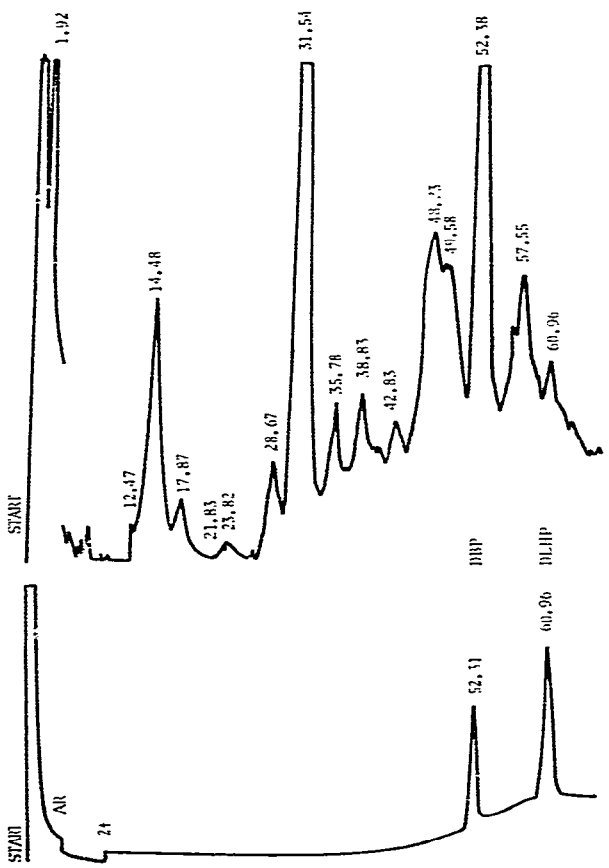

Fig. 1. A gas chromatogram of standard solutions of dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) is shown at the bottom. The gas chromatogram at the top is the fatty acid ester fraction of our patient in whom plasticizers were detected.

observation of the possible presence of DBP and another plasticizer, di-(2-ethylhexyl) phthalate (DEHP), in other patients (Fig. 1).

The use of plastic devices in medical care has increased tremendously. Surgical practice requires exposure of the patients to multiple uses of such materials; in many instances exposure is via the parenteral route and for extended periods of time. Various investigators have demonstrated plastic contamination of biological material and the ability of fluids contained in plastic containers used for parenteral therapy to leach out the plasticizer used in the manufacture of the containers [1—9]. Ono et al. [10] detected measurable levels of the plasticizer DEHP in peripheral blood samples of hemodialysed patients immediately after dialysis therapy. Hillman et al. [11] used GC—MS to identify and measure the same plasticizer in autopsy tissues (heart and small intestine) of infants who had had umbilical catheters inserted and had received varying amounts of blood products. Aronson et al. [12] reported that DEHP significantly decreased spontaneous heart rate, coronary flow and isometric tension but elevated diastolic tension in isolated perfused heart. In addition, significant concentration changes were noted for tissue glycogen, ATP, creatine phosphate, etc.

EXPERIMENTAL

Patient selection

Four critically ill patients in the Surgical Intensive Care Unit and two other subjects were selected for study. They were selected with respect to the duration of parenteral therapy, volume of blood and blood products infused, and the presence of renal dysfunction. One subject was selected as a control.

Control subject

The newest (less than two months) member of the Surgery Department's secretarial staff volunteered for the study. She had no previous history of intravenous infusions or contact with any medical environment and had minimal exposure to the surgical research facility's environment.

Preparation of sample

Peripheral venous blood samples were drawn using glass syringes and aluminum-coated needles and immediately transferred to rubber-stoppered glass tubes. The serum was separated from the clotted red cells and then frozen prior to analysis. Aliquots (1 ml) of the serum were extracted with either (A) 2.5 ml of Dole's mixture (2-propanol—*n*-heptane—sulphuric acid, 40:10:1, v/v) [13], or (B) 10 ml of absolute alcohol and 10 ml of *n*-heptane to denature the proteins and separate the lipids [10]. The remainder of the serum was then refrozen. The heptane layer containing the lipid fraction was separated, washed with water and then evaporated to dryness under a stream of nitrogen. The residue was redissolved in benzene and the fatty acids methylated at 60°C using BF_3—methanolic solution kits (Applied Science Labs., State College, PA, U.S.A.). The benzene layer, containing the lipids, was separated by centrifugation, dried over anhydrous sodium sulphate and evaporated to dryness under nitrogen. The methylated lipids were redissolved in 100 μl of acetone. The plasticizers were quantified by GC and their presence confirmed by GC—MS.

Gas chromatographic and gas chromatographic—mass spectrometric analysis

Aliquots of the processed extracts were analysed on a dual-column Hewlett-Packard 5830A gas chromatograph (with an auto sampler and data system) and flame ionization detectors using a 1.83 m × 2 mm I.D. glass column packed with 10% Silar-10C on Gas-Chrom Q (100—120 mesh) (Applied Science Labs.). The compounds were identified by comparing their retention times and mass spectra with those of authentic samples of DBP and DEHP (Applied Science Labs.). The column oven was held at 145°C for 25 min after injection and then programmed to 225°C at 2.5°C/min, and finally held at 225°C for 45 min. These conditions were used because the methyl esters of fatty acids are resolved at lower temperatures, and the phthalate esters are resolved at higher temperatures.

The mass spectra were obtained with a DuPont 21-492 double-focusing mass spectrometer coupled to a Varian 1400 gas chromatograph. Electron-impact ionization was used and the mass spectrometer was operated at a resolution of 1000, an ionization potential of 120 V and an accelerating voltage of 1800 V. Data were collected with a VG 2040 data system. GC peaks were identified by plots of total ion current or of selected ion currents. The GC column and GC conditions described above were used in some experiments. In other experiments, which were undertaken to analyse only the plasticizer, the GC oven was operated isothermally at 200°C.

RESULTS

GC and GC—MS proved suitable for examining the presence of plasticizers in blood extracts. The background levels in the ethanol-extracted samples were too high to be of value, and only the Dole's solution extract proved suitable for evaluation. DBP and DEHP were identified by their retention times and mass spectra in which prominent ions were observed at *m/e* 149 and *m/e* 169 for DEHP and at *m/e* 149 for DBP. Fig. 2 shows a plot of selected ion currents of a typical GC—MS run of DBP and DEHP. In the 200°C isothermal GC run an extra GC peak eluted between DBP and DEHP. To identify this unknown com-

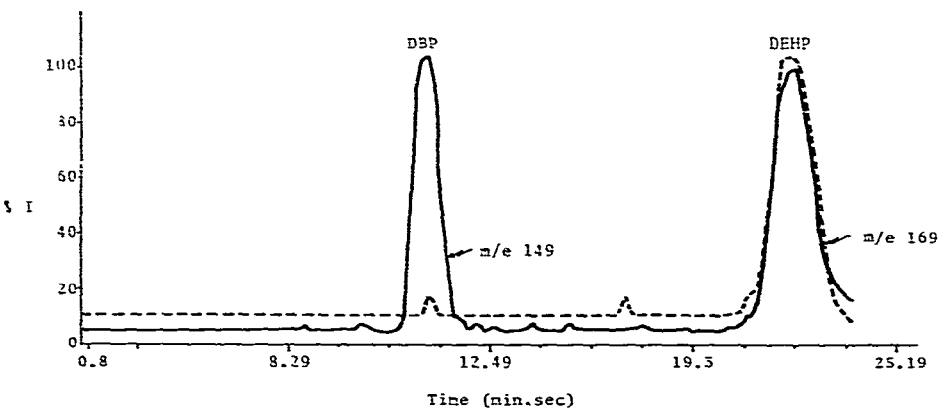

Fig. 2. The plot of *m/e* 149 and *m/e* 169 ion currents in a GC—MS analysis of a standard solution of dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP).

pound we used the Cornell University Probability Based Matching System [14]. The compound was identified as cholesta-3,5-diene with a confidence index $K = 96$ and K of 11. It is possible that cholesta-3,5-diene was formed by dehydration of cholesterol, and further experiments are under way to investigate this. Complete identification of the chromatograms was not attempted and the results of fatty acids will be the subject of another communication.

The levels of plasticizers in the water blank and the serum samples extracted by Dole's solution are summarized in Table I and compared to the duration of intravenous therapy, the quantity of blood transfused, and the time between the sampling and blood transfusion. The distilled-water blank did not contain DBP but did contain some DEHP. Similar DEHP contamination of water has been reported by Ishida et al. [9]. The control subject and patient No. 1, who received only 2000 ml of intravenous fluids 37 days before sampling, have almost the highest levels of DBP and relatively low levels of DEHP. Since these two subjects had received little or no surgical treatment near the time of sampling, the plasticizer found in their blood could have come from their normal environment and not from medical devices. Patient No. 2, who received the largest volume of crystalloid replacement over 43 days of complete intravenous replacement, showed relatively small levels of DBP and DEHP. Patient No. 3 had the highest levels of DBP and DEHP when sampled after having received three units of blood within 24 h in the diuretic phase of renal failure, but showed clearance 15 days later when renal function had improved and no further transfusions were required. Patient No. 4, who received the largest volume of transfused blood, 17 units, still showed some evidence of DBP and DEHP 21 days after having received the blood in spite of adequate urinary function. Patient No. 5 with oliguria showed a high level of DEHP.

TABLE I

SERUM PLASTICIZER LEVELS MEASURED IN SURGICAL PATIENTS

Sample*	Duration of intravenous therapy (days)	Volume of blood transfused (liters)	Interval between transfusion and sampling (days)	DBP (μg/ml)	DEHP (μg/ml)
Distilled water	—	—	—	0	(0—4)**
Control subject	—	—	—	37	3
Patient 1	12 (hours)	—	—	35	8
Patient 2	43	2	3	0.2	4
Patient 3	9	3	24	41	14
Patient 3	24	3	15	17	7
Patient 4	45	17	21	0.3	3
Patient 5	8	5	7	0	6

*Patient 1 was sampled 37 days after receiving intravenous therapy. Patient 2 required total parenteral nutrition therapy. Patients 3 and 5 had an element of renal failure. Patient 5 required subtotal gastrectomy for hemorrhage and 17 units of blood replacement.

**Range of the levels of the blanks from our laboratory.

DISCUSSION

Our attention was directed toward the confirmation and measurement of circulating plasticizer levels after identification of DBP in the lipid fraction of one of our patients. This patient had developed intestinal fistulas and complete wound disruption secondary to a colostomy for perforated diverticulitis. She required intravenous therapy throughout her 3.5-month course of treatment in the hospital because she was unable to tolerate an oral elemental diet. Total parenteral nutrition failed as she had a serum albumin of 1.5 g% and developed a large tracheoesophageal fistula secondary to tracheostomy damage. She was then selected for a complete nutritional evaluation, including analysis of serum unesterified fatty acids and amino acids. GC analysis of the fatty acids revealed an exceptionally large unidentified peak eluting after identifiable fatty acids. This sample was then examined by GC—MS and two components, *p*-nonylphenol and DBP, were identified in addition to the fatty acids. Subsequent GC revealed that there were really three GC peaks which could be resolved at an oven temperature of 200°C. The first of these peaks was identified as DBP by GC and GC—MS. The second peak, a very broad GC peak, was tentatively identified as cholesta-3,5-diene. The third GC peak was identified as DEHP. Unfortunately, further investigation of this patient was precluded by her death three days after the blood sample had been drawn; the blood sample was not drawn with a glass syringe so the results cannot be compared to the selected patients.

The phthalate ester plasticizers represent a large family of plasticizers which are approved by the Food and Drug Administration (Federal Register, 15 October, 1968, 33 F.R. 15281) for use in packaging materials for food intended for human consumption. The plastic resin (e.g. polyvinyl chloride) is combined with a plasticizer and stabilizer before it is finally manufactured into the item destined for medical use. The plasticizer DEHP is most commonly used in medical-grade plastic devices. Other investigators have measured this plasticizer in blood and tissues, which we have identified together with a second plasticizer, DBP, in the serum of five of the six subjects studied.

The use of plastics in various aspects of medical care continues to increase. Plastic devices are disposable and their use results in a savings of labor costs and avoids contamination between patients. The decrease in the costs makes their use more economically feasible. A survey of the parenteral solution devices used in our hospital revealed extensive use of plastic materials with potentially leachable plasticizers. Blood is stored in plastic bags, as are most of the crystalloid solutions; intravenous solutions are administered via plastic administration sets and catheters. The total parenteral nutrition amino acid solutions are, however, stored in glass containers. Patient No. 2, on parenteral fluids for 43 days, was primarily on these hyperalimentation fluids which may explain why the plasticizer was not detected in his blood. Our investigations of the solutions stored in plastic bags have revealed the presence of DEHP in stored blood, and both DEHP and DBP in the crystalloid solutions [15]. This may partially account for the presence of these plasticizers in our patients.

Teflon devices do not contain these plasticizers. Another possible source of DBP identified so far is the alcohol sponges used to wash skin; analysis of six

separate sponges revealed an average of 1.4 μg/ml if all of the DBP in the sponge contaminated the blood sample. The DBP levels are low in the hospitalized patients but highest in the subjects not hospitalized, suggesting contamination from environmental sources. Further studies of the source of the DBP are being pursued.

The circulating levels of DEHP were highest in patients soon after receiving blood transfusions and when there was associated renal dysfunction. Blood stored in plastic bags has high levels of plasticizers because the blood proteins are able to bind plasticizers. Once absorbed the plasticizers need to be metabolized by de-esterification to a water-soluble form which can be excreted in the urine. Absorbed plasticizers may also be deposited in tissues and may persist there for an unknown length of time. Surgical patients are exposed to contamination via the parenteral route. Special consideration may have to be given to reducing this contamination, especially for patients with renal dysfunction.

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