

notes on methodology

Loss of lipid to plastic tubing

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SUMMARY ^{14}C -labeled oleic acid and ^3H -labeled monoether in a bile salt solution were perfused through three types of plastic tubing. Large proportions of lipid were lost to the walls of silicone rubber and polyvinyl chloride tubes. The major portion of the lipid lost was recoverable only when chloroform-methanol was perfused through the tubings. On the other hand, very little lipid was lost to the wall of polyethylene tubing. Polyethylene tubing should therefore be used in perfusion studies involving lipid-soluble compounds.

SUPPLEMENTARY KEY WORDS adsorption · silicone rubber · polyvinyl chloride · chloroform-methanol · polyethylene

PLASTIC TUBING is used extensively for the infusion of lipids into experimental animals. It is important that the tubing does not appreciably alter the amount of lipid present in the infusion mixture, by adsorption on the wall or solution in the plasticizer. The following experiments show that very considerable losses may occur with some commonly used types of tubing.

Materials and Methods. All solvents were of analytical grade. All lipids and bile salts used were checked for purity by TLC using appropriate solvents; all compounds chromatographed as one spot. The composition of the buffer used in preparation of the emulsions was: HPO_4^{2-} , 7.5 mM; H_2PO_4^- , 15 mM; Cl^- , 137 mM; Ca^{2+} , 1 mM; K^+ , 7.5 mM; Na^+ , 157 mM; glucose, 1 mM. The pH was 6.4. The scintillation fluid used in counting contained 4 g of 2,5-diphenyloxazole and 0.05 g dimethyl-1,4-bis[2-(5-phenyloxazolyl)]benzene in 1 liter of toluene.

Oleic acid- $1\text{-}^{14}\text{C}$ (Radiochemical Centre, Amersham) and glyceryl-1-monooleyl-9,10- ^3H -ether (gift of Dr. A. F. Hofmann, Mayo Clinic, Rochester, Minn.) were dissolved, with carrier, in chloroform to give final specific activities of 50,000 dpm/ μmole and 240,000 dpm/ μmole , respectively. The appropriate volumes of solution were placed in a graduated cylinder and solvent was removed by evaporation under nitrogen. Bile salts (taurocholate-taurodeoxycholate, 4:1 molar ratio), and then the buffer,

Abbreviations: TLC, thin-layer chromatography.

were added. The mixture was sonicated with a Branson Sonifier at 40 w, 20,000 cycles/sec. The final concentrations were oleic acid, 1 mM; monoether, 1 mM; and bile salts, 5 mM. Ultracentrifugation showed that 86% of the oleic acid was solubilized in this mixture.

Three types of tubing were tested: (a) polyethylene (Sterivac, Allen and Hanbury's Ltd., London), I.D. 1.0 mm, O.D. 1.5 mm; (b) silicone rubber (Esco [Rubber] Ltd., London), quality TC 156, I.D. 1.0 mm, O.D. 2.0 mm; and (c) polyvinyl chloride (Irvington Division, 3M Co., Irvington, N.J.), catalogue no. 3002, I.D. 1.0 mm, O.D. 1.5 mm.

Two 60-cm lengths of each type of tubing were cut. One piece was weighed and then refluxed in chloroform-methanol 2:1 (v/v) for 6 hr at 60°C. This tubing was then dried and reweighed.

Through the second piece of tubing emulsion was perfused for 4 hr at a rate of 3 ml/hr. The perfusate was collected hourly and analyzed for radioactivity. At the end of the perfusion the tube was washed with saline and the washings were collected. Saline was then perfused through the tube at 3 ml/hr for 2 hr. Following these procedures a mixture of unlabeled oleic acid, 1.0 mM, unlabeled monoether, 1 mM, and bile salts, 5 mM, was perfused through the tubes for 4 hr at the same rate as before. Hourly collections were made and analyzed for radioactivity. At the end of perfusion the tubes were washed with solvent containing diethyl ether-petroleum ether (bp 40-60°C)-redistilled ethanol 1:1:1 (v/v/v) (triple solvent). The tubes were then perfused with chloroform-methanol 2:1 (v/v) at a rate of 12 ml/hr for 2 hr. Solvents were evaporated and the radioactivity was determined.

Results and Discussion. Percentage weight losses of polyethylene, silicone, and polyvinyl chloride tubings after refluxing in hot chloroform-methanol 2:1 (v/v) were 0.3, 3.2, and 15.3%, respectively. These losses are probably due to extraction of plasticizer or monomer from the walls of the tubes.

Percentages of hourly perfused radioactivity emerging from the tubings are indicated in Table 1. All aliquots of perfusate were extracted in duplicate and all duplicates agreed to within 3%.

TABLE 1 HOURLY RECOVERY OF ISOTOPE*

Hour	Polyethylene		Silicone Rubber		Polyvinyl Chloride	
	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H
1	84.4	96.3	49.7	72.6	49.2	70.1
2	87.0	95.5	53.7	76.9	49.1	66.7
3	80.5	97.3	55.3	79.4	47.2	62.1
4	89.5	95.2	56.2	83.5	47.0	61.7

* Expressed as % of label infused hourly. ^{14}C , oleic acid- $1\text{-}^{14}\text{C}$; ^3H , 9,10- ^3H monoether.

TABLE 2 RECOVERY OF RADIOACTIVE LABEL*

	Polyethylene		Silicone Rubber		Polyvinyl Chloride	
	¹⁴ C	³ H	¹⁴ C	³ H	¹⁴ C	³ H
Pooled perfusion collections	87.0	95.3	53.8	77.9	47.8	64.6
Wash and perfusion†	8.7	4.8	17.2	14.3	9.8	9.0
Chloroform-methanol perfusion	0.4	0.0	29.8	8.4	40.0	26.1
Total recovery	96.1	100.1	100.8	100.6	97.6	99.7

* Results are expressed as percentage of total radioactivity that was infused. ¹⁴C and ³H, as in Table 1.

† Includes saline wash, saline perfusion, unlabeled lipid perfusion, and triple solvent wash.

Percentages of infused radioactivity recovered by the procedures described under Materials and Methods are shown in Table 2. It is clearly apparent that polyvinyl chloride and silicone rubber tubings remove lipid from the perfusion medium to a marked extent. This lipid is fully recoverable only after perfusion of chloroform-methanol 2:1 (v/v), which also destroys the tubing. More oleic acid is lost than monoether in all three tubes.

It was notable that the greater the percentage of lipid-soluble compounds in the tubing material (as shown by

percentage weight loss after refluxing in chloroform-methanol), the greater the loss of perfused lipid to the tubing.

These experiments indicate that care must be taken in the selection of tubing used for infusion of lipids into experimental animals. This is especially true when low concentrations of lipid are being infused. Polyethylene appears to be the most suitable type of tubing for infusion of lipids. Another inert tubing, Teflon, could also be suitable. Work by Minder, Weder, and Bickel (1) has indicated that Teflon tubing does not adsorb lipophilic drugs. In that study it was also shown that silicone rubber and polyvinyl chloride tubings did take up lipophilic drugs to a marked extent.

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REFERENCES

1. Minder, R., H. J. Weder, and M. H. Bickel. 1970. Experimental errors resulting from uptake of lipophilic drugs by soft plastic materials. *Biochem. Pharmacol.* **19**: 2179-2180.