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Platelet Function and Platelet Lipid Biosynthesis in Rats and Rabbits Fed the Plasticizer DEHP

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Widespread use of polyvinylchloride (PVC) plastic bags in the storage of blood and blood products intended for human use is controversial (AUTIAN 1973, AUTIAN *et al.* 1978, BELL 1976) because of the contamination of the stored materials by di-(2-ethylhexyl) phthalate (DEHP) which leaches from the plastic (JAEGER & RUBIN 1970, MARCEL & NOEL 1970). The DEHP extracted by whole blood is found associated with the plasma proteins (GILBO & COLES 1975, VESSMAN & RIETZ 1974, 1978), lipoproteins (JAEGER & RUBIN 1970, 1972), and formed elements (CONTRERAS *et al.* 1974, JAEGER & RUBIN 1970, 1972, 1973). Of particular interest to us was the observation that platelets are able to concentrate DEHP in plasma (JAEGER & RUBIN 1973). Although little is known concerning the effect of DEHP on the function of blood cells (BELL 1976), DEHP has been implicated in the modification of platelet adhesion to artificial surfaces (KIM *et al.* 1973) and an increased formation of microaggregates in stored blood (RUBIN & JAEGER 1973). In view of these observations with platelets exposed to DEHP *in vitro*, we undertook to study the function and biochemistry of rat and rabbit platelets exposed to DEHP *in vivo* through the addition of DEHP to the diet (STEIN *et al.* 1974, TANAKA *et al.* 1975, IKEDA *et al.* 1978). In addition to studying platelet aggregation and platelet lipid biosynthesis, we examined prothrombin time and plasma cholesterol.

MATERIALS AND METHODS

Animals and diets: Male New Zealand rabbits (2.5-3 kg) and male Sprague-Dawley rats (Upjohn:TUC(SD)spf), designated as Young (6 wk old, 150-160 g) or Mature (5 mo old, 550-600 g), were fed Purina Chow containing DEHP (Eastman Kodak) (BELL & NAZIR 1976, BELL *et al.* 1978) at a level of 1 or 0.5%, respectively, for up to 32 days; control diets consisted of Purina Chow alone.

Blood Collection. Rats were anesthetized by i.p. injection of sodium cyclopal and bled via the abdominal aorta into sodium citrate (2.2%, 1 part to 9 parts blood). Rabbit blood was collected via the orbital sinus and diluted with sodium citrate (3.8%, 1 part to 9 parts blood) (LUMSDEN *et al.* 1974).

Platelet-Rich Plasma. Blood samples were centrifuged 250 x g for 10 min at 10C. Platelet-rich plasma (PRP) was removed by aspiration and the remaining sample (lower phase) centrifuged 700 x g for 30 min to obtain platelet-poor plasma (PPP).

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Platelet Counting. Platelets in PRP were counted with a Coulter Counter, Model B, using platelet reference standard (Technicon Instrument Corp.)

Platelet Aggregation. Platelet aggregation was studied using the turbidometric method of BORN (1962). Platelets were mechanically stirred at 37 C and aggregation observed by the change in light transmission which was recorded. Rat PRP was adjusted to 10^6 platelets/mL with homologous PPP and diluted 1:1 (v/v) with modified Tyrode's solution (glucose, Ca^{++}- and Mg^{++}-free). Rabbit PRP was used undiluted. The aggregating agents were ADP and collagen (Type II, bovine). Collagen was suspended in modified Tyrode's solution at pH 3.0. Both stimuli were titrated to give submaximal aggregating response.

One-Stage Prothrombin Time. Coagulation was assessed in the PPP with a prothrombin time test using reconstituted Simplastin A®.

Plasma Analyses. Blood glucose (GERRITSEN & DULIN 1965), plasma insulin (ZAHARKO & BECK 1968), and plasma glucagon (FALSOONA & UNGER 1974) were measured in fasted (18 h) rats. Plasma cholesterol (RUDEL & MORRIS 1973) and plasma Ca^{++} (GITELMAN 1967) were measured in blood taken at 9 a.m. in animals with free access to food.

Lipid Biosynthesis *In Vitro*. PRP (2 mL) was incubated at 37 C for 2 h with either 1 µCi sodium acetate-1-^{14}C (sp. act. 58.3 mC/mM) or DL-mevalonic-5-^{3}H(N) acid, dibenzylethylenediamine salt (sp. act. 5C/mM). Incubations with ^{14}C-acetate were extracted with 1:1 (v/v) chloroform:methanol (BELL *et al.* 1970) and the lipid extracts washed according to FOLCH *et al.* (1957). The phospholipid fraction of the lipid extract was isolated by thin-layer chromatography as described in detail elsewhere (BELL & NAZIR 1976) and assayed for radioactivity (BELL *et al.* 1970). Incubations with ^{3}H-mevalonate were digested in alcoholic-KOH (BELL 1976) and the non-saponifiable lipid fraction, containing the sterols, was extracted into n-hexane and assayed for radioactivity as previously described (BELL 1976).

RESULTS AND DISCUSSION

Table 1 summarizes the data obtained on platelet counts, platelet aggregation, prothrombin time, and plasma cholesterol from rabbits and Young rats fed DEHP for 28 or 32 days, respectively. No statistically significant effect of DEHP was found in either species with respect to platelet counts or with respect to collagen-induced aggregation. In addition, ADP-induced aggregation (performed only with rat platelets) was not statistically significantly altered by DEHP feeding. Although platelets appear to be unaffected by the DEHP treatments in these studies, several other blood parameters were affected. In the rat, but not the rabbit, plasma cholesterol was significantly reduced (92 vs 75 mg/dL, $P<0.01$) by DEHP feeding, thus confirming our previous observations in the rat (BELL *et al.* 1978b) and suggesting a species difference in response to DEHP. There was also a small but significant ($P<0.02$) decrease in prothrombin time (12.7 vs 11.6 sec) in the rat. It is doubtful, however, that such a small change could be of physiological significance. Fasting blood glucose, plasma insulin, plasma glucagon, and plasma

TABLE 1

Effect of DEHP Feeding on Platelet Aggregation, Prothrombin Time, and Plasma Cholesterol in Rats and Rabbits[a]

	Rabbit		Rat	
	Control (n=6)	DEHP (n=7)	Control (n=6)	DEHP (n=7)
Platelet count (10^6/mm^3)	0.61± 0.05	0.60±0.04	1.57±0.03	1.66±0.09
Collagen-induced platelet aggregation	18.0 ± 3.6[b]	17.9 ±1.9	30.7 ±6.1	35.7 ±4.9
ADP-induced platelet aggregation	-	-	27.8 ±1.9	30.2 ±2.6
Prothrombin time (sec)	5.5 ± 0.1	5.6 ±0.1	12.7 ±0.3	11.6 ±0.3[c]
Total cholesterol (mg/dL)	39 ±10	34 ±5	92 ±5	75 ±2[d]

[a]The rabbits received 1% DEHP in the diet for 28 days; rats (beginning at 150 g) received 0.5% DEHP in the diet for 32 days.

[b]Units of aggregation.

[c]Significantly different from the corresponding control values by Student's independent t-test, $P<0.02$; [d]$P<0.01$.

Ca^{++} were also measured in the rats and rabbits (data not shown) and found to be unaffected by DEHP feeding. Table 2 summarizes the *in vitro* lipid biosynthesis studies in platelets from Young and Mature rats fed DEHP. These studies were prompted by the fact that platelets possess the capability to synthesize all major lipid classes (MAJERUS *et al.* 1969) and by our previous observations that DEHP significantly modifies lipid metabolism in rat tissues (BELL 1976, BELL & NAZIR 1976, BELL & GILLIES 1977, BELL *et al.* 1978a,b). Biosynthesis of phospholipids (from ^{14}C-acetate) and sterols (from ^{3}H-mevalonate) was studied because these two lipids constitute the major lipids of the platelet. Neither the incorporation of ^{14}C-acetate into phospholipids nor the incorporation of ^{3}H-mevalonate into sterols was significantly affected ($P>0.05$) by DEHP feeding to either the Young or Mature rats. The average values for ^{3}H-mevalonate incorporation into sterols did, however, tend to be lower in all the DEHP-fed animals, perhaps suggesting a weak inhibitory effect of DEHP on platelet sterologenesis. This might be expected in view of the fact that DEHP inhibits sterologenesis in other rat tissues (BELL 1976, BELL *et al.* 1978a,b). The platelet, however, readily accepts cholesterol from the plasma lipoproteins (COOPER 1969; SHATTIL *et al.* 1977) making it unlikely that DEHP feeding would result in a deficiency of platelet cholesterol.

The data presented gives no indication that platelet function and platelet lipid metabolism are modified with DEHP fed at the substantial levels of 0.5% (rats) or 1.0% (rabbits) in the diet despite the fact that the same levels of DEHP modify lipid metabolism in various organs of the rat and rabbit (BELL 1976, BELL & NAZIR 1976, BELL & GILLIES 1977, BELL *et al.* 1978a,b). The results suggest that reports of increased platelet adhesion to artificial surfaces *in vitro* (KIM *et al.* 1976), the possibility of increased platelet aggregation in blood stored in PVC bags (RUBIN & JAEGER 1973), and the decreased survival of platelets stored frozen in PVC bags (KIM & BALDINI 1973) may not reflect effects of DEHP that can be extrapolated to platelets exposed to DEHP *in vivo*.

TABLE 2

Incorporation of ^{14}C-Acetate and ^{3}H-Mevalonate into Lipids of Platelet-Rich Plasma (PRP) from Rats Fed DEHP (dpm/mL PRP)

Duration of Feeding (days)	Group[a]	^{14}C-Acetate into phospholipids		^{3}H-Mevalonate into sterols	
		Control	DEHP	Control	DEHP
9	Y (n=8)	3549±627	5028±912	504±75	393±66
9	M (n=4)	3454±748	3104±593	498±38	408±81
22	Y (n=9)	936± 75	1338±168	129±48	72±27
22	M (n=4)	1445±314	1636±166	174±31	123±20

[a]Y=Young, M=Mature

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