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Inhibition of Sterologenesis in Rat Liver Minces by the Addition of Di-2-ethylhexyl Phthalate

Frank P. Bell and Edna V. Hubert

The Upjohn Company, Diabetes and Atherosclerosis Research, Kalamazoo, MI 49001

Esters of phthalic acid are widely used as plasticizers in the production of polyvinylchloride plastics (GRAHAM 1973). Recent reports that phthalate esters can leach from plastic hemodialysis tubing (EASTERLING *et al.* 1974) and plastic bags used to store blood and blood products for patient use (JAEGER & RUBIN 1972, 1973, CONTRERAS *et al.* 1974) have prompted investigations into the biochemical effects of phthalates. Our own studies have shown that numerous disturbances in lipid metabolism arise in rats fed various phthalate esters (BELL & NAZIR 1976, BELL 1976a, BELL & GILLIES 1977, BELL *et al.* 1978a,b). In view of the fact that phthalates have also been shown to exert toxic effects when added to various cultured cell systems (DILLINGHAM & AUTIAN 1973, KASUYA 1973,1974, JACOBSON *et al.* 1974, JONES *et al.* 1975), we have studied sterologenesis from ^{14}C-acetate and ^{3}H-mevalonate in rat liver minces incubated *in vitro* in the presence of di-2-ethylhexyl phthalate (DEHP). The results indicate that DEHP is inhibitory to hepatic sterologenesis when added directly to the medium in liver incubation systems.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Upjohn:TUC(SD)spf) weighing 275-300 g were used in the studies. The rats were maintained on a stock chow diet (Purina Laboratory Chow) and individually housed with free access to food and water. The rats were killed between 9 a.m. and 10 a.m. and liver minces prepared (BELL *et al.* 1978b). The liver minces (500 mg) were incubated for 3 h in Krebs-Ringer bicarbonate buffer (KRB), pH 7.4, which contained 2 µCi of acetate-1-^{14}C, sodium salt (Sp. act. 58.3 mCi/mM) or a combination of 4 µCi each of ^{14}C-acetate and DL-mevalonic acid-5-^{3}H (DBED salt, sp. act. 5.0 Ci/mM). Both radiochemicals were purchased from New England Nuclear Corporation. Di-2-ethylhexyl phthalate (DEHP) was introduced into the incubation medium as a sonicated dispersion or as a Tween 20-stabilized suspension. Sonicated dispersions of DEHP were prepared by adding DEHP to KRB and sonicating at 20,000 cps for 3 min at 10°C (Branson Sonifier). Stable suspensions of DEHP in KRB were prepared as above by the addition of Tween 20 to a final concentration of 0.16%; these suspensions were stable beyond 1 week at 10°C. Termination of the incubations, saponification of the samples, and recovery of non-saponifiable lipids and digitonin-precipitable sterols have been described in detail previously (BELL 1976a,b, BELL *et al.* 1978a,b). Radioactivity was assayed by liquid scintillation counting as previously described (BELL *et al.* 1978a).

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RESULTS AND DISCUSSION

We have previously demonstrated in the rat and rabbit that DEHP administered in the diet results in an inhibition of hepatic sterologenesis from labeled acetate and mevalonate (BELL 1976a, BELL *et al.* 1978a,b). In view of the reports that DEHP is toxic to cultured cells when present in the culture medium (DILLINGHAM & AUTIAN 1973, KASUYA 1973,1974, JACOBSON *et al.* 1974, JONES *et al.* 1975) it seemed important to investigate the effects of the direct addition of DEHP on hepatic sterologenesis since it is possible that inhibition of sterologenesis is a toxic manifestation of DEHP in cultured cell systems. The results of our studies are given in Table 1. The incorporation of ^{14}C-acetate into digitonin-precipitable sterols

TABLE 1. Inhibition of the incorporation of ^{14}C-acetate and ^{3}H-mevalonate into digitonin-precipitable sterols and total non-saponifiable lipids in rat liver minces in the presence of DEHP.

DEHP conc. (mM)	Incorporation into digitonin-precipitable sterols (rel %)	Incorporation into total non-saponifiable lipids (rel %)	
	^{14}C-Acetate[a]	^{14}C-Acetate[b]	^{3}H-Mevalonate[b]
0.0	100± 0 (9)[c]	100± 0 (3)	100± 0 (3)
0.25	92±15 (6)	83± 9	107± 8
0.5	75±12 (6)	92±11	109±12
1.0	87± 7 (4)	64±11	112± 3
2.5	54± 6 (8)	62±25	108±12
5.0 [d]	51± 8 (2)	-	-

[a]Incubations contained DEHP as a Tween 20-stabilized dispersion and 2 µCi acetate-1-^{14}C, sodium salt.
[b]Incubations contained DEHP as a sonicated dispersion and 4 µCi each of acetate-1-^{14}C, sodium salt and DL-mevalonic-5-^{3}H acid (DBED salt).
[c]Values are means ± SEM; the number of animals is given in parentheses.
[c]In incubations employing sonicated dispersions of DEHP, DEHP tended to separate out with time so that the effective concentrations in the buffer phase may be less than indicated.

(3β-hydroxysterols [BELL 1976b]) was investigated in incubations with various levels of DEHP present as a Tween 20-stabilized suspension. At levels of up to 1 mM, sterologenesis was affected either slightly or not at all. However, at 2.5 or 5.0 mM, DEHP resulted in an inhibition of sterologenesis of about 50%. Similar results were obtained when DEHP was added to the incubations as a sonicated dispersion and the incorporation of ^{14}C-acetate into

total non-saponifiable lipids (sterols plus squalene [BELL 1976a]) examined; inhibition of about 40% was observed at 1 mM and 5 mM DEHP. Incorporation of ^{3}H-mevalonate, which was present with the ^{14}C-acetate in the incubations, was not affected by DEHP, suggesting that inhibition of sterologenesis by DEHP occurs prior to the rate limiting step which is the conversion of 3-hydroxy-3-methyl-glutaryl CoA to mevalonic acid (MCNAMARA & RODWELL 1972). The present results confirm our previous results in rats fed DEHP in which we observed an inhibition of hepatic sterologenesis from ^{14}C-acetate within 48 hours after the initiation of DEHP feeding, whereas inhibition of sterologenesis from ^{3}H-mevalonate was not observed until 2 to 4 days later (BELL 1976a). The eventual inhibition of ^{3}H-mevalonate incorporation *in vivo* may reflect a decline in the activity of substrate-induced enzymes subsequent to a decreased production of mevalonate (BELL *et al.* 1978a).

The present data establish that DEHP, when added *in vitro* to incubations of normal tissue, inhibits sterologenesis. Although phthalates are capable of other modifications of lipid metabolism, it is conceivable that the toxicity of DEHP to cells in culture (DILLINGHAM & AUTIAN 1973, KASUYA 1973,1974, JACOBSON *et al.* 1974, JONES *et al.* 1975) could relate to alterations in sterologenesis.

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