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Effect of Di-2-Ethylhexyl Phthalate in the Female Rat: Inhibition of Hepatic and Adrenal Sterologenesis *In Vitro*

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In previous reports from this laboratory, we have shown that feeding the plasticizer di-2-ethylhexyl phthalate (DEHP) to rats results in inhibition of sterologenesis in liver and various other tissues (BELL 1976, BELL *et al.* 1978a, BELL *et al.* 1979). Most of these studies, however, have been conducted in male animals. The present report describes the additional observations that DEHP feeding inhibits hepatic sterologenesis in normal female rats and that the pregnant rat may be less sensitive to the effects of DEHP because of DEHP distribution to the developing fetal tissue. In addition, this report gives evidence that DEHP feeding inhibits sterologenesis in adrenal glands from female, as well as male, rats.

MATERIALS AND METHODS

Male and female rats of the Sprague-Dawley strain were individually housed with free access to food and water. The animals received either a stock chow diet (control diet, Purina Laboratory Chow) or the stock diet supplemented with 0.5% or 1.0% DEHP (BELL & NAZIR 1976). Pregnant female rats, bred as previously described (BELL *et al.* 1979), were given the DEHP-containing diets from the fifth to eighteenth day of gestation (13 days). Liver minces (500 mg), prepared as previously described (BELL *et al.* 1979), were incubated for 3 hr in Krebs-Ringer-bicarbonate buffer (KRB), pH 7.4, at 37°C containing RS-mevalonic acid-2-^{14}C, DBED salt (spec. act. 40.8 mCi/mM, New England Nuclear Corporation). Adrenal glands were dissected free of adhering tissue and capsular material and incubated for 3 h at 37°C in 1.52 ml of KRB containing 1.0 µCi ^{14}C-labeled mevalonic acid as above. All incubations were terminated by the addition of ethyl alcohol and KOH sufficient to yield final concentrations of 82% and 11%, respectively, and the samples saponified for 2 h at 60°C (BELL 1976). The non-saponifiable lipids were extracted from the samples with n-hexane (BELL 1976) and later fractionated by thin layer chromatography to separate the sterols and squalene as previously described (BELL 1976, BELL *et al.* 1978a). Statistical analyses of data were performed using Student's independent *t*-test. Significance was given by values of $P \leq 0.05$.

RESULTS AND DISCUSSION

The effect of phthalate feeding on the incorporation of ^{14}C-mevalonic acid into sterols and squalene by liver minces from non-pregnant female rats is shown in Table 1. Incorporation of the label into both the sterol and squalene fractions was significantly reduced below control values ($P<0.05$ and $P<0.001$, respectively).

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TABLE 1. Incorporation of ^{14}C-mevalonate into non-saponifiable lipids (dpm/g wet wt) in liver from female rats fed DEHP.[a]

Group	Sterols	Squalene
Control (n=6)	40520[b]±2440	56870 ±5170
DEHP (n=6)	29640[c]±3640	24360[d]±2490

[a]Female rats (n=12, 251±3 g) were fed either the stock diet (control, Purina Laboratory Chow) or the chow diet supplemented with 1.0% DEHP for 13 days. Liver minces (500 mg) were incubated for 3 h at 37°C in 3.4 ml KRB, pH 7.4, containing 1 µCi of RS-mevalonic-2-^{14}C acid, DBED salt. The non-saponifiable lipids were extracted, fractionated, and assayed for radioactivity as described under Materials and Methods.
[b]Values are means ± SEM of the number of animals given in parentheses.
[c,d]Significantly different from control values by Student's independent *t*-test (c,$P<0.05$; d,$P<0.001$).

In addition to studying the effect of dietary DEHP in the normal female rat, studies of hepatic sterologenesis from ^{14}C-mevalonate were carried out using liver minces from pregnant rats fed 0.5% DEHP in the diet from the fifth to eighteenth day of gestation (Table 2).

TABLE 2. Incorporation of ^{14}C-mevalonate into non-saponifiable lipids (dpm/g wet wt) in liver from pregnant rats fed DEHP.[a]

Group	Sterols	Squalene
Control (n=6)	67460[b]±9490	41150±2230
DEHP (n=6)	53020 ±3350	35630±2720
	N.S.[c]	N.S.

[a]Pregnant rats were fed the stock chow diet (control, Purina Laboratory Chow) or the chow diet supplemented with 0.5% DEHP from the fifth to eighteenth day of gestation (13 days). Liver minces (500 mg) were incubated for 3 h at 37°C in 3.4 ml KRB, pH 7.4 containing 2 µCi RS-mevalonic-2-^{14}C acid, DBED salt. Analysis of the samples was as described in the footnote to Table 1.
[b]Values are means ± SEM of the number of animals given in parentheses.
[c]Not significantly different from control values by Student's independent *t*-test.

A dietary level of 0.5% DEHP was selected on the basis of our previous studies (BELL 1976) in which male rats receiving 0.5% DEHP in the diet for about the same length of time (11 days) demonstrated statistically significant reductions (35-40%) in hepatic sterologenesis. Although ^{14}C-mevalonate incorporation into sterols and squalene tended to be lower on an average (20% and 14%, respectively) in the livers from DEHP-fed pregnant rats (Table 2), no statistically significant differences were observed between the control and DEHP-fed groups. The failure of this level of DEHP to affect sterologenesis from ^{14}C-mevalonate in the pregnant female could be a result of a partitioning of the DEHP between maternal and fetal tissues *in utero*, thereby reducing the effective level of DEHP to which maternal tissues are exposed. This is a likely possibility given that placental transfer of DEHP occurs in the rat (SINGH *et al.* 1975). Evidence for the partitioning of DEHP is strengthened by our observation that sterologenesis in brain and liver from the fetuses removed from the female rats reported in Table 2 was statistically significantly inhibited ($0.05>P<0.001$) (BELL *et al.* 1979).

The effect of DEHP feeding on adrenal gland sterologenesis was also examined in the female rats reported in Table 1. The results are given in Table 3. Feeding 1% DEHP for a period of 13 days resulted in a significant reduction in the incorporation of ^{14}C-mevalonate into adrenal sterols (ca. 25%, $P<0.01$). Incorporation of

TABLE 3. Incorporation of ^{14}C-mevalonate into non-saponifiable lipids (dpm/g wet wt) of adrenal glands from female and male rats.[a]

	Female		Male	
	Sterols	Squalene	Sterols	Squalene
Control	3520±230[b]	1240±100	3320±200	840± 40
DEHP	2660± 80[c]	1050± 70	2640±120	660±100
	$P<0.01$[c]	N.S.[d]	$P<0.02$[c]	N.S.

[a]Female rats (251±3 g, n=12) and male rats (320±20 g, n=10) were fed the stock chow diet (control, Purina Laboratory Chow) or the chow diet supplemented with 1.0% DEHP for either 13 days or 73 days, respectively. The two adrenal glands from each animal, prepared as described under Methods, were incubated for 3 h at 37°C in 1.52 ml KRB, pH 7.4, containing 1 µCi RS-mevalonic-2-^{14}C acid, DBED salt. Analysis of the samples was as described in the footnote to Table 1.

[b]Values are means ± SEM of 5 (males) or 6 (females) animals.

[c]Significantly different from control values by Student's independent *t*-test.

[d]N.S. denotes differences not statistically significant.

label into squalene was lower on the average but was not significantly reduced ($P>0.05$). The data suggest that the effect of DEHP in blocking adrenal sterologenesis from mevalonate occurs at a point beyond squalene in the biosynthetic pathway for sterols. Data are also included in Table 3 from male rats fed 1% DEHP for a considerably longer period (73 days); it is clear that DEHP feeding inhibits sterologenesis significantly ($P<0.02$) in male rat adrenal tissue as well. Just as observed in the female tissue, the incorporation of ^{14}C-mevalonate into squalene in male adrenals tended to be reduced but not significantly ($P>0.05$) (Table 3).

This report extends and compliments our previous studies on the effects of phthalate esters on lipid metabolism in the rat (BELL 1976, BELL *et al.* 1978a,b, 1979, BELL & NAZIR 1976) and shows that the response of the normal female rat to DEHP feeding is similar to that of the male rat with respect to hepatic adrenal sterologenesis. Of additional interest is the observation that, in the pregnant rat, 0.5% DEHP feeding, which significantly reduces hepatic sterologenesis in the male (BELL 1976), did not significantly reduce the incorporation of ^{14}C-mevalonate into hepatic tissues. The fact that hepatic sterologenesis was significantly reduced in livers of fetuses from those rats (BELL *et al.* 1979), however, suggests that the attenuated response of the pregnant females may have been the result of an *in vivo* dilution effect of DEHP between maternal and fetal liver.

An observation of the biological effects of phthalates in animals raises the question of the possible biological effects of phthalates in man, particularly since phthalates have been found in various tissues from man (JAEGER & RUBIN 1972, RUBIN & NAIR 1973, MES *et al.* 1974, HILLMAN *et al.* 1975). At this time, the toxicity of phthalates in man is essentially an unknown quantity with but a few cases of accidental acute ingestion being reported (SCHAFFER 1945 and LEFAUX 1978). Concern has been expressed over the introduction of phthalates into patients during exposure to DEHP-containing plastic devices used in medical procedures (JAEGER & RUBIN 1972, HILLMAN *et al.* 1975, GIBSON *et al.* 1976, AUTIAN 1978). In addition, chronic exposure of rhesus monkeys to DEHP has been reported to result in abnormalities in liver function and histopathology (JACOBSON *et al.* 1977).

REFERENCES

AUTIAN, J.: Vox Sang. 34, 244 (1978).

BELL, F.P.: Lipids 11, 769 (1976).

BELL, F.P., M. MAKOWSKE, D. SCHNEIDER, and C.S. PATT: Lipids 14, 372 (1979).

BELL, F.P. and D.J. NAZIR: Lipids 11, 216 (1976).

BELL, F.P., C.S. PATT, and P.J. GILLIES: Lipids 13, 673 (1978a).

BELL, F.P., C.S. PATT, B. BRUNDAGE, P.J. GILLIES, and W.A. PHILLIPS: Lipids 13, 66 (1978b).

GIBSON, T.P., W.A. BRIGGS and B.J. BOONE: J. Lab. Clin. Med. 87, 519 (1976).

HILLMAN, L.S., S.L. GOODWIN and W.R. SHERMAN: N. Engl. J. Med. 292, 381 (1975).

JACOBSON, M.S., S.V. KEVY and R.J. GRAND: J. Lab. Clin. Med. 89, 1066 (1977).
JAEGER, R.J. and R.J. RUBIN: N. Engl. J. Med. 287, 1114 (1972).
LEFAUX, R.: Practical toxicology of plastics. Cleveland, OH: CRC Press 1968.
MES, J., D. COFFIN and D.S. CAMPBELL: Bull. Environ. Contam. Toxicol. 12, 721 (1974).
RUBIN, R.J. and P.P. NAIR: N. Engl. J. Med. 288, 915 (1973).
SCHAFFER, C.B., C.P. CARPENTER and H.F. SMYTH JR.: J. Ind. Hyg. Toxicol. 27, 130 (1945).
SINGH, A.R., W.H. LAWRENCE, and J. AUTIAN: J. Pharmaceut. Sci. 64, 1347 (1975).