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Effect of Di(2-Ethylhexyl)phthalate Administration on Rat Sperm Count and on Sperm Metabolic Enzymes

Abrar Siddiqui and Satya P. Srivastava

Industrial Toxicology Research Centre, Post Box 80, M. G. Marg, Lucknow-226, 001, India

Di(2-ethylhexyl)phthalate (DEHP) is extensively used in the manufacture of flexible plastics. It has been widely distributed in the environment and its potential health hazards have been extensively reviewed (Peakall 1975; Tomita et al., 1982; Thomas and Thomas, 1984; Pollack et al., 1985). Recent interest in DEHP toxicity has focused on the male gonads (Curto and Thomas 1982). DEHP is known to produce testicular atrophy and antifertility effects in experimental animals (Gray and Butterworth, 1980; Autian 1982; Gangolli 1982; Fukucka et al. 1989). Morphological and biochemical studies have revealed that DEHP damages seminiferous tubules, reduces the epididymal sperm count and alters the activity of testicular enzymes sorbitol dehydrogenase (SDH), lactate dehydrogenase (LDH), γ-glutamyl transpeptidase, β-glucuronidase and acid phosphatase, related to specific events of spermatogenesis (Parmar et al.1986). Alterations in the serum gonadotropins, testosterone levels and testicular zinc and testosterone contents have also been reported in DEHP induced toxicity (Foster 1980; Oishi and Hiraga 1980). The reduced epididymal sperm count may not be the only factor responsible for reported antifertility effects of DEHP, because other factors such as sperm movement would also affect the fertility. It is also possible that the biochemical and morphological changes occurring in the spermatozoa as a result of exposure to DEHP may affect their fertilizing capacity. The present study was undertaken to evaluate the effect of DEHP on the activity of certain energy-linked enzymes in rat spermatozoa as little is known about the influence of DEHP on sperm energy-metabolism..

Send reprint requests to Dr. S.P. Srivastava at the above address.

Table 1. Effect of DEHP on the weight of epididymis and sperm count in rat

Treatment	Absolute weight (g)	Relative weight (g/100 g body weight)	Sperm count (X 10^6)
Control	0.50±0.01	0.29±0.02	8.20±0.39
500 mg/kg	0.46±0.04	0.28±0.01	6.82±0.43*
1000 mg/kg	0.43±0.02*	0.23±0.01*	3.61±0.33*

Values are mean ± S.E. from six animals.
*P < 0.05 when compared to controls by student's 't'test.

Table 2. Effect of DEHP on the activity of lactate dehydrogenase, sorbital dehydrogenase and aldose reductase in spermatozoa of rat

Treatment	Aldose reductase (n mole NADPH oxidised/min/mg protein)	Sorbitol dehydrogenase (n mole NADPH oxidised/min/mg protein)	Lactate dehydrogenase (n mole NADPH oxidised/min/mg protein)
Control	1.61±0.12	5.47±0.23	45.2±2.83
500 mg/kg	1.28±0.10	5.01±0.26	51.6±2.94
1000 mg/kg	0.85±0.03*	3.92±0.19*	69.5±5.37*

Values are mean ± s.e. from six animals.
*P < 0.001 when compared to control by student's 't' test.

MATERIALS AND METHODS

Male albino rats (150-200 g) from the Industrial Toxicology Research Centre animal breeding colony were housed in three groups of six animals each and were given a commercial pellet diet (Hindustan Lever, Bombay) and water ad libitum throughout the experiment. DEHP dissolved in groundnut oil was administered orally with the help of a feeding cannula at a dose of 500 or 1000 mg/kg body weight per day for 15 days. The control animals were given an equivalent amount of groundnut oil in an identical manner. The animals were sacrificed by cervical decapitation 24 hrs after the last dose. The epididymis was removed promptly and weighed.

Spermatozoa were isolated from the epididymis according to the method described by Brooks (1976). A portion of the spermatozoal suspension was diluted 10 times with 2% formalin and sperm were counted by the method described by Freund and Carol (1965). The remaining spermatozoal suspension was centrifuged at 800 g for 10 min. The sedimented spermatozoa were resuspended in 0.25 M sucrose, sonicated and used for assay of enzymes. The activity of lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH) and aldose reductase (ALRD) were determined by the method described earlier by Srivastava et al. (1989) and Tanimoto et al. (1983) respectively. Protein was estimated according to the method of Lowry et al. (1951) using bovine serum albumin (Sigma Chemical Co.,St.Louis,MO,USA)as the standard

RESULTS AND DISCUSSION

There were no deaths or signs of overt toxicity in DEHP exposed rats. The exposure to DEHP significantly decreased the absolute and relative weight of epididymis only at higher dose tested (Table 1). Administration of DEHP was found to reduce the sperm count at both the dose levels studied (Table 1). The effect of DEHP on the activity of LDH, SDH and ALRD is shown in Table 2. The activity of LDH was increased significantly while that of SDH and ALRD decreased in animals exposed to 1000 mg/kg DEHP only.

Consistant with our previous report, the present study showed a decrease in epididymal sperm count in a dose dependent manner. This may be due to the effect of DEHP on various morphological and biochemical events related to spermatogenesis (Parmar et al. 1986). The decrease in epididymal weight observed may have been due to reduced number of sperm.

The energy requirements of the spermatozoa for the metabolic reactions are met by the reversible oxidation

and reduction reactions catalyzed by dehydrogenases (Bishop, 1968). Fructose being the chief energy source for spermatozoa (Mann, 1964), its decreased production or altered oxidation would affect the bioenergetics of spermatozoa. ALRD catalyses the conversion of glucose to sorbitol and SDH catalyses the conversion of sorbitol to fructose which is subsequently oxidized through the glycolytic pathway. A decrease in the activity of ALRD and SDH, as observed in the present study, might lead to reduced production of fructose. An accompanying increase in LDH activity would therefore enhance the rate of oxidation of the already depleted fructose reserves thereby promoting the generation of nonviable, poorly motile spermatozoa (Mann, 1964; Bishop, 1968).

The current investigation demonstrated that DEHP or its metabolites may interfere with bioenergetics of spermatozoa of rat. Further research is needed to show a relationship with decreased sperm function.

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