EFFECT ON THE OFFSPRING OF REPEATED CAFFEINE ADMINISTRATION TO PREGNANT RATS

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(Received 21st August 1972)

Summary. Repeated intraperitoneal injections of caffeine given to rats in dosages ranging from 4 to 16 mg daily throughout pregnancy resulted in significant resorptions and decrease in the birth weight of the offspring. These effects were dose-related. Developmental malformations, however, were not observed in the offspring at these dosage levels.

These results indicate that caffeine, even in low doses which at the lower levels of the experiment may approximate to the daily human intake of a heavy coffee consumer, suppresses growth of embryonic and fetal tissues.

INTRODUCTION

Since caffeine is a universal beverage and is consumed in large quantities by the human race, its effect during gestation on the developing offspring is important. There is good evidence that caffeine is mutagenic in bacteria (Demerec and his co-authors, 1950; Demerec, Bertani & Flint, 1951; Gezelius & Fries, 1952; Novick, 1955; Greer, 1968), fungi (Fries, 1950; Zetterberg, 1959) and plants (Kihlman & Levan, 1949; Kihlman, 1952), as well as in *Drosophila* (Andrew, 1959; Ostertag, 1963; Ostertag & Haake, 1966; Mittler, Mittler & Owens, 1967) and in human cells *in vitro* (Ostertag, Duisberg & Stürmann, 1965; Ostertag, 1966a, b; Ostertag & Greif, 1967). Although there have been some conflicting reports on the mutagenicity of caffeine in higher organisms (Adler, 1970; Epstein, 1970), chromosomal breakage has been induced in human cell cultures as well as in *Drosophila* (Kuhlmann, Fromme, Heege & Ostertag, 1968).

Caffeine has been proven to be teratogenic in the offspring of mice following a single large intraperitoneal injection (Nishimura & Nakai, 1960; Fujii & Nishimura, 1969), but the effect of relatively low doses has not been studied. In the present paper, the effect of repeated caffeine injections on the offspring of pregnant rats given a dose approximating to that of a high daily coffee consumption of humans is examined.

MATERIALS AND METHODS

Forty female Holzer rats, each weighing approximately 200 g, were mated. Following observation of spermatozoa in the vaginal smears, each rat was given intraperitoneal injections of caffeine at 6-hr intervals. Taking the figure of 100 mg caffeine in an average cup of coffee, the daily equivalents per 50 kg (the average weight of an adult human female) were calculated from the dosage schedule shown in Table 1.

Table 1. Dosage schedule of caffeine given to mated female rats

Group*	Caffeine†	Total daily dose	Daily caffeine equivalent as coffee/50 kg
A	1 mg	4 mg	10 cups
B	2 mg	8 mg	20 cups
C	4 mg	16 mg	40 cups
D (control)	—	—	—

* Each group contained ten rats.

† Given intraperitoneally every 6 hr; the control group received 1 ml normal saline intraperitoneally every 6 hr.

The dosage was continued from Day 0 to Day 20, at which time all animals were killed. Random serum caffeine levels were determined by the method of Axelrod & Reichenenthal (1953) in each group. The peak of caffeine concentration occurred 30 min after caffeine injection. The highest serum levels of caffeine reached in the experimental groups were: Group A, 2.8 mg/100 ml; Group B, 6.6 mg/100 ml; Group C, 14.6 mg/100 ml.

The litter size, number of resorptions, fetal weights and placental weights were determined. The fetuses were fixed in 10% buffered formalin and examined for anomalies. Attempts to identify skeletal malformations were made by staining in alizarine-KOH and clearing soft tissues in glycerol.

RESULTS

Table 2 summarizes the average litter size, number of resorptions, fetal weight and placental weight in each group. A 20% decrease in litter size was observed in Group A given 4 mg caffeine/day and 40% decrease in Groups B and C.

Table 2. Effects of caffeine administration on the offspring of pregnant rats

Group	Daily dose of caffeine (mg)	Average litter size	Average no. of resorptions	Average fetal weight (g)	Average placental weight (g)
A	4	8	2.1	4.20	0.6
B	8	6	4.0	3.50	0.5
C	16	6	4.0	3.20	0.4
D	Control	10	1.2	4.60	0.7

Likewise, the average number of resorptions was twice as great in Group A (4 mg caffeine/day) as compared with the control group, and was quadrupled in Groups B and C.

The average fetal weight was significantly decreased in all experimental groups, the decrease appearing to be dose-related. The placental weight generally corresponded to the weight of the fetus and the placentae showed no significant gross or histological changes.

No significant developmental malformations or skeletal defects were observed in the fetuses after dissection and staining.

DISCUSSION

Caffeine is a purine-1,3,7-trimethyl xanthine. It has been observed recently that inhibition of DNA polymerase activity is effected in human cell cultures at concentrations of caffeine above 500 $\mu\text{g/ml}$ after 48 hr (Wragg, Carr & Ross, 1967). When 1% caffeine is added to Hela cell cultures, extensive changes in the fine structure of the cells occurs with expulsion of nuclear material into the cytoplasm (Ostertag & Fromme, 1967). In addition, breakage has been reported in human chromosomes, even in doses below 500 $\mu\text{g/ml}$ (Kuhlmann *et al.*, 1968).

More recently, a high frequency of chromatid aberrations, predominantly of the unstable type, has been induced with caffeine in human leucocytes and human embryonic cells in culture (Lee, 1971). Studies on the fate of radiation-induced chromosome aberrations (Buckton, Jacobs, Court Brown & Doll, 1962; Norman, Sasaki, Ottoman & Fingerhut, 1966; Sasaki & Norman, 1967; Leonard, 1970) have shown that such unstable aberrations do not survive cell divisions due to the genetic imbalance caused by the abnormal distribution of genetic material to the daughter cells. Furthermore, the absence of exchange aberrations and the restriction of damage to the DNA synthesis phase which Lee (1971) observed may relate to the ambiguous results on the effectiveness of caffeine in the induction of dominant lethal mutations in higher organisms.

A change in sex ratio has been observed in female larvae of *Drosophila* exposed to 1% caffeine for 1 hr (Zetterberg, 1959). After hatching, there was a shift of sex ratio in favour of females. Previous investigators have shown for X-rays (Oster, 1958; Ostertag & Muller, 1959; Ostertag, 1963) as well as for chemical mutagens (Ostertag, 1963) that this is related to genetic damage induced by chromosomal breakage. Loss of the X chromosome in male cells is lethal whereas a single X chromosome loss in female cells can be tolerated.

In the dog, Axelrod & Reichenthal (1953) have shown that caffeine is distributed freely in the intracellular water of all tissues. Furthermore, Goldstein & Warren (1962) have demonstrated that caffeine administered to the human adult gains access to the gonads and fetal tissues and reaches concentrations substantially the same as those in the plasma. It can, therefore, be concluded that the consumption of coffee several times daily by the human pregnant female allows constant exposure of the fetal tissues to the effects of the caffeine. Fetal gonadal tissue is also exposed, with the potential hazard of a deleterious effect on the germ cells.

Congenital malformations in the offspring of mice treated with a single large

(250 mg/kg body weight) intraperitoneal injection of caffeine have been observed by Nishimura & Nakai (1960) and Fujii & Nishimura (1969). When the compound was administered between the 9th and 14th days, malformations, predominantly in the skeletal system, were found. Haematomata were also frequently observed.

The present experiments utilizing relatively small dosages of caffeine indicate that although fetal malformations are not produced at this low concentration, there is a significant degree of fetal loss and decrease in birth weight.

ACKNOWLEDGMENT

This work was supported by a General Institutional Support Grant from the University of Wisconsin School of Medicine.

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