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Influence of nicotine and caffeine on rat embryonic development

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Summary. The influence on embryonic development of nicotine and caffeine at dose levels approximating human consumption was investigated in Sprague-Dawley rats. One group of animals received nicotine administered subcutaneously by an Alzet mini-osmotic pump from gestational day 6 through 12 (25 mg over 7 days; rate 149 µg/hr). Control animals received physiological saline in a similar manner. A second group received a single intravenous injection of caffeine (25 mg/kg) on gestational day 6. Control animals were treated with physiological saline. A further group received both nicotine and caffeine on gestational day 6 as described for the two previous groups. There were no significant differences among any of the groups with respect to maternal weight gain, litter size, embryolethality, fetal weight, or crown-rump length. The offspring of nicotine treated animals showed a significantly higher incidence of hydrocephaly when compared to the controls, but in the combined treatment group no malformed fetuses were observed. Light microscopic examination of maternal liver, kidney and placentas revealed changes in the hepatic sinusoids, glomeruli and intervillous spaces after nicotine and combined treatment. In addition, the decidua basalis was poorly developed compared to the controls. Chorionic villi and fetal kidney appeared normal in all groups. A coteratogenic effect is not evident from these findings.

Key words: Nicotine, Caffeine, Embryonic development

Introduction

Nicotine and caffeine represent two of the most common pharmacologically active substances used by pregnant women. Exposure of the conceptus to these drugs occurs primarily as a result of maternal cigarette smoking and consumption of caffeine containing beverages, especially coffee.

The adverse effects of cigarette smoking during pregnancy are well documented. However, no clear association between nicotine use and developmental defects has to date been established (Kelsey et al., 1978; Christianson, 1980; Lindenschmidt and Persaud, 1980; Persaud, 1982; Hemminki et al., 1983; Shiono et al., 1986). Though several studies have demonstrated the teratogenicity of caffeine in laboratory animals (Fujii and Nishimura, 1969; Fujii et al., 1969; Fujii and Nishimura, 1972; Palm et al., 1978; Lee et al., 1982; Gilani et al., 1983; Scott, 1983; Tanaka et al., 1984), experimental results can not be applied to humans because of the variability of caffeine dose, exposure time and species differences. Nonetheless, the Food and Drug Administration of the United States urged recently that «pregnant women should avoid caffeine-containing food and drugs, if possible, or consume them sparingly» (FDA, 1981).

At this time, only a single study involving the combination of nicotine and caffeine has been reported in the literature (Gilani and Persaud, 1986). In view of increasing concern regarding the potential deleterious effects of these agents on the embryo, the influence of nicotine and caffeine at dose levels approximating human consumption was investigated in the pregnant rat.

Materials and methods

Virgin Sprague-Dawley rats (200-300 g) were used. All animals were housed in an environmentally controlled room, in spacious wire mesh cages. The temperature was kept constant at 22°C (± 1°C.) with a relative humidity of 50% (± 10%). A cycle of twelve hours of light, from 0800 to 2000 hours, and twelve hours of darkness, from 2000 to 0800 hours was also maintained throughout this study.

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Male and female animals were mated overnight and copulation was confirmed the following morning by the presence of spermatozoa in the vaginal smear. The day on which this occurred was designated day 1 of gestation. The pregnant animals were randomly assigned to one of six treatment groups: untreated controls (Group I), nicotine treated (Group II), control to nicotine (Group III), caffeine treated (Group IV), control to caffeine (Group V), caffeine and nicotine treated (Group VI). Each treatment group consisted of 10 dams, for an overall total of 60 experimental animals. The pregnancy rate was 100%. In all cases, the animals were treated at 0900 hours on gestational day 6.

Nicotine was administered to the pregnant animals over a 7 day period, from gestational day 6 through 12, via an «ALZET mini-osmotic pump» (Alza Corporation) implanted subcutaneously. The osmotic mini-pump is a miniature self-powered pump designed to deliver test agents to experimental animals at a constant controlled rate (2001 Model 1 µl/hr), over a fixed period of time. The dosage was calculated to ensure that animals would receive a total dose of 25 mg nicotine over the seven days treatment period. The mini-pump delivery rate of nicotine was 149 µg/hr. Caffeine (25 mg/kg B.W) was given to the experimental animals as a single, intravenous injection via the tail vein, on the morning of the 6th day of pregnancy. In addition to an untreated control group of animals, there were corresponding control groups for the nicotine and caffeine treated animals, respectively. A further group of 10 animals received a combined treatment of caffeine and nicotine. Both drugs were administered on the morning of the 6th day of pregnancy. The animals received a single injection of caffeine intravenously into the dorsal tail vein (25 mg/kg of body weight). Nicotine (25 mg) was administered by means of an osmotic mini-pump as previously described.

For the duration of the treatment period experimental animals were pair-fed. On the morning of treatment, the nicotine treated animals received 50 g of rodent pellets. The following morning, the amount of food remaining in the trough was weighed again to calculate the amount of food eaten by the animal over a 24 hour period. This procedure was repeated for the 7 day treatment period. After implantation of the mini-pump or the saline injection, control animals received a 24 hour food allocation equal to the amount eaten by the treated animal with which it was paired. Because each treatment animal was matched with a specific control, a lag time of at least 24 hours was necessary between treatment and control experiments for all experimental procedures. Food pellets *ad libitum* were available for the remainder of the experimental period.

The animals were killed on gestational day 20 and the uterine horns were examined *in situ* for resorption sites. Fetal position within the uterine horns, as well as the number of live and dead fetuses, was also recorded. At the time of recovery, each fetus was scrutinized for external anomalies, and then fixed in Bouin's solution for a minimal period of 2 weeks. After this period any remaining membranes were debrided, and the umbilical cords removed. Following this, individual crown-rump measurements and fetal weights were recorded. The placentas were also weighed. Internal organs were studied by the Wilson technique (Wilson, 1964).

Samples of the placenta, fetal liver and kidney, randomly selected from each group, were routinely processed for examination by light microscopy.

Data corresponding to fetal weight and crown rump length were collected on 685 fetuses. Maternal weight gain, placental weight, litter size, embryolethality and developmental defects were also recorded. A one-way analysis of variance and Multiple Range Test were performed, using a designed computer program. Data relating to embryolethality and the incidence of developmental defects were subjected to Chi-square (X^2) tests, and multiple comparisons made between the groups.

Results

Embryolethality (Table 1) and fetal growth (Table 2) were not significantly affected by maternal exposure to nicotine, caffeine or the combination of these two substances. No significant differences were found among any of the treatment groups with respect to the incidence of resorptions, fetal weight, crown-rump length or placental weight.

The incidence of developmental defects was low (Table 3). Hydrocephalus occurred to varying degrees in all treatment groups, except for the combined nicotine and caffeine treated group. The incidence of hydrocephalus was significantly increased ($P < 0.05$) in the fetuses of animals treated with nicotine, compared to both untreated and nicotine controls, as well as to the nicotine and caffeine treated animals (Fig. 1). There were no visceral anomalies observed in any of the treatment groups.

In mothers treated with nicotine and nicotine and caffeine combined, several glomeruli appeared as dense disorganized basophilic bodies, congested with red blood cells (RBC's), but devoid of any recognizable microscopic features. Other glomeruli revealed fragmented capillaries enclosed within a Bowman's capsule. In the control animals, Bowman's capsule enclosed a well defined urinary space and distinct glomerular capillaries were observed, with occasional RBC's, in their lumina. Dense, basophilic nuclei were present within numerous glomerular capillary tufts in tissue sections from animals of all groups, and were therefore not considered to be abnormal.

Proximal and distal convoluted tubules were normal in all treatment groups, displaying cuboidal epithelial cells with prominent round nuclei. The granular cytoplasm of the proximal tubule epithelial cells demonstrated the deeper acidophilia and brush border characteristic of cells in this location, while distal tubular epithelial cells stained less intensely with eosin and demonstrated well defined intact cell borders. At the cortico-medullary junction, the ascending and descending limbs of the loop of Henle were not well

preserved or visualized, due to tissue processing and shrinkage. Flat squamous to cuboidal epithelial cells appeared clustered together, while the lumina were generally collapsed.

The collecting tubules and ducts were easily recognizable in the renal medulla. Well defined cuboidal to columnar epithelial cells with distinct borders enclosing a prominent nucleus were seen surrounding relatively wide, more irregular lumina. There were no intraluminal precipitates present in any part of the renal tubular system, nor any section showed evidence of the vacuolar degeneration of epithelial cell linings often indicative of functional disturbances.

Blood vessels throughout the kidney from animals of all treatment groups demonstrated normal connective tissue and smooth muscle walls lined by simple squamous endothelium.

Fetal kidneys were developmentally immature, but appeared normal in all treatment groups.

Tissue sections of maternal liver displayed in all cases varying degrees of cellular degeneration. Focal cellular degenerative changes were evidenced by the appearence of scattered cells with pyknotic and sometimes fragmented nuclei. The cytoplasm appeared highly vacuolated in comparison to the majority of normal appearing hepatocytes surrounding them. These cells were pale staining and easily identified. In many instances, degenerative changes involved the majority of hepatocytes constituting an entire hepatic lobule. This hepatocellular degeneration resulted in the section having a «mottled» appearance, with pale and darker staining areas. No appreciable difference could be identified in the degree of these degenerative changes among any of the treatment groups, approximately 50% of the mothers in each group being equally affected.

Seven of the 10 mothers treated with nicotine and caffeine (group VI) demonstrated dilatation of hepatic sinusoids and vascular congestion when compared with any other group. The sinusoids were excessively wide, especially in the area approximating the central veins and appeared congested with RBC's. In addition, the central veins in the majority of these animals were completely occluded with blood. These findings were also seen in the nicotine treated mothers, but they occurred to a lesser degree and central venous congestion was not as apparent. The hepatic sinusoids and central veins of caffeine treated mothers did not differ in appearance from those of their corresponding controls.

Hepatic cellular organization of fetal liver appeared normal. However, the sinusoidal dilatation and congestion observed to occur in maternal liver was also seen in the fetal liver of the nicotine and caffeine treated group primarily, as well as in animals treated with nicotine.

The placentas from all treatment groups revealed variable degrees of degenerative changes, most evident in the decidua basalis and stem (anchoring) villi. Many of the decidual cells were pale staining, with highly vacuolated or «empty» looking cytoplasm surrounding a pyknotic nucleus. These findings were considered to be the result of normal degenerative changes occurring in the near term placenta. In general, the number of anchoring villi seen in the placentas of nicotine treated animals was diminished relative to other treatment groups.

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Table 1. Influence of nicotine and caffeine on rat embryonic development.

Treatment groups	No. of mothers*	Total implantations*	Resorptions (%)*	Live fetuses (%)*
I Untreated Controls	10	143	7 (4.9)	136 (95.1)
II Nicotine Treatment	10	132	4 (3.0)	128 (97.0)
III Control to Nicotine	10	133	4 (3.0)	129 (97.0)
IV Caffeine Treatment	10	146	7 (4.8)	139 (95.2)
V Control to Caffeine	10	131	5 (3.8)	126 (96.2)
VI Nicotine and Caffeine Treatment	10	135	4 (3.0)	131 (97.0)

* Data subjected to Chi-Square (x^2) test: no significant differences between groups.

Table 2. Influence of Nicotine and Caffeine in the Pregnant Rat.

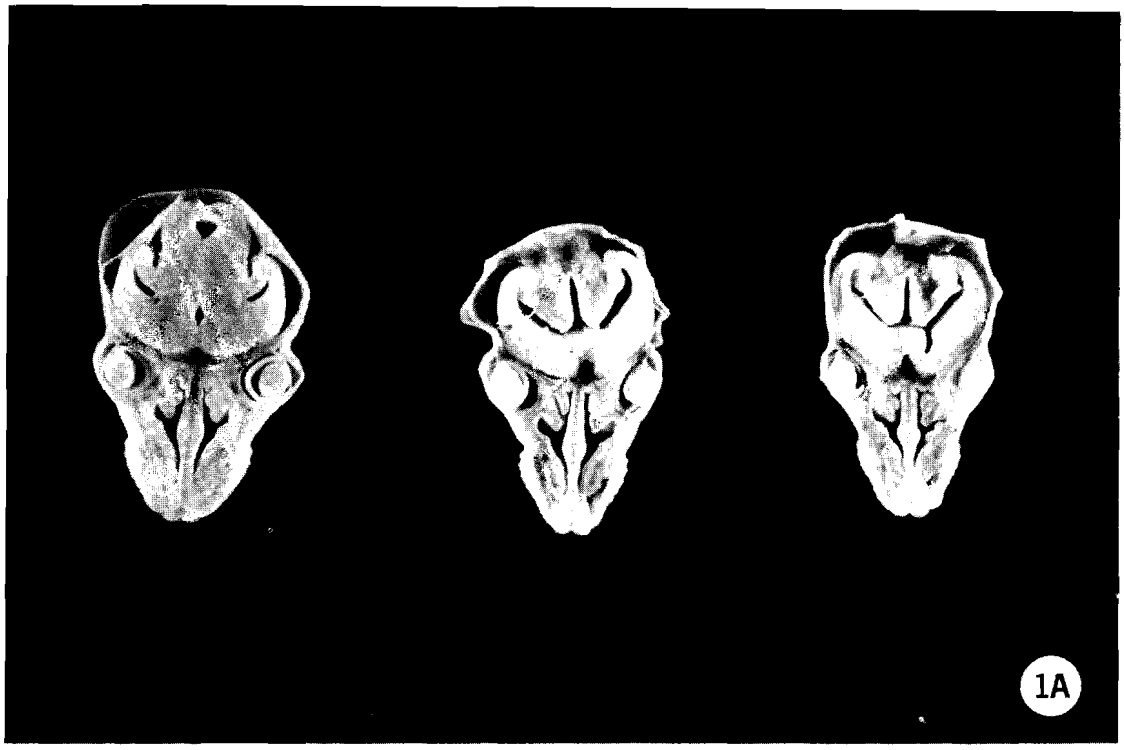
Treatment groups	No. of animals	Maternal wt. gain* (mean ± SDM)	Litter size* (mean ± SDM)	Fetal weight* (mean ± SDM)	Placental weight* (mean ± SDM)	Crown rump* (mean ± SDM)
I Untreated Control	10	117.3 ± 18.3	13.6 ± 1.0	1.95 ± 0.06	0.34 ± 0.02	2.62 ± 0.03
II Nicotine Treatment (149 µg/hr × 7 days)	10	112.8 ± 5.1	12.8 ± 1.3	2.08 ± 0.04	0.33 ± 0.01	2.61 ± 0.03
III Control to Nicotine	10	90.3 ± 5.7	12.9 ± 1.3	1.93 ± 0.09	0.35 ± 0.02	2.62 ± 0.05
IV Caffeine Treatment (25 mg/kg)	10	120.1 ± 5.0	13.9 ± 0.75	1.96 ± 0.04	0.35 ± 0.007	2.67 ± 0.03
V Control to Caffeine	10	126.1 ± 15.0	12.6 ± 1.3	1.86 ± 0.05	0.34 ± 0.007	2.60 ± 0.03
VI Nicotine & Caffeine Treatment (149 µg/hr × 7 days & 25 mg/kg)	10	113.5 ± 7.0	13.1 ± 0.8	1.97 ± 0.05	0.36 ± 0.01	2.67 ± 0.03

Weights recorded in grams; crown-rump recorder in cm.
*Duncan's New Multiple Range Test: No significant differences among the groups ($p < 0.05$).

Table 3. Frequency of developmental defects following administration of nicotine and caffeine to pregnant rats*.

Treatment groups	Total no. of live fetuses	Abnormal** (% of fetuses affectet)	Types of defects
I Untreated Controls	136	3 (2.2)	hydrocephalus (3)
II Nicotine Treatment	128	7 (5.5)*** ($p < 0.05$)	hydrocephalus (6) digital anomalies; short forepaw digits (1)
III Control to Nicotine	129	2 (1.6)	hydrocephalus (2)
IV Caffeine Treatment	139	3 (2.2)	hydrocephalus (1) digital anomalies; missing forepaw digts (1); microcephaly (1)
V Control to Caffeine	126	1 (0.8)	hydrocephalus (1)
VI Nicotine & Caffeine Treatment	131	0 (0.0)	nil

* Each treatment group consisted of 10 pregnant females.
** Includes external and visceral anomalies.
*** Significant differences between group II and III, II and VI, and II and I (X^2-test).



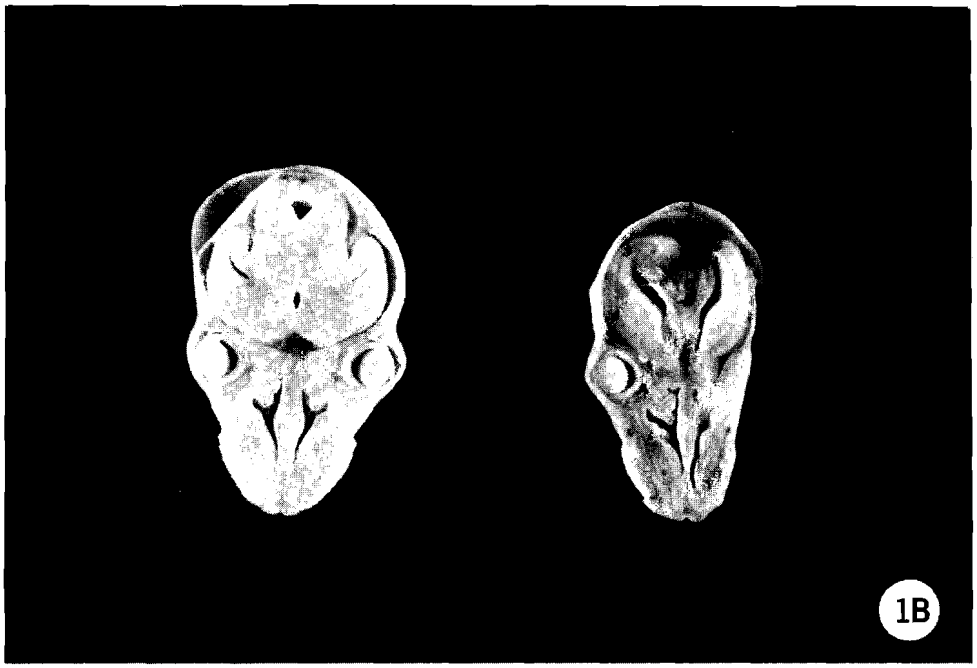
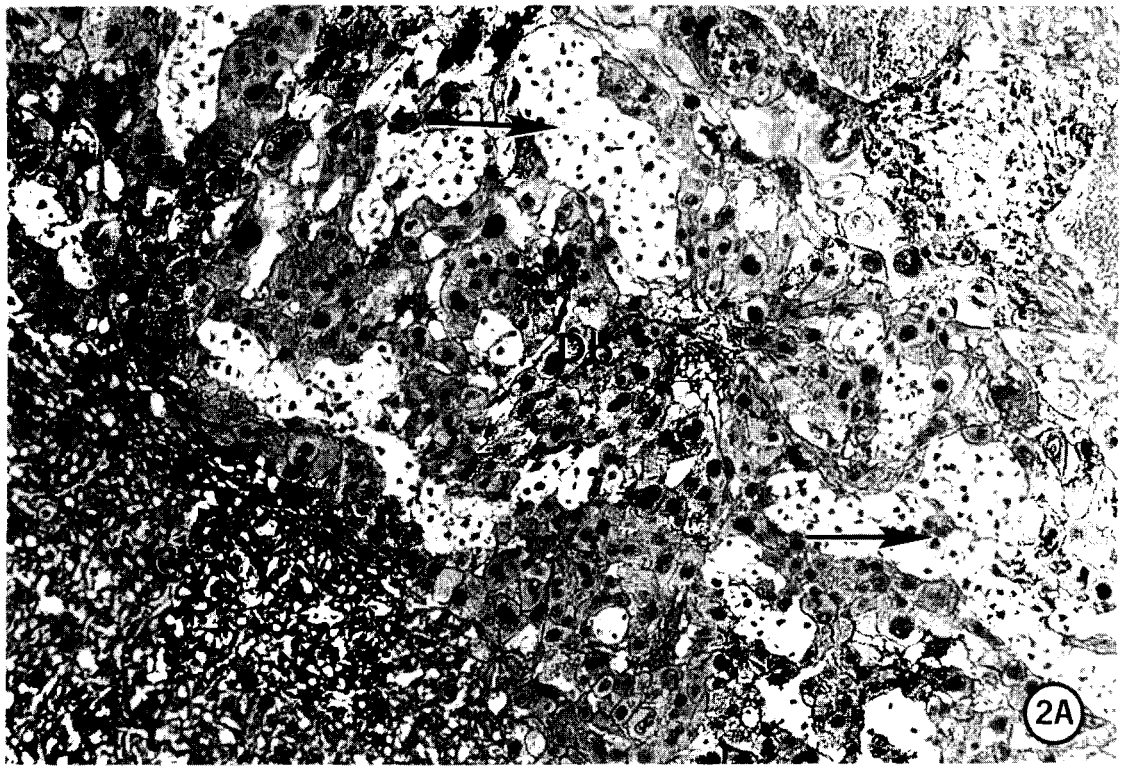


Fig. 1A. Wilson's section through the head region of 20 day rat fetuses. Control fetus (extreme left); nicotine treated fetuses showing dilatation of the lateral ventricles (right). **Fig. 1B.** Control fetus (left); nicotine treated fetus revealing hydrocephalus in an oblique section (right).



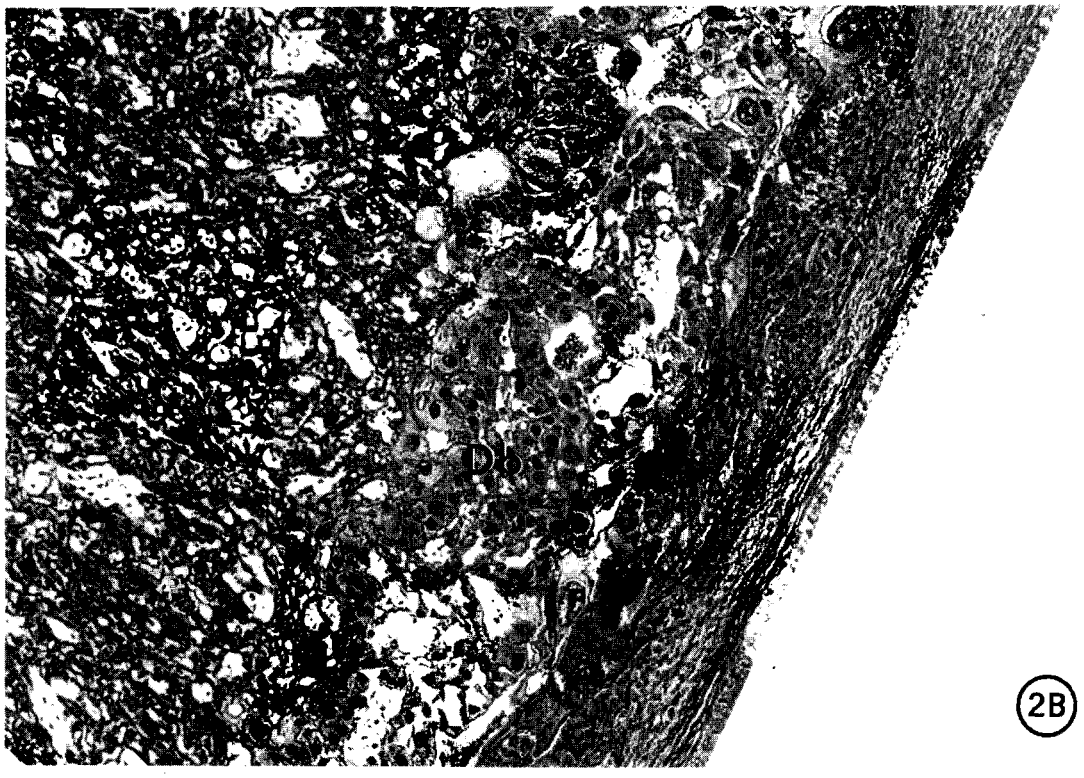


Fig. 2A. Placenta of control animal showing a well developed decidua basalis (Db) with varying degrees of degeneration (arows). Chrorionic villi (Cv) demarcating the fetal portion of the placenta. H & E., × 113. **Fig. 2B.** Placenta of nicotine treated animal. Note the thinner decidua basalis (Db) as compared with the control. Chorionic villi (Cv) demarcating the fetal portion of the placenta. H & E., × 113

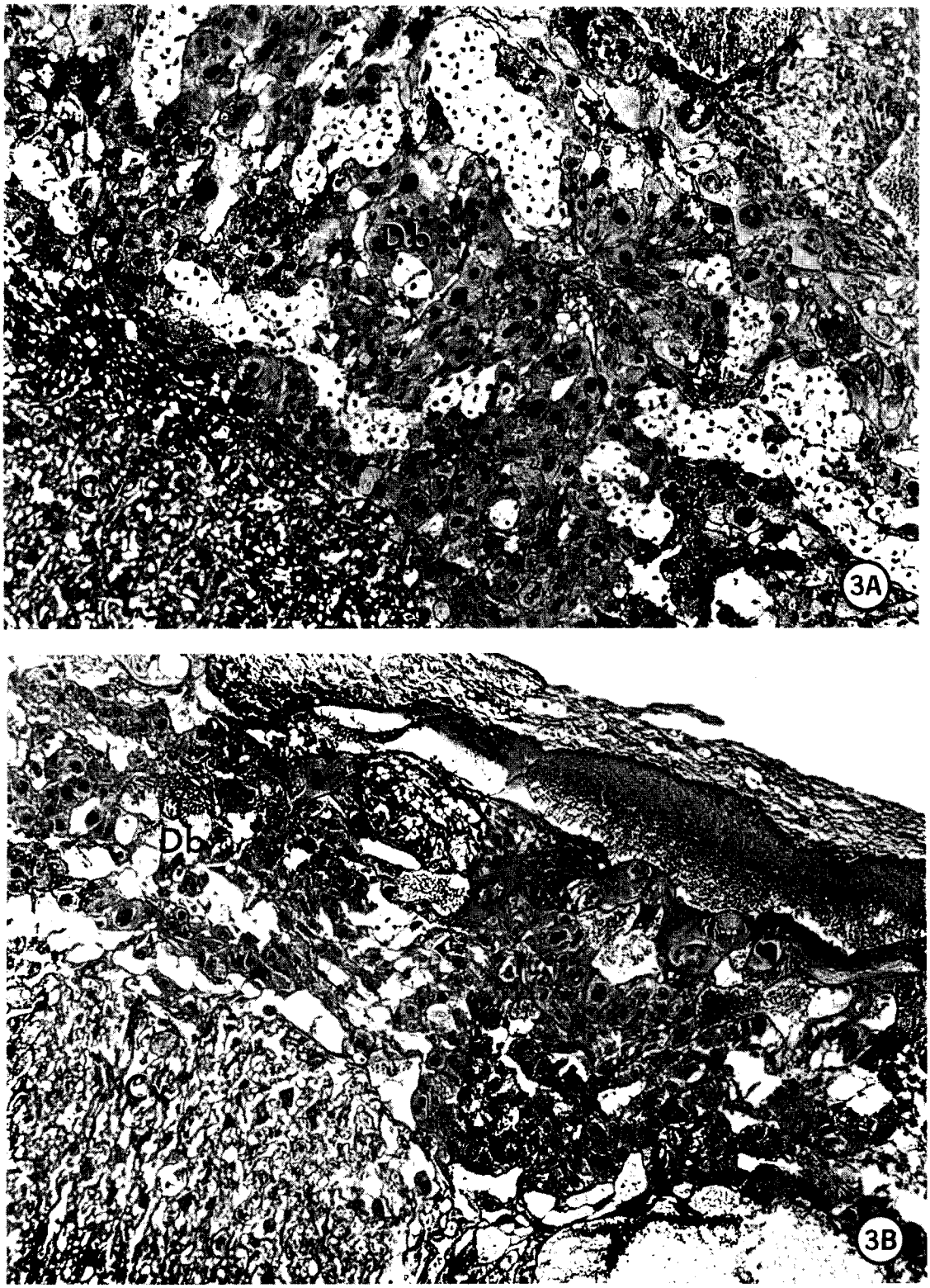
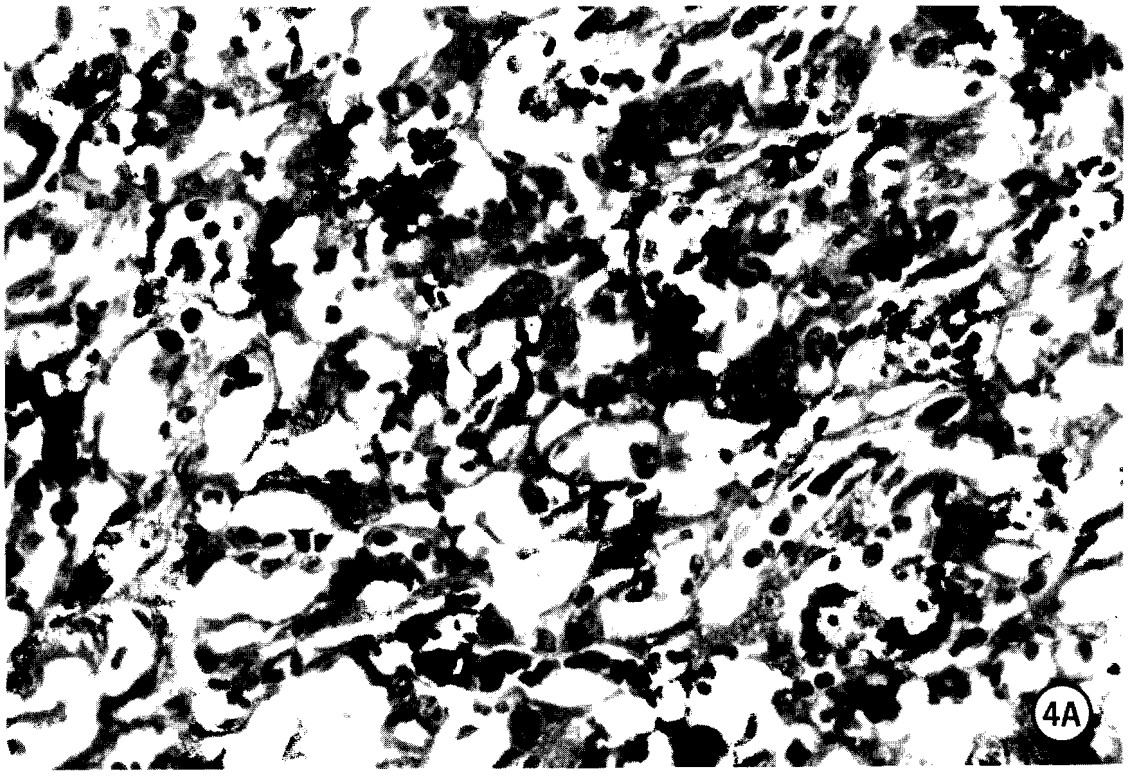


Fig. 3A. Placenta of control animal showing decidua basalis (Db) and chorionic villi (Cv). H & E., × 113. **Fig. 3B.** Placenta of nicotine and caffeine treated animal. The chorionic villi (Cv) are evident and decidua basalis (Db) is notably thinner than that of the control. H & E., × 113



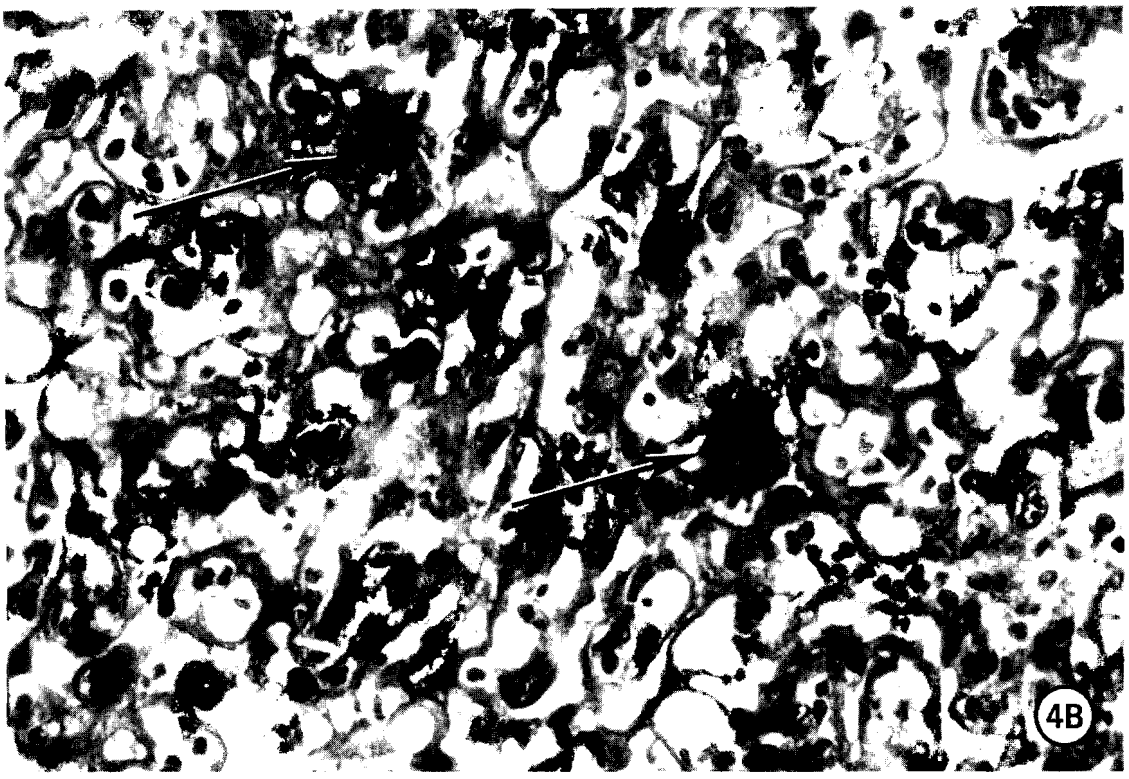


Fig. 4A. Placenta of a control animal showing chorionic villi and extensive intervillous spaces H & E., × 450. **Fig. 4B.** Placenta of a nicotine and caffeine treated animal showing congestion of intervillous spaces with red blood cells (arrows). H & E., × 450

caffeine treated animals, was less developed, when compared with respective controls. Both of these groups were affected to a similar degree. Whereas the decidual cells generally appeared normal in all treatment groups, the decidua was notably thinner following nicotine and both nicotine and caffeine treatment (Figs. 2, 3). Similar findings were observed in the placentas of the caffeine treated animals; however these were minimal in comparison with those already mentioned.

Both nicotine treated and nicotine and caffeine treated animals also demonstrated congestion of intervillous spaces. This was most prominent at the placental margins, and at the interface between the maternal and fetal portions of the placenta, occurring in eight of the ten nicotine treated, and all ten of the combined treated animals. Definitive intervillous dilatation was not evident in these sections (Fig. 4).

No evidence of pathological changes or abnormal development were observed in the fetal components of the placenta in any of the treatment groups. Branch villi appeared normal, with fetal capillaries identified by the presence of RBC's in their lumina.

Discussion

Wilson (1974) referred to developmental toxicity as «encompassing all deviations in developmental processes originating between fertilization and postnatal maturity, including fetal death, malformation, growth retardation and functional deficiency». Identification of environmental teratogens represents a complex toxicological problem, in that the response of the developing conceptus is influenced by multiple other factors acting either individually or collectively (Persaud, 1985). These include the type of drug, level and duration of exposure, maternal modulation of dosage, access to the conceptus, developmental stage at time of treatment, disposition within the conceptus and susceptibility of species and the individual. In today's society, pregnant women are frequently exposed to a wide variety of different chemical agents, including industrial pollutants, over-the-counter pharmaceutical preparations and socially abused drugs. Thus it is difficult to attribute deleterious effects on development to any single agent.

One of the major difficulties encountered in the interpretation and subsequent practical application of the research literature relates to the dosages of nicotine and caffeine utilized to induce teratogenic effects in laboratory animals. This is particularly evident with regards to caffeine. Whereas numerous investigators have demonstrated various teratogenic effects of caffeine in rodents (Fujii and Nishimura, 1969; Fujii et al., 1969; Palm et al., 1978; Kimmel et al., 1982; Scott, 1983), the dosages utilized were far in excess of those generally consumed by humans.

According to a recent report by Lelo et al. (1986), the average daily human caffeine intake of moderate to heavy consumers ranges from approximately 300 to 600 mg/day, or from 3 to 6 cups of coffee (assuming 100 mg/cup). The dosage level therefore in a person weighing 70 kg ranges from approximately 4.3 to 8.6 mg/kg/day. In comparison, caffeine doses administered to laboratory animals ranged from 30 mg/kg (Palm et al., 1978) to 250 mg/kg (Fujii and Nishimura, 1969). Even when species variation is taken into account, the practical application of the results obtained from many of these animal experiments to the human condition is unrealistic due to the excessive dose levels administered.

Other important considerations in studying the teratogenic potential of test agents include the mode of administration (Fujii et al., 1969) and the developmental stage during which the conceptus is exposed (Wilson, 1964). In the present study, a total dose of 25 mg nicotine was administered to pregnant rats over a seven day period. The mode of administration was via continuous subcutaneous infusion from an osmotic mini-pump. Nicotine treatment commenced on gestational day 6 to coincide with implantation of the blastocysts, and continued through the 12th day of pregnancy. In the rat, the 9th to 12th gestational days are developmentally critical, as they represent the major period of organogenesis. During this time, differentiation of nearly all organ primordia occurs (Hebel and Stromberg, 1986). Also, humans consume nicotine in small continuous doses, over an extended period of time through inhalation of cigarette smoke. Thus, the rat embryos were exposed to nicotine during their critical stages of development in a manner similar to human consumption. At an infusion rate of 149 μg/hr, each animal received approximately 3.6 mg nicotine/day. Based on an average maternal weight of 300 g, this can be translated to a dose of 0.5 mg/kg/hr, or the nicotine equivalent of 12 cigarettes, assuming each cigarette contains 1.0 mg nicotine (Taylor, 1985).

A moderate dosage level of 25 mg/kg caffeine was administered as a single intravenous injection on gestational day 6. Fujii et al. (1969) demonstrated that in mice, whereas embryolethality is related to the duration of caffeine exposure, teratologic effects are more dependent on a sufficiently high concentration of the drug. Though intravenous injection does not simulate human caffeine consumption the method of caffeine administration in the present study was the most expedient and in accordance with that utilized by others.

In the rat, fertilization usually occurs in the uppermost part of the oviduct at the time of coitus. Tubal passage of the fertilized ova into the uterine horns requires three days. During this time, the conceptus begins its segmentation and differentiation, and enters the uterus at the late morula stage (Hebel and Stromberg, 1986). Caffeine can thus act as a mitotic poison in early mammalian embryos (Spindle and Wu, 1985). Furthermore, Fabro and Sieber (1969) demonstrated that caffeine penetrates the preimplantation blastocysts in rabbits. In the present study, caffeine was administered to the pregnant rats on the 6th gestational day in order to study the teratogenic effect on early embryonic development.

Nicotine has long been considered to be the primary substance in tobacco smoke responsible for the pharmacological response to smoking, although carbon monoxide, carbon dioxide and cyanide have also been implicated (Kelly et al., 1984; Phillip et al., 1984; Luck et al., 1985). The most consistently observed effect of maternal smoking during pregnancy is intrauterine growth retardation, with a clear dose-response relationship existing between the number of cigarettes smoked and the birth weight deficint (Lubs, 1973; Spira et al., 1975; Meyer et al., 1976; Harrison et al., 1983; Mochizuki et al., 1984; Phillip et al., 1984; Cnattingius et al., 1985; Nieburg et al., 1985).

In all of the above mentioned studies, observations are based on retrospective data from large human populations. Babies were evaluated at birth, and the mothers smoked cigarettes throughout the entire gestational period. While the growth retardant effect of nicotine on fetal growth cannot be disputed under these circumstances, questions remain as to when does this effect occur and what is (are) the mechanism (s) involved.

In the present experiments, maternal exposure to nicotine during the early stages of pregnancy had no significant effect on fetal growth evaluated near term, either alone or in combination with caffeine. These results are in accordance with those reported by Meyer et al. (1976) who revealed that mothers who stopped smoking during prenancy gave birth to babies of normal weight and length. Thus, I.U.G.R. secondary to nicotine consumption (smoking) during pregnancy is dependent at least in part upon fetal exposure throughout the entire gestational period.

One of the proposed mechanisms responsible for the retarded fetal growth in smokers is impairment of uteroplacental circulation secondary to the vasoconstricting effect of nicotine (Suzuki et al., 1980; Mochizuki et al., 1984; Phillip et al., 1984). Despite discontinuation of nicotine treatment after the 12th gestational day, histologic changes observed in maternal liver, kidney and placenta on the 20th day of gestation were suggestive of some degree of functional circulatory disturbances secondary to nicotine. This was evidenced by dilatation and congestion of hepatic sinusoids, glomerular congestion associated with degenerative changes and congestion of placental intervillous spaces. The poor development of the decidua basalis observed in the nicotine and the nicotine and caffeine treated animals may be the result of impaired uteroplacental circulation. The dilatation and congestion of hepatic sinusoids observed to occur in fetal liver are in support of the hypothesis that nicotine has a direct effect on the fetus, causing circulatory responses similar to those observed in the mother (Suzuki et al., 1980; Eriksen and Marsal, 1984).

Of practical importance is the observation that although histological changes were observed in the maternal component of the placenta, the chorionic (branch) villi of the fetal compartment appeared normal. Whereas fetal plasma concentrations of nicotine have been shown to surpass those of the mother, the amount of nicotine in the entire fetus accounts for only 1 to 4% of the total dose administered to the mother (Suzuki et al., 1980). Maternal metabolic processes and placental metabolism play an important role in determining the dosage of a drug to which the fetus is exposed (Wilson, 1974; Yaffe and Juchau, 1974; Persaud, 1985). Though based on limited numbers, the histological changes noted in this investigation suggest that even a moderate dose of nicotine administered over a limited time period has a persistent adverse effect on the circulatory function of maternal tissues. While some evidence suggesting a direct effect of nicotine on the fetus was observed, the normal appearance of the kidney, and in particular the fetal component of the placenta, indicates that the functional development of fetal tissues was not adversely affected by nicotine.

The acute effect of nicotine on fetal tissues cannot be assessed from this study, because development was allowed to continue to near term. Factors such as maternal modulation of dosage and the highly proliferative nature of embryonic tissues may account for the lack of observed effects of nicotine treatment. The compensatory placental hypertrophy reported by Spira et al. (1975) was not evident in this investigation.

To date, evidence associating maternal nicotine consumption and embryolethality (early fetal loss) remains inconclusive. Whereas several studies on human populations reported a higher incidence of spontaneous abortion in mothers who smoked (Hemminki et al., 1983; Kline et al., 1977,1983), all of the confounding factors known to influence human embryonic development could not be statistically controlled, nor were the reported increases statistically significant. Nasarat et al. (1986) reported a higher incidence of perinatal mortality in mice, associated with maternal exposure to nicotine. In this study, however, perinatal mortality was defined according to the observed number of neonatal deaths and stillborn infants, and embryolethality, as evidenced by the number of resorption sites in the uterine horns, was not evaluated. In the present study, nicotine had no effect on embryolethality. Similar results were reported by Persaud (1982) and Lindenschmidt and Persaud (1980).

Whether or not cigarette smoking during pregnancy represents significant teratologic risk to the fetus remains a controversial topic. Factors such as conflicting definitions and classification of various birth defects, lack of control for potential risk factors in the human population and species variation make the interpretation of research literature very difficult. With regards to human populations, recent studies indicate that moderate doses of nicotine (<1 package/day) do not have a teratogenic effect on the developing conceptus (Christianson, 1980; Hemminki et al., 1983; Shiono et al., 1986).

The overall low incidence of birth defects observed throughout this investigation are likely due to the normal low incidence of birth defects in the Sprague-Dawley strain of rats. Thus, extremely large study

populations would be required to detect any significant increase in these defects. In animals treated with nicotine, however, a significantly higher incidence of hydrocephalus was observed relative to any other group which may represent a manifestation of observations reported by Lajtha and Sershen (1986) and Seidler et al. (1986). These authors found that early fetal exposure to nicotine causes interruption of both DNA synthesis and subsequent cell replication (Seidler et al., 1986), and protein metabolism and synthesis in central nervous system neurons (Lajtha and Sershen, 1986). They propose that the biochemical interference with early developmental events may contribute to the formation of some central nervous system defects.

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