THE EFFECT OF CHRONIC MATERNAL MARIHUANA INHALATION DURING PREGNANCY ON NEUROCRANIAL AND MAXILLOFACIAL GROWTH AND DEVELOPMENT OF RAT OFFSPRING

by

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Abstract

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Currently, a very important question dealing with causative factors in congenital malformations concerns marihuana and its possible effects during pregnancy and on offspring exposed in utero. To date, no specific studies have been conducted to characterize precisely the nature of neurocranial and maxillofacial growth and development effects that might relate to this substance.

The present study was performed to evaluate the effect of marihuana on the neurocranial and maxillofacial growth of 150 rats whose dams had been exposed to marihuana smoke for intermittent periods throughout gestation; 119 rats served as controls.

Data were collected in two categories: (1) general appearance, physical condition, and body weight of the newborn rats; and (2) roentgenographic cephalometric measurements of the neurocranial and maxillofacial areas of the offspring exposed in utero.

1. In general, the experimental animals did not reveal any gross distortion or malformation due to marihuana at any of the dosage levels.

2. Analysis at thirty days revealed an increase in snout length and body weight for animals exposed *in utero* to the low dose containing 0.1 mg/kg concentration of marihuana smoke, and a decrease in snout length and body weight for animals exposed *in utero* to higher doses of 0.2, 0.4, 1.0 mg/kg concentrations of marihuana smoke.

3. Further analysis at sixty days after birth, however, revealed no significant differences in neurocranial and maxillofacial dimensions or body weight between control and marihuana-exposed animals at any of the dosage levels.

Further studies are indicated to investigate possible effects due to any type of smoke during pregnancy, and also the physiologic basis for the 30 day and 60 day effects noted in this investigation.

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DEDICATED

TO

My wife, Susan, for her love

and understanding.

My parents, who made my

education possible.

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INTRODUCTION

Human congenital malformations have been of interest for centuries. Experimental teratology, however, gained its greatest impetus from studies in the 1940's that linked maternal rubella infections with malformations and from the thalidomide disaster of the early 1960's. Currently, a very important question in this biologic area concerns marihuana and its possible effects during pregnancy on offspring exposed in utero. This question urgently needs to be answered in view of the widespread use of derivatives from this plant.

The hemp plant, Cannabis Sativa, has been used as a source of fiber, a drug in tribal religious ceremonies, and as a medicine. With the explosion of its use as a recreational drug in the 1960's, at first on college campuses followed by a spread downward to secondary schools and upward to a portion of the established middle class, controversy over the dangers of marihuana generated a desire for more objective information about the drug. Currently, Cannabis has approximately 33,500,000 users in the United States according to a 1978 Harris Poll, and some 9.1% of U.S. high school seniors smoke it daily. Marihuana is so widely used that its effects need to be investigated as thoroughly as possible.

Since the developing fetus may be especially vulnerable to drugs consumed by the mother during fetal development, the effects on the fetus of chronic marihuana inhalation by pregnant mothers

merits thorough study. Any effect, large or small, that produces a documented deviation from the normal pattern of growth needs to be understood. Specifically in the field of orthodontics, any factor that causes a divergence from the regular pattern of craniofacial development is a matter of concern and must be considered.

It is the purpose of this study to investigate, using the Sprague-Dawley rat, the effect of chronic maternal marihuana inhalation on the neurocranial and maxillofacial growth and development of offspring. Particular attention will be paid to cranial and midfacial dimensional changes. Using radiographic cephalometrics, specific neurocranial and maxillofacial measurements will be taken to determine any significant changes in pattern and amount of growth and development.

REVIEW OF THE LITERATURE

Pharmacologic studies of Cannabis indicate that the tetrahydrocannabinol fraction of the resin is the active component. Since the discovery that delta-9-tetrahydrocannabinal (Δ^9-THC) is the principal constituent responsible for psychological, pharmacological, and behavioral effects in man, more specific research has been possible. The importance of investigating marihuana during pregnancy was realized when radioactive studies established that Δ^9-THC readily diffuses across the placenta (Idänpään Heikkilä, et al., 1969; Harbison and Mantilla-Plata, 1972; Kennedy and Waddell, 1972; Freudenthal, et al., 1972). This substance has also been shown to appear in milk (Jakubovic, et al., 1973).

Although much research has been conducted concerning marihuana during pregnancy, conflicting and contradictory results presently exist. The lack of consensus may be due to numerous factors, including species of animal used, form of drug used (naturally occurring plant, natural and synthetic tetrahydrocannabinol (THC) preparations), purity of the drug, dosage, and route of administration.

Literature Suggesting No Adverse or Minimal Effects of Marihuana

Borgen et al. (1971) used a daily subcutaneous dose in pregnant rats and found no stunting or malformations of offspring al-

though the period of gestation was prolonged 1-2 days from 21-22 days to 23-24 days. When exposing rats to a subcutaneous dose of Δ^9-THC on gestational days 6-15, Banerjee et al. (1975) found a significant fetal weight reduction of offspring but no morphological anomalies. Again using a prenatal exposure to Δ^9-THC by subcutaneous injection, but on days 10, 11, and 12, Borgen, Davis and Pace (1973) reported changes in the physical and behavioral development of neonatal rats at a dose level that neither kills or stunts the fetuses, nor causes any observable changes in the morphology of the newborn. Gianutsos and Abbatiello (1972) used a subcutaneous dose in rats on days 8-11 of pregnancy and found no significant weight difference at birth and no gross malformations, but did find a retarded ability in maze learning in experimental offspring. Harbison and Mantilla-Plata (1972), using mice and intraperitoneal injections during pregnancy, found reduced fetal body weight but no congenital anomalies. Manning and McDonough Jr. (1971) found that oral and intraperitoneal doses of Δ^9-THC given to newborn rats from days 11-40 resulted in decreased food intake and decreased weight gain. Weisz, Fazel and Rawitch (1976) reported that oral administration of Δ^9-THC to rats throughout pregnancy showed no teratogenicity or stunting and no significant weight difference in offspring. In an earlier study Miras (1965) found that rats impregnated after being fed a diet containing 0.2% marihuana extract for several months showed reduced fertility, but the offspring pro-

duced were normal. A more recent investigation into smoking of marihuana by Fried (1976), using Cannabis smoke and inhalation in pregnant rats, found there was an increase in fetal resorptions, but that litter size, length of gestation, sex ratio, live births and external appearance of offspring did not differ between experimental and control groups. Birth weights of experimental offspring were significantly reduced, however.

Literature Suggesting More Severe Effects Due to Marihuana

Geber and Schramm (1969) found congenital malformations and abnormal fluid accumulations in fetal hamsters and rabbits after maternal subcutaneous administration of large doses of marihuana resin on gestation days 6-8 and 7-10, respectively. They also noted an increase in runts for both animals. More specifically, malformations of the brain, spinal cord, foreleg and liver, as well as edema of the head and spinal region were seen in the offspring. Joneja (1975) utilized maternal intravenous, subcutaneous and intragastric administration in mice and found that the intragastric route produced significant fetal growth retardation. Live fetuses were deformed and skeletal anomalies involving accessory ribs were found using a single intragastric dose of 200 mg/kg on day 10. The intragastric route also produced asynchronous vertebral ossification, fused ribs, and poorly ossified cranial bones suggesting some effect on skeletal development at higher doses. Persaud and

Ellington (1967) injected gravid mice intraperitoneally with 16 mg/kg of Cannabis resin on day 6 of gestation and produced offspring that were stunted but not malformed. The same dose given on days 1-6 caused a 13% increase in fetal resorptions. In a second experiment by Persaud and Ellington (1968) using rats, an intraperitoneal injection of 4.2 mg/kg of Cannabis extract on days 1-6 resulted in a high frequency of malformed progeny. Mantilla-Plata et al. (1975) found a significant incidence of in utero death, reduction in body size and offspring with cleft palate from pregnant mice given intraperitoneal doses of Δ^9-THC. Most recently, Siegel et al. (1977) used oral doses of Δ^9-THC in pregnant rats and found an increased, fluctuating dental asymmetry in the mandibular dentition leading to the conclusion that stressful fetal effects occurred.

In summary, definite conclusions concerning marihuana exposure during pregnancy and its effects on growth and development in offspring are difficult to arrive at. Depending on the timing of the drug exposure, the effects seen may be due to direct effect during gestation through placental transfer or to residual effect during nursing by transfer of marihuana metabolites to the offspring through milk. To date there have been no studies employing chronic inhalation during pregnancy and with the utilization of cephalometrics to determine precisely what changes in neurocranial and maxillofacial growth and development might be occurring.

Human Studies

Because the Food and Drug Administration has, until recently, forbidden controlled marihuana studies on women, the effects during pregnancy in humans remains obscure at best. Although consistent and corroborating results from controlled experimental conditions are lacking for humans, some information is available on the human effects of marihuana and its metabolism.

McKinley (1978) has reviewed the effects of Cannabis, including marihuana, hashish, and hash oil. Although marihuana strength, as judged by content of THC, ordinarily ranges from 0.5 - 7% THC, some varieties may contain 10-20% THC. Hashish usually has approximately 8-10% THC, and hash oil around 60% THC. The short term effects of smaller doses of marihuana are relaxation, euphoria, altered perception, fascination with visual and auditory phenomena, and laughter. Short term effects of larger amounts of marihuana are confusion of time sense, inability to carry out mental tasks, a sense of strangeness and unreality about one's self and surroundings, fear of dying, anxiety and panic. The long term effects of chronic use of abuse are very contradictory, but such use of very heavy doses have been reported to cause impairment of concentration, memory, alertness, and the ability to perform complicated tasks.

Kolansky and Moore (1972) reported on toxic effects of chronic use in humans and found that symptoms were related to duration and frequency of smoking. Their clinical findings suggested a temporary

toxic cerebral state on a biochemical basis. Cerebral atrophy was noted in the examination of subjects who were heavy users for 3 to 11 years. They suggested that biochemical and structural changes in the central nervous system (possibly the cerebral cortex) with intense use seem probable. Their general feeling was that, especially in developmental stages, the cerebral cortical cells may be damaged by chemical changes due to Δ^9-THC leading to deleterious effects on the development of the adolescent personality. Even though there is a general acknowledgement that marihuana may be harmful to adolescents, there is little aggreement that Cannabis can be harmful to adults. Kolansky and Moore do point out that persistent and intense marihuana or hashish smoking may affect the cerebral functioning of any individual in much the same way regardless of age or psychological maturity.

Investigation into minimum effective doses of inhaled marihuana range from 6 µg/kg (Kiplinger, et al.: cited by Lemberger, et al., 1971) to 70 µg/kg (Hollister, 1971) of the principal active ingredient of THC. Lemberger and Tamarkin (1971) have found that long-term marihuana smokers can obtain pharmacologic effects from smoking a marihuana cigarette delivering a dose of Δ^9-THC in the order of 6.25 µg/kg.

The Metabolism of Marihuana

Some of the growth effects to be described in the present report could be a result of marihuana metabolites. THC is a lipid-

soluble, nonpolar compound and is a substrate for the mixed-function oxidase system (Cohen, et al., 1971).

The actual mechanism of the metabolism of THC is explained by Harbison, Mantilla-Plata and Lubin (1972) as follows:

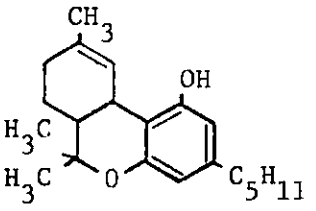
> The salient features of THC metabolism are schematized in figure 1; these are: (1) a rapid allyl hydroxylation, primarily at the 11-methyl group but also at C8, yielding the active primary metabolites, 11-hydroxy-THC and 8α, 8β-hydroxy-THC; (2) deactivation of 11-hydroxy-THC occurs by further hydroxylation to 8α-11-hydroxy-THC, or by (3) oxidation to 11-oxo and 11-carboxy-THC; (4) hydroxylation of the pentyl side chain at C1' or C2' (this reaction can occur before or after the formation of the acid form); and (5) conjugation, the full pattern of which is not yet clear.

Several studies have shown that the THCs are the principal metabolites (Christensen, et al., 1971; Freudenthal, et al., 1972). The 11-hydroxy-THC metabolite appears to the major fraction in most species. In human studies by Lemberger and Tamarkin (1971) considerable quantities of metabolites were present in the plasma, including about 25% 11-hydroxy-THC, which was detectable within 10 minutes after administration of Δ^9-THC.

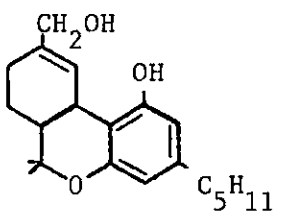
Once in the body, marihuana is virtually totally metabolized. Negligible amounts of unchanged Δ^9-THC appear in urine indicating complete body transformation, according to Lemberger and Tamarkin (1971). Klausner and Dingell (1971) indicate confirmation of earlier findings that THC is excreted almost completely in the feces as polar metabolites. It has been found by Lemberger and Tamarkin (1971) that although the mode of total metabolism and ex-

FIGURE 1

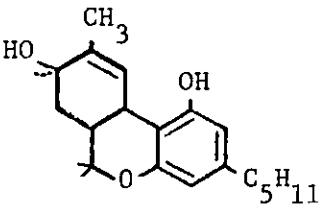
PROPOSED SCHEME OF METABOLISM OF THC



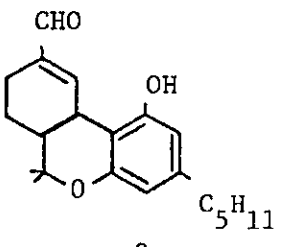
Δ^9-THC



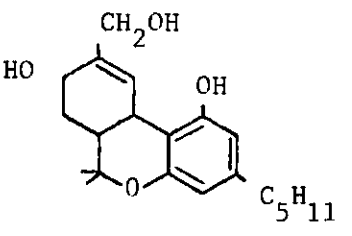
11-hydroxy-Δ^9-THC



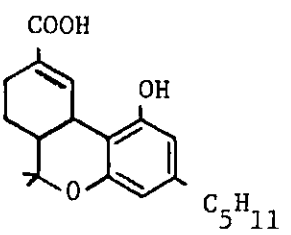
8α(or 8β)-hydroxy-Δ^9THC



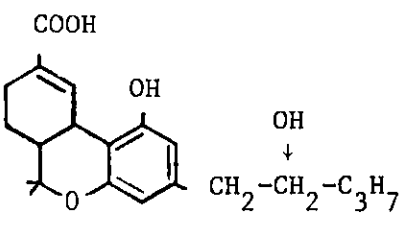
11-oxo-Δ^9-THC



8α, 11 dihydroxy-Δ^9-THC



11-carboxy-Δ^9-THC



1'(or 2')-hydroxy, 11-carboxy-Δ^9-THC

After Harbison *et al*. (1977), Figure 1.

cretion is the same in smokers and nonsmokers, the process occurs more rapidly in chronic users.

Although THC passes through many organs and tissues, metabolism occurs mainly in the liver, but also in the lungs, spleen, and blood (Christinsen, et al., 1971). After exposure to labeled THC and inhalation administration, Ho et al. (1970) found that high and persistent levels occurred in brain, lungs and liver. There the brain levels were especially slow in being dissipated; even after 7 days. This may account for the biphasic disappearance from the body where there is a rapid decline in the first few hours, but after this there is a half-life of about 21 hours (Klausner and Dingell, 1971). It has been shown by Lemberger and Tamarkin (1971) that Δ^9-THC persists in human plasma for several days and is excreted in feces and urine for 8 days or more. Klausner and Dingell (1971) have also shown that 60% is excreted by 5 days, and total excretion is complete within about 144 hours in rats.

Placental Transfer of Tetrahydrocannabinol

It has been established that THC and its metabolites cross the placenta (Heikkila, et al., 1969; Harbison and Mantilla-Plata, 1972; Weisz, et al., 1976).

After administration of Δ^9-THC by various routes, placental transfer takes place rapidly and can be measured in the amniotic fluid and fetus for a number of different species. The amounts in the amniotic fluid and fetus are much less than in maternal

TABLE I

Levels of Δ^9-THC plus metabolites in mice following intraperitoneal injection of 100 mg./kg. Δ^9-THC (disintegrations/min./100 mg. of tissue)

Hours after Injection	Days of Gestation	Placenta	Fetus	Amniotic Fluid
4	14 and 15	570	200	250
8	14 and 15	230	75	180
16	14 and 15	250	70	50
24	14 and 15	180	30	40
36	14 and 15	200	40	30
48	14 and 15	250	30	10

After Harbison and Mantilla-Plata, 1972, Figure 7.

tissues but are still significant.

Heikkila et al. (1969) injected Δ^9-THC intraperitoneally in pregnant hamsters and found placental transfer taking place within 15 minutes, although peak levels in the fetus were attained at 30 minutes. The fetal Δ^9-THC was still present after 24 hours. Using both subcutaneous and intraperitoneal administration, the placental uptake was found to be 2-3 times higher than the fetal uptake indicating that the fetus is protected to some degree by the placental barrier. In comparing administration in early and late pregnancy, it was found that fetuses contained three times as much Δ^9-THC when exposed in earlier stages of pregnancy than fetuses exposed in late pregnancy.

Harbison and Mantilla-Plata (1972) exposed pregnant mice to intraperitoneal injections of 200 µg/kg Δ^9-THC on gestational days 8-13 and found small amounts in placental tissues. Although no difference was found between fetal and amniotic fluid levels of Δ^9-THC, the placenta contained three times the fetal level. This indicated that approximately 30% of the Δ^9-THC in the placenta was passed to the fetus.

From the references previously reviewed, it can be concluded that THC passes from the mother to the fetus and amniotic fluid via the placenta, and as a result, could conceivably create adverse effects upon the developing fetus.

STATEMENT OF THESIS

The purpose of this investigation is to analyze the neurocranial and maxillofacial form of thirty-day-old and sixty-day-old Sprague-Dawley rats that were exposed to marihuana for intermittent periods during gestation via maternal marihuana smoke inhalation. Specifically, it will be determined whether there was any change in size, shape, or relative positions of the cranial vault and nasomaxillary complex in the experimental animals.

METHODS AND MATERIALS

SAMPLE

Two hundred sixty-nine Sprague-Dawley rats from twenty-three litters were utilized to ascertain any neurocranial and maxillofacial form and pattern effects from exposure to marihuana. The breeding stock was purchased from Zivic Miller, Allison Park, Pennsylvania.

Pregnant rats were obtained by housing three females with one male in 21 x 35 x 44 cm plastic cages with a twelve-hour light and dark cycle. Daily observations for vaginal plugs were made within two hours after the dark cycle. The presence of a plug was taken as the midpoint of the first twenty-four hours of gestation (Kalter, 1968). At that time, all the females were placed in separate 20 x 25 x 40 cm metal cages, identified according to the estimated time of conception, and provided with water and Purina rat-mouse chow *ad libitum*.

Experimental Design

A total of twenty-three litters were used in this investigation to analyze neurocranial and maxillofacial growth and development and were divided into the following groups and subgroups:

1. Thirteen litters (one hundred fifty rats) were exposed to marihuana via maternal marihuana smoke inhalation for intermittent periods from the first through the twenty-first day of gestation.

The thirteen litters were subdivided into four dosage groups

of three litters each (thirty, thirty-six, and thirty-two rats) in the lower three dosage groups, and four litters (fifty-two rats) in the highest dosage group.

2. Ten litters (one hundred nineteen rats) served as controls. Their mothers were not placed in the chamber and therefore inhaled no marihuana smoke.

Marihuana Exposure Levels

The exposure levels of 0.1, 0.2, 0.4, and 1.0 mg/kg/day were chosen to determine any differences in effects at different dosage levels. Dams were placed in the chamber long enough to inhale the desired dose and provided with water and Purina rat-mouse chow *ad libitum* before and after exposure to the marihuana smoke.

The marihuana used in this experiment was of Mexican origin, grown on the University of Mississippi Campus, and certified to contain 1% Δ^9-THC. Cigarettes containing 0.85 g of marihuana were rolled in a hand operated rolling machine and marked with an ink pen to subdivide the cigarette into the different dosages.

The times necessary to burn the marihuana cigarette and produce the quantity of smoke for the desired dosage groups were one half, one, one and one half, and two minutes. In all groups this was followed by a two minute period during which the airflow was adjusted so that it bypassed the cigarette chamber, but continued directly to the animals. In this way the cigarette was extinguished. Immediately following this, the airflow was readjusted to flow

TABLE II

Dosage Groups and Inhaled Amounts

Dosage Group	Amount of Marihuana Burned (g.)	Calculated Inhaled Dose Δ^9-THC(mg./kg.)
1	0.05	0.10
2	0.10	0.20
3	0.20	0.40
4	0.50	1.00

through the cigarette chamber and then to the animals for two minutes to clear all the smoke produced for each group.

Care of Litters for Growth and Development Analysis

For the growth and development analysis, all newborn were left in isolated cages with their mothers until twenty-five days postnatal. In this way, a natural weaning could take place at approximately twenty-one days with minimal disturbance to both mother and offspring. The litters were handled as little as possible to minimize maternal cannibalism. All cages were changed at least every ten days to maintain sanitary conditions. At twenty-five days and fifty-five days tetracycline was administered to all offspring. At thirty and sixty days the rats were sedated, labeled and radiographed. At sixty-one days all animals except those in the highest dosage group were killed with an overdose of pentobarbital. It is planned that the highest dosage animals will be utilized to examine possible second generation effects in a follow-up study.

Exposure to Marihuana

Inhalation Chamber. In order to obtain marihuana exposure at constant, significant levels, an inhalation chamber was devised. The choice of an inhalation route as opposed to subcutaneous, intragastric or intraperitoneal was selected to resemble as closely as possible the normal human exposure to the drug.

The inhalation chamber utilized in this study was approximately 2260 cubic centimeters in volume and constructed of clear plexiglass. Up to 4 rats at a time could be accomodated in the chamber, each rat with its own tunnel connected to the main exposure chamber.

The combustion chamber which held the cigarette was airtight and connected to the inhalation chamber with tygon tubing. A respirator circulated room air through the combustion chamber to maintain a draw through the marihuana cigarette and produce smoke. This smoke was then diluted by 50% with more room air, and then circulated in the inhalation chamber. A vacuum line completed the airtight system and removed the smoke-air mixture after circulation around the rats.

In order to confirm the adequacy of ventilation, eight test rats were placed in the chamber for fifteen minutes per day for 8 days with the same airflow, but no smoke, and exhibited no adverse effects from being in the chamber. This same procedure, using eight additional test rats and a burning tobacco cigarette for fifteen minutes per day for 8 days also showed no adverse effects.

Data Collection

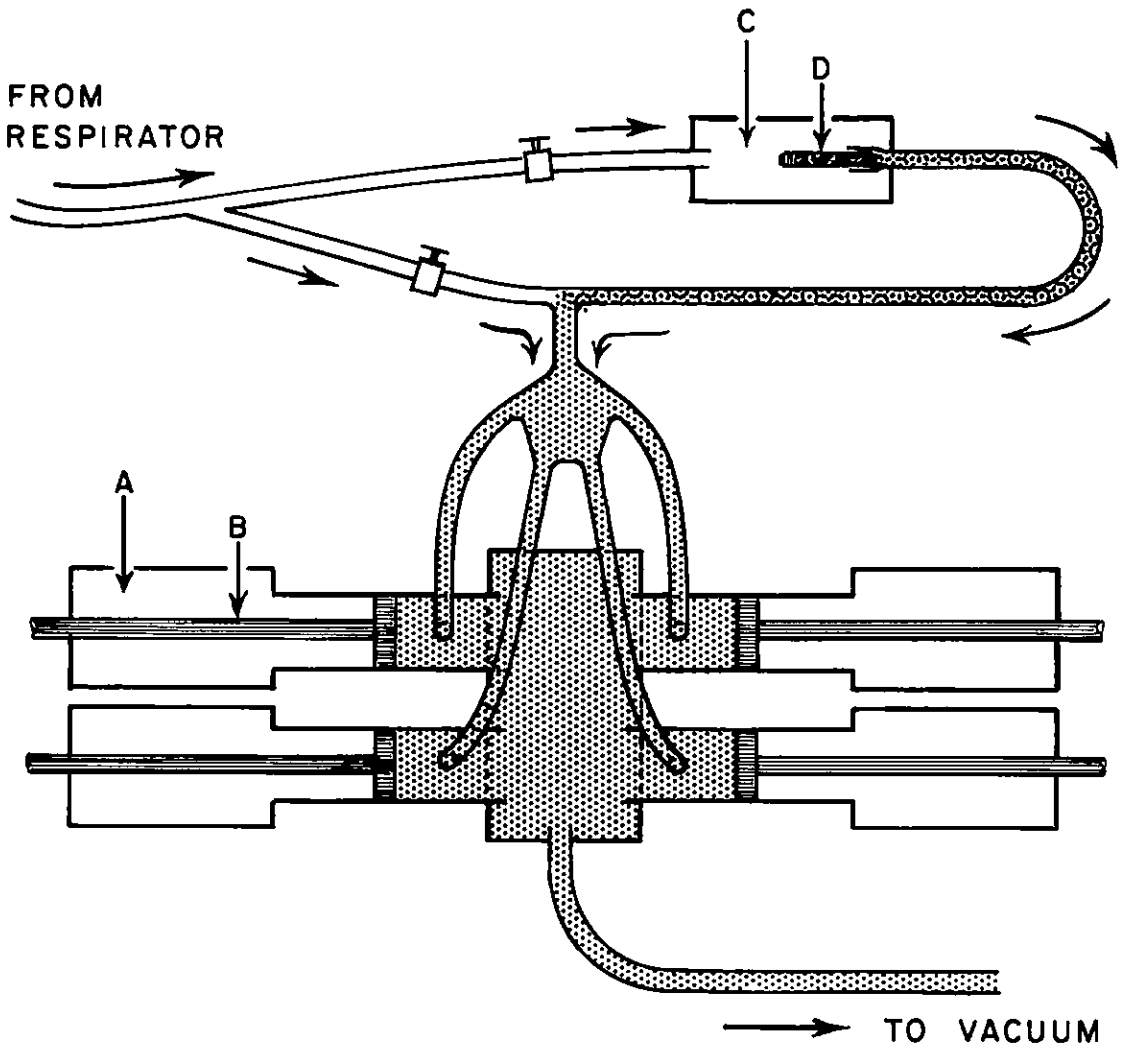
Twenty-five and fifty-five days after birth, the rats were given intramuscular injections of tetracycline to label current osteogenesis.

Thirty and sixty days after birth, the rats were sedated with

FIGURE 2

INHALATION CHAMBER

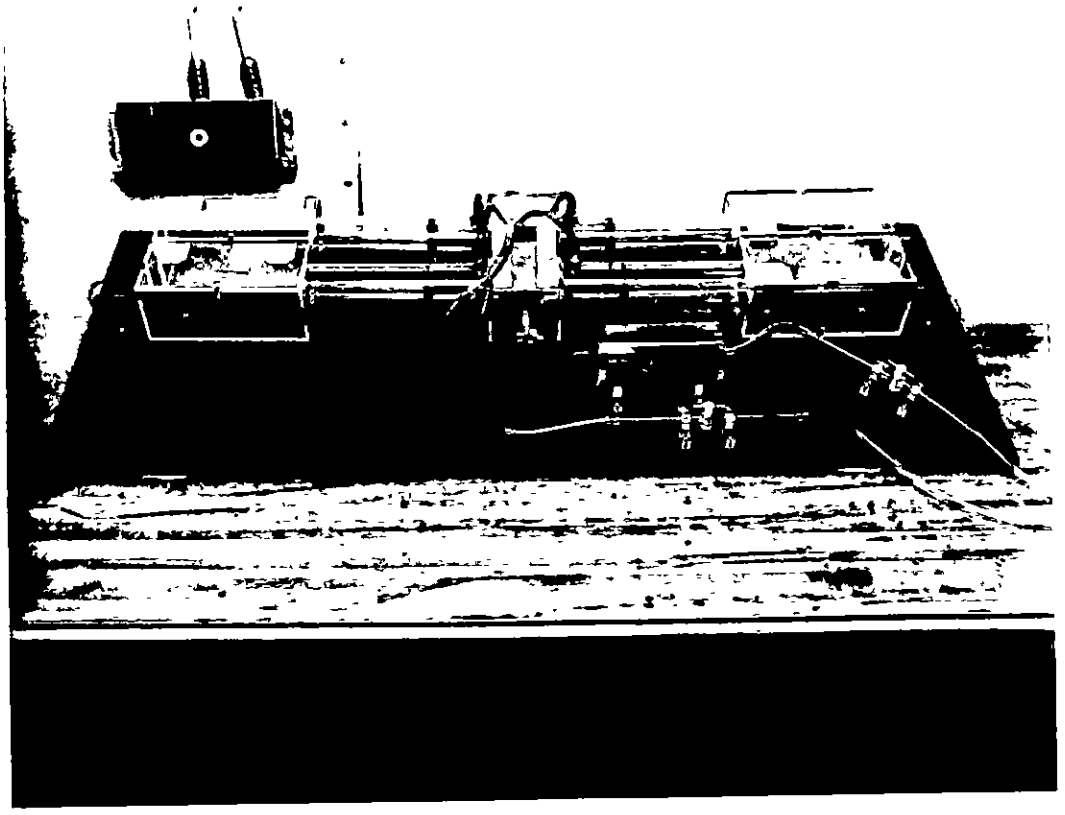
SCHEMATIC DIAGRAM OF INHALATION CHAMBER



A ENTRANCE
B PLUNGER
C COMBUSTION CHAMBER
D CIGARETTE
STOPCOCK
MIXTURE OF SMOKE AND AIR
DIRECTION OF AIR FLOW

PLATE I

INHALATION CHAMBER



pentobarbital, weighed to the nearest tenth of a gram on an analytical balance (700, Ohaus Scale Corporation, Florham Park, New Jersey) and then radiographed.

Cephalometric Technique

Cephalograms. A specially constructed cephalostat, used by Palmer (1978), and held in a Broadbent-Bolton cephalometer (Series 1 K 550, Bolton Fund, Cleveland, Ohio) was used to immobilize the animal (Plates II and III).

The radiographs were taken on standard dental occlusal film (Kodak DF-46) at 1.5 seconds, 10 milliamperes and 90 kilovolts. Lateral radiographs were taken on all animals and dorso-ventral radiographs were taken on all control and highest dosage group animals. The central ray was perpendicular to the film and passed through the transporionic axis, and the source-to-film distance was held constant at five feet. All films were developed in an automatic processor to insure consistency of technique.

Cephalometric Landmarks. With the aid of a magnifying glass, the following points were punched directly on the radiograph, (Figure 3).

Dorso-Ventral

1. The most posterior point on the external occipital protuberance (O);
2. A point in the median plane that lies on a line tangent to the

PLATE II

RAT MOUNTED IN THE CEPHALOSTAT ON THE BROADBENT-BOLTON CEPHALOMETER SHOWING THE LATERAL PROJECTION

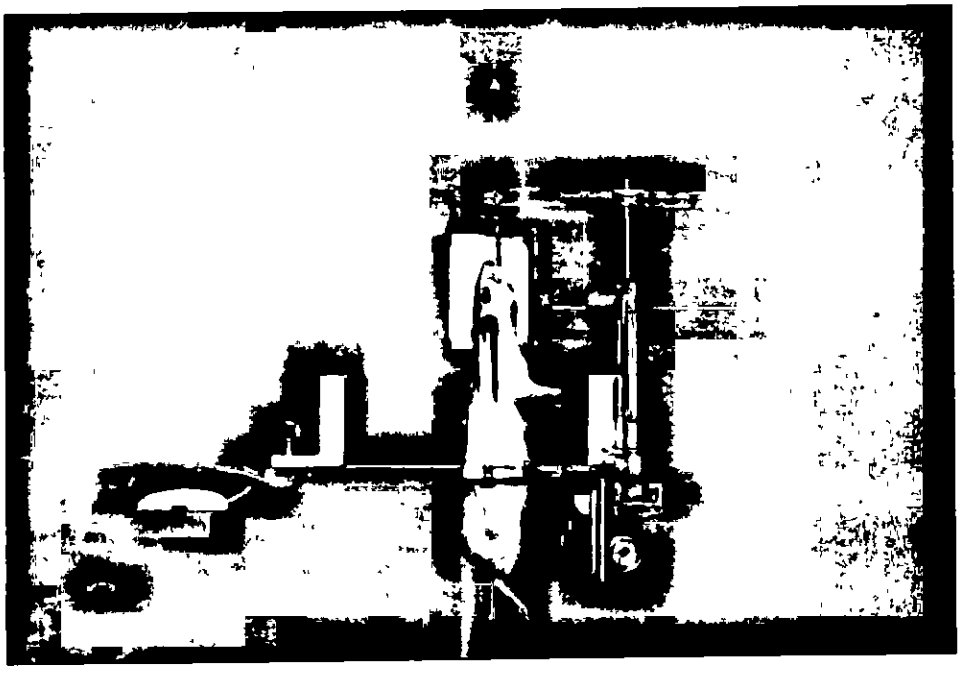


PLATE III

RAT MOUNTED IN THE CEPHALOSTAT ON THE BROADBENT-BOLTON CEPHALOMETER SHOWING THE DORSO-VENTRAL PROJECTION.

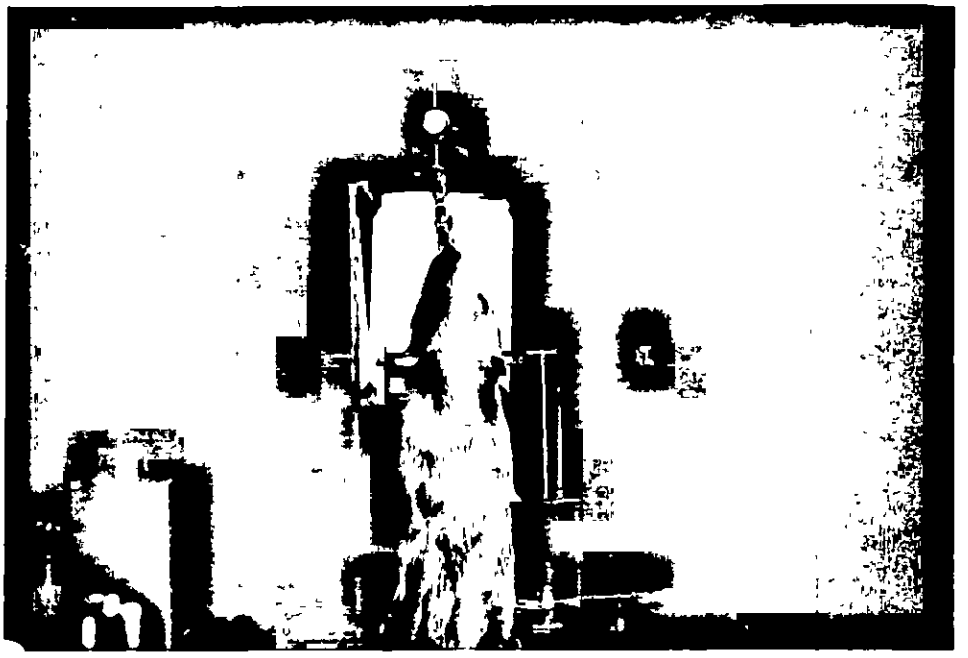


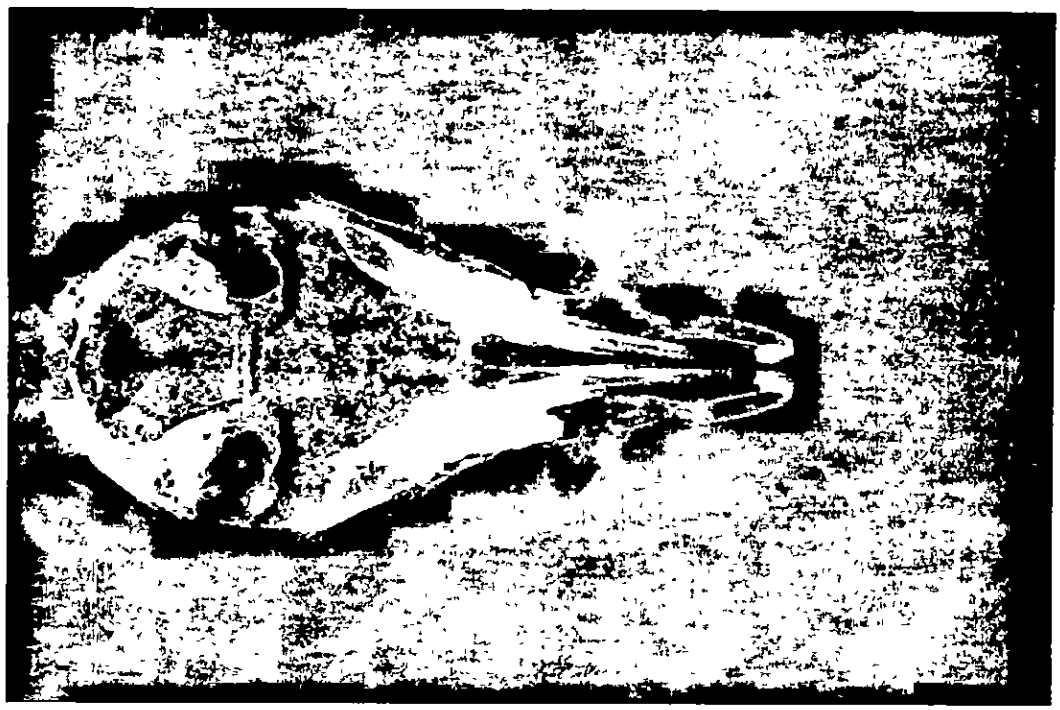
PLATE IV

CEPALOGRAMS

THE LATERAL PROJECTION (3X)

THE DORSO-VENTRAL PROJECTION (3X)





inner curvature of the maxillary incisor (I);

3. The most anterior point on the nasal bone (N);
4. The most lateral point on the left side of the skull (L);
5. The most lateral point on the right side of the skull (R).

Lateral

1. The intersection of the lingual periodontal ligament of the maxillary incisors and the superior (dorsal) plane of the bony palate (i);
2. The interface between the maxilla and the mesial surface of the maxillary first molar root (um);
3. The most anterior point on the nasal bone (n);
4. The most posterior point on the external occipital protuberance (o).
5. The most inferior (ventral) point on the image of the jugum limitans fossa olfactoria (jl);
6. A point similar to Bregma and located at the most superior point on the skull (b).

Cephalometric Analysis. Six direct linear measurements were performed on the radiographs to characterized any dimensional differences among control and treatment groups (Figure 4). The measures used are listed in Table III.

Measurements were taken to the nearest hundredth of a millimeter with a dial vernier calipers (Helios Calipers, DCA Corpora-

FIGURE 3

CEPHALOMETRIC LANDMARKS

LATERAL PROJECTION

DORSO-VENTRAL PROJECTION

NOTE: for legend see pages 25 and 32

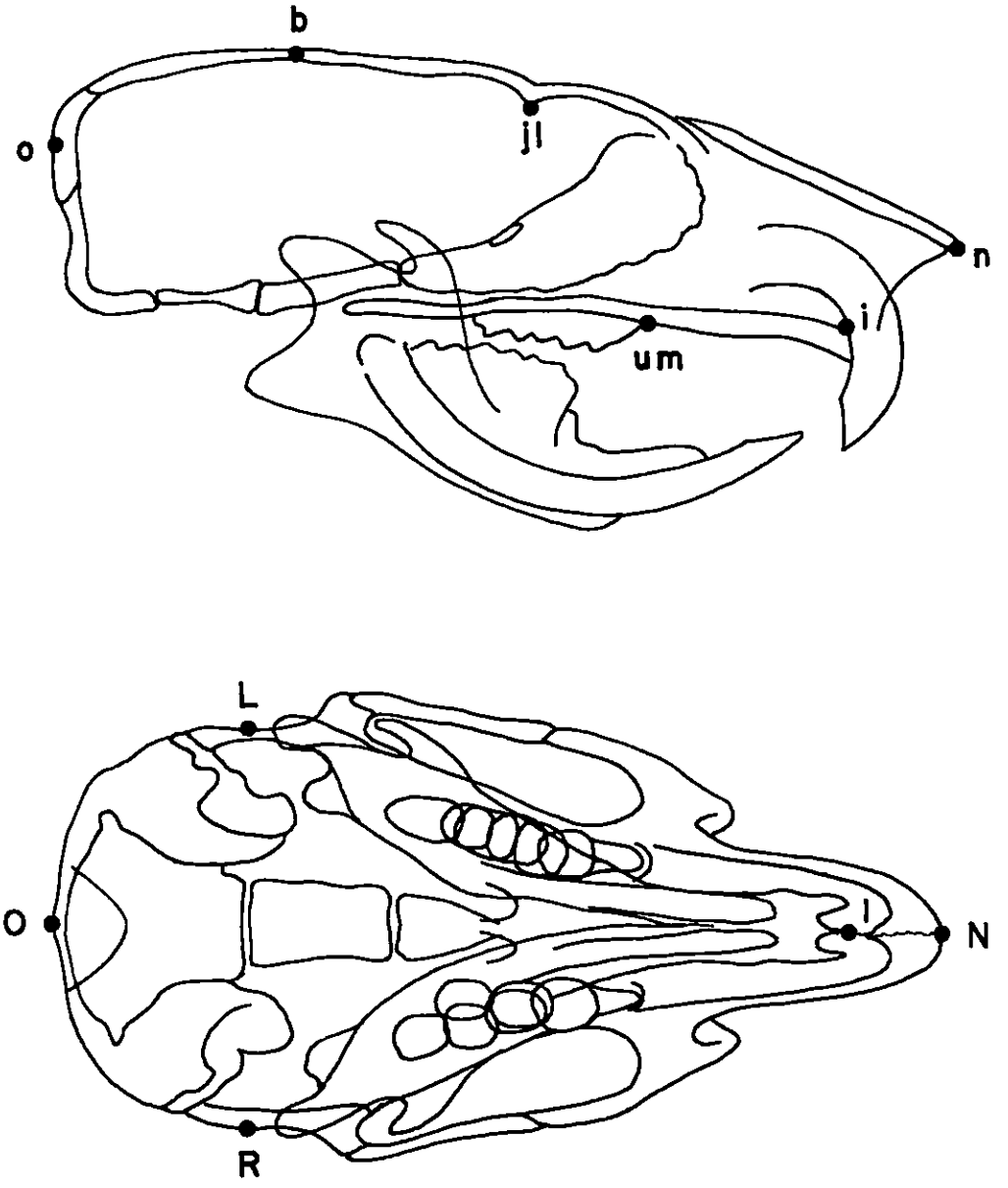

b
o
jl
n
i
um
L
O
l
N
R

FIGURE 4

CEPHALOMETRIC MEASUREMENTS

LATERAL PROJECTION: ORIENTATION ON um-i PLANE

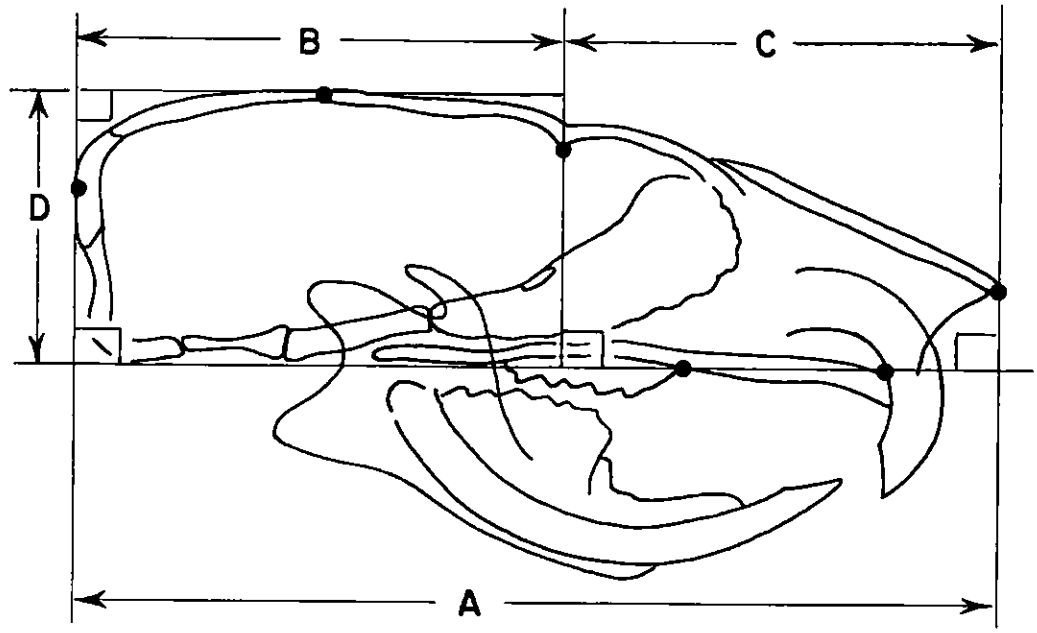
Direct Measures

A Total Skull Length (o-n)
B Neurocranium Length (o-jl)
C Snout Length (jl-n)
D Neurocranium Height (um-i plane-b)

DORSO-VENTRAL PROJECTION: ORIENTATION ON O-N PLANE

Direct Measures

E Neurocranium Width (L-R)
F Total Skull Length (O-N)


B
C
D
A

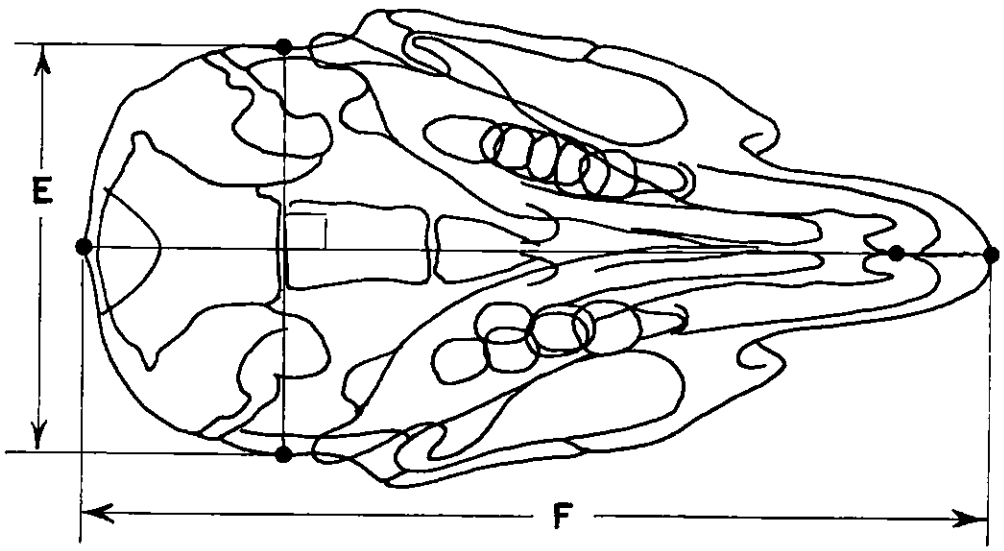

E
F

TABLE III

CEPHALOMETRIC ANALYSIS

LATERAL VIEW

Anatomical Dimensions - Direct Measures

Lateral Orientation -- (um-i) Plane

Measure		Landmarks
A	Total Skull Length	o-n
B	Neurocranium Length	o-jl
C	Snout Length	jl-n
D	Neurocranium Height	(um-i) plane - b

DORSO-VENTRAL VIEW

Anatomical Dimensions - Direct Measures

Dorso-Ventral Orientation -- (O-N) Plane

Measure		Landmarks
E	Neurocranium Width	L-R
F	Total Skull Length	O-N

tion, Washington, D.C.), and calculations were performed on an electronic calculator (Model F 4902, Commodore, Palo Alto, California).

Data Reduction. Treatment effects were examined by an analysis of variance and a multiple range test consisting of a Scheffe procedure.

Error Study. Previous studies utilizing the same cephalometric apparatus and technique used in the present study have been conducted (Palmer, 1978; Pooler, 1979). These studies have verified the high degree of reproducibility and reliability associated with the cephalometric radiographs and measurements obtained by the methods used in the present experiment.

RESULTS

General Appearance, Physical Condition, and Body Weight

Examination of the experimental animals revealed no gross deformities in the shape of the head and snout. The incisors were in proper occlusion, and coat, eyes, and color appeared normal.

The means and standard deviations for body weights of the five groups are shown in Tables IV and V for thirty and sixty days, respectively. The analysis of variance presented in Table VIII revealed significant differences at thirty days for all dosage groups ($p < .05$). The group 1 animals that received the lowest dose were significantly heavier than control animals, and group 2, 3, and 4 animals that received higher doses weighed significantly less than control animals. At sixty days, the differences were not shown to be significant for any of the dosage groups ($p < .05$).

Cephalometric Measurements

Descriptive Statistics. Means and standard deviations for the six neurocranial and maxillofacial dimensions are presented in Tables VI and VIII for thirty and sixty days, respectively. For all dimensions, at thirty days, the group 1 animals that received the lowest dose had larger measurements than control animals, and group

2, 3, and 4 animals that received higher doses had measurements smaller than control animals. At sixty days, these relationships were not apparent for any of the dosage groups.

Inferential Statistics. The results of the analysis of variance are presented in Table IX. Measurements A, C, and F showed differences that were significant for all dosage levels at thirty days ($p < .05$). The group 1 animals that received the lowest dose had significantly larger measurements than control animals, and group 2, 3, and 4 animals that received higher doses had significantly smaller measurements (figure 5, 6, and 7).

The length of the neurocranium (measure B), the height of the neurocranium (measure D), and the width of the neurocranium (measure E) could not be shown to be significantly different for any of the dosage levels at thirty days ($p < .05$). At sixty days these findings were not apparent and in all areas of measurement the differences could not be shown to be significant for any of the dosage levels ($p < .05$).

TABLE IV

BODY WEIGHT OF 30-DAY POSTNATAL RATS

	Animal Group				
	Control	1	2	3	4
mean (gms.)	108.6	117.4	94.7	103.1	101.0
S.D.	14.13	12.25	10.62	12.47	13.64

TABLE V

BODY WEIGHT OF 60-DAY POSTNATAL RATS

	Animal Group				
	Control	1	2	3	4
mean (gms.)	254.5	251.3	250.3	254.4	253.6
S.D.	39.11	51.55	37.69	50.16	40.19

TABLE VI

MEANS AND STANDARD DEVIATIONS 30-DAYS

(in millimeters)

Measure	Animal Group				
	Control	1	2	3	4
A	38.76 (1.00)*	38.95 (0.92)	37.94 (0.83)	38.51 (0.88)	37.97 (1.06)
B	20.22 (0.62)	20.18 (0.80)	20.02 (0.49)	20.18 (0.61)	19.86 (0.63)
C	18.53 (0.70)	18.77 (0.40)	17.92 (0.76)	18.33 (0.59)	18.10 (0.77)
D	11.54 (0.66)	12.29 (0.38)	11.49 (0.50)	11.75 (0.51)	11.80 (0.45)
E	16.80 (0.38)	---** ---	--- ---	--- ---	16.45 (0.33)
F	37.92 (0.83)	--- ---	--- ---	--- ---	37.46 (1.20)

* Numbers in parentheses are standard deviations.

** Dorso-ventral radiographs were not taken on animals in groups 1, 2, and 3, therefore data was not available.

Measure	Definition
A	Total Skull Length-Lateral
B	Neurocranium Length
C	Snout Length
D	Neurocranium Height
E	Neurocranium Width
F	Total Skull Length-Dorso-Ventral

TABLE VII

MEANS AND STANDARD DEVIATIONS 60-DAYS

(in millimeters)

Measure	Animal Group				
	Control	1	2	3	4
A	44.37 (0.85)*	44.40 (1.01)	44.33 (0.11)	44.42 (1.42)	44.35 (0.16)
B	21.54 (0.48)	21.55 (5.35)	21.46 (0.48)	21.53 (0.71)	21.50 (0.92)
C	22.84 (0.64)	22.90 (6.36)	22.87 (0.73)	22.84 (0.84)	22.79 (0.82)
D	12.72 (0.70)	12.77 (0.49)	12.71 (0.57)	12.74 (0.54)	12.67 (0.50)
E	17.05 (0.45)	---** ---	--- ---	--- ---	16.99 (0.29)
F	43.90 (0.97)	--- ---	--- ---	--- ---	43.82 (1.13)

* Numbers in parentheses are standard deviations.

** Dorso-ventral radiographs were not taken on animals in groups 1, 2, and 3, therefore data was not available.

Measure	Definition
A	Total Skull Length-Lateral
B	Neurocranium Length
C	Snout Length
D	Neurocranium Height
E	Neurocranium Width
F	Total Skull Length-Dorso-Ventral

TABLE VIII

STATISTICAL ANALYSIS: BODY WEIGHT

Analysis of Variance

30 days			60 days		
Sum of Squares	Standard Error	F	Sum of Squares	Standard Error	F
11154.75	1.6219	.006*	14415.81	1.8321	.318

* Significant at the 95% level, $F_{(.95)} = 0.050$.

TABLE IX

STATISTICAL ANALYSIS: CEPHALOMETRIC MEASUREMENTS

	Analysis of Variance					
	30 days			60 days		
Measure	Sum of Squares	Standard Error	F	Sum of Squares	Standard Error	F
A	39.66	.1197	.001*	21.32	.0993	.213
B	3.85	.0993	.311	4.65	.0886	.323
C	19.14	.0844	.003*	34.09	.0656	.201
D	15.58	.0693	.800	26.31	.0811	.309
E	4.30	.0360	.202	17.15	.0436	.406
F	7.89	.0956	.002*	9.82	.0969	.331

* Significant at the 95% level, $F_{(.95)} = 0.050$.

Measure	Definition
A	Total Skull Length-Lateral
B	Neurocranium Length
C	Snout Length
D	Neurocranium Height
E	Neurocranium Width
F	Total Skull Length-Dorso-Ventral

FIGURE 5

30 DAY RESULTS :

TOTAL SKULL LENGTH – LATERAL

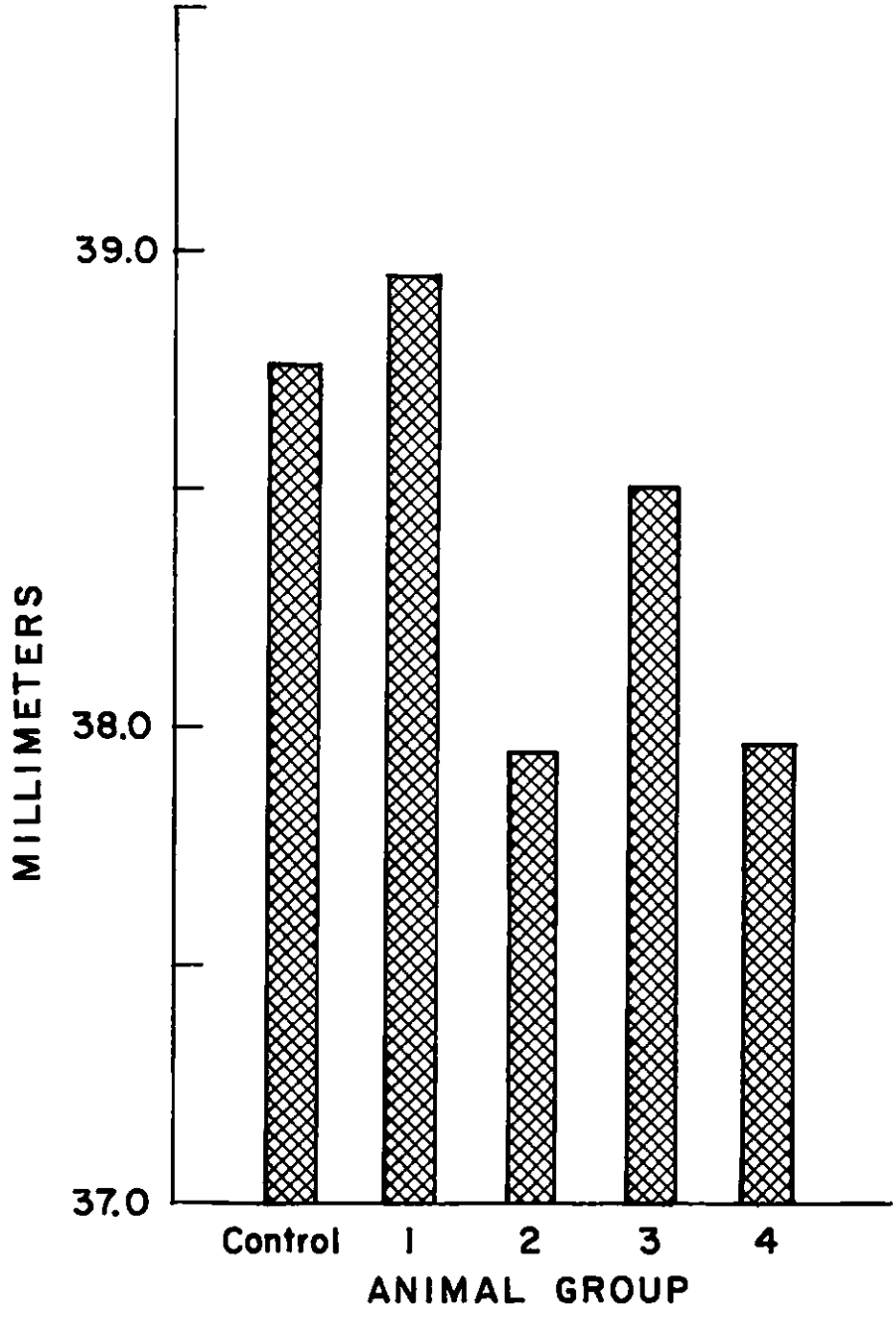


FIGURE 6

30 DAY RESULTS :
SNOUT LENGTH

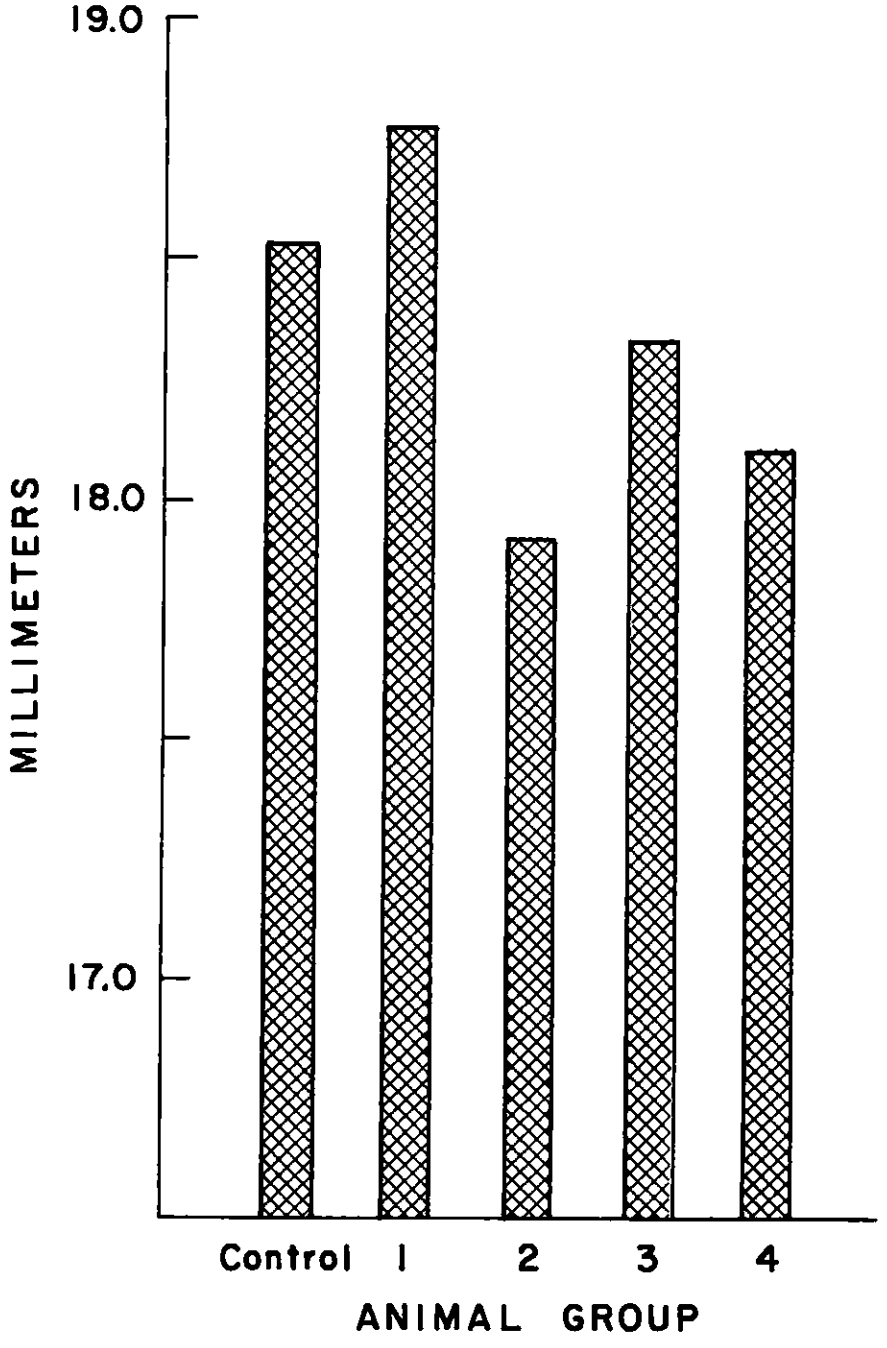
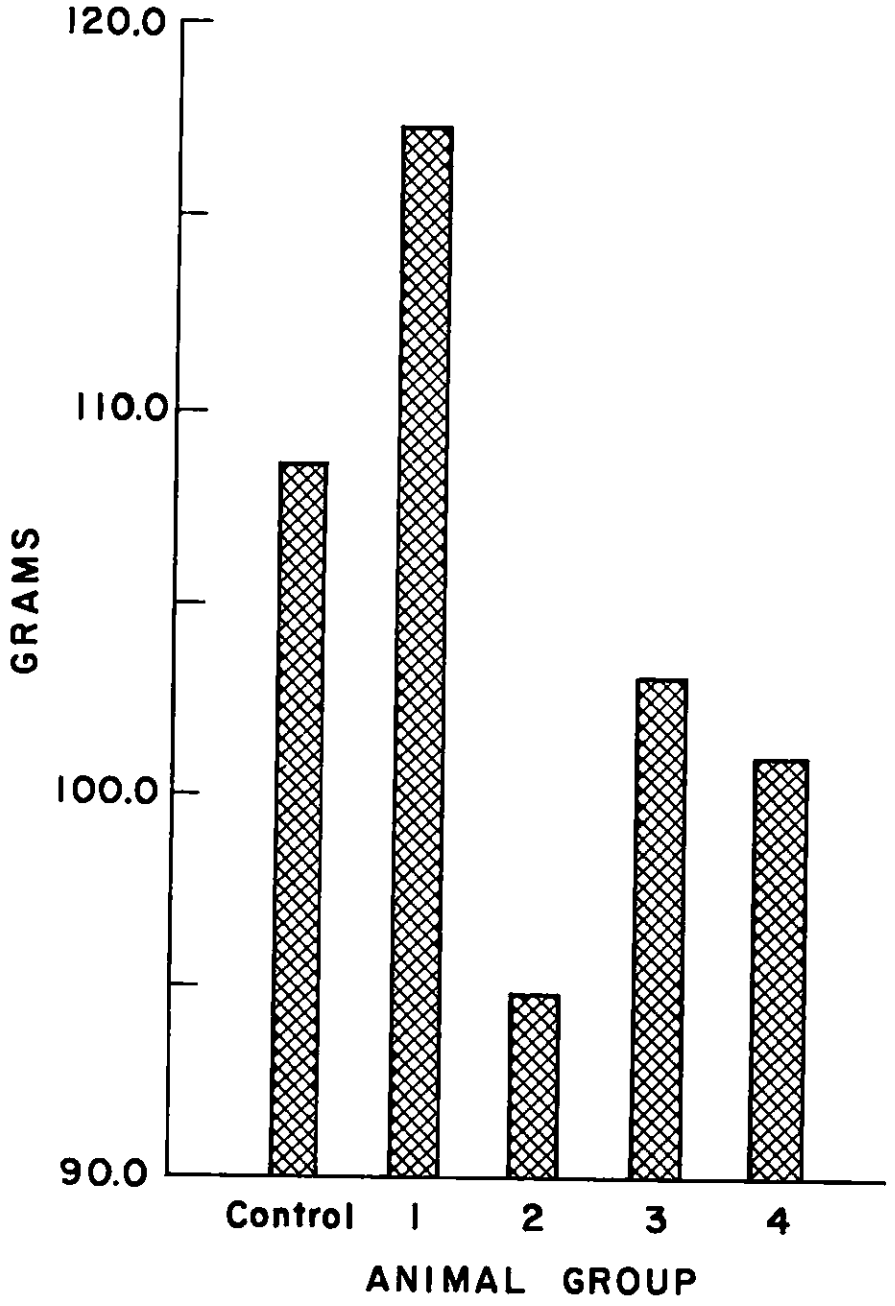


FIGURE 7

30 DAY RESULTS:
BODY WEIGHT



DISCUSSION

The purpose of this investigation was to determine the effect of inhaled marihuana smoke by pregnant rats on the neurocranial and maxillofacial growth and development of their offspring. To determine the significance of treatment effects between control and experimental groups, an analysis of variance was used.

At 30 days, the analysis of variance showed all dosage groups to be significantly different from the control group in the measured areas of weight, snout length and overall skull length as influenced by snout length ($p < .05$). At 30 days, in the measured areas of neurocranium length, width, and height, no significant differences were found between any dosage groups and the control group ($p < .05$). At the 30 day period, however, a definite dose-effect relationship was apparent. When compared to control animals, the lower-dose group of animals (0.1 mg/kg) appeared to have a growth stimulating response, as evidenced by their increased weight and cranial measurements. When compared to control animals, the group 2, 3, and 4 animals (0.2, 0.4, and 1.0 mg/kg), in contrast, appeared to have a growth retarding response to these higher doses of marihuana, as shown by their decreased weight and cranial measurements. It is interesting that in all categories the same pattern of results (with growth stimulation at a low dose and growth retardation at higher doses) was seen at thirty days, even in the measurements determined not significantly different.

At 60 days, there appeared to be no significant differences in body weight and neurocranial and maxillofacial measurements be-

tween control animals and all 4 dosage levels in the experimental animals ($p < .05$).

Marihuana Effects

The results of the present investigation show that with the marihuana exposure the offspring exhibited effects, although the measurements employed suggest that the effects are mild and transitory. The fact that the thirty day data show a stimulating growth response at a low dose and a retarding growth response at higher doses may be due to a number of factors.

The daily exposure to marihuana was *in utero* and therefore subject to the chronic metabolism of the drug by the mother. It is known that Δ^9-THC and metabolites are stored in the body, and with repeated use of the drug, accumulate over time. It is possible that the fetal effects occur only after a threshold level is reached in the mother's body. This effect on the fetus may be active during the gestation period when marihuana exposure is ongoing and also during the weaning period after birth due to the latent release of THC and metabolites through the milk. It is conceivable that at the low dose used in this experiment a significant body buildup in the mother was not achieved, but did cause an increase in food consumption resulting in offspring that weighed more at thirty days. It is also possible that while under the effects of a low dose of marihuana certain growth processes were temporarily accelerated leading to an increased body weight and

increased snout growth. These effects may have been either so small as to have been eliminated by normal growth, or directly linked to the presence of marihuana and reversed by eliminating exposure.

The higher doses and the growth retarding effect seen may be due to definite maternal thresholds being reached and constantly being delivered to the developing fetus during gestation and also through milk during the suckling period. These higher levels may have caused the retarding growth response seen in groups 2, 3, and 4 at thirty days, but may be reversible with continued growth in a non-marihuana environment after weaning.

The eating and breathing habits of the offspring may also have been affected by residual maternal milk levels of THC during weaning. An increase in appetite at low levels of THC may account for higher body weights in these animals, and an increase in respiration rate could lead to greater snout length. These occurrences might show transitory effects at thirty days. Conversely, high doses of THC through milk may lead to decreases in appetite or respirations and a lower body weight with smaller snout size, again reversible upon removal of the THC in the milk.

It is known that human babies born to tobacco smoking mothers are smaller and have lower birth weights associated with some form of fetal malnutrition (Crosby, et al., 1977; Andrews and McGarry, 1972). Some areas implicated in fetal malnutrition are impairment

of maternal protein metabolism, and lower levels of maternal blood amino acids, vitamins B_{12} and C, glucose, and fatty acids. The fetus may also be deprived of oxygenation due to the higher levels of carboxyhemoglobin and nicotine in the smoking mother.

Further experiments and more discriminating procedures will now be necessary to determine if the responses seen are due to the effect of smoke in general, or specifically to the effect of smoke containing marihuana. The procedures used in this experiment compare control animals, whose mothers were exposed to a smoke-free environment throughout gestation, to experimental animals, whose mothers were exposed to various levels of marihuana smoke during gestation. Testing with either tobacco smoke or marihuana smoke devoid of active ingredients may point out the effect of different combustions on the offspring when exposed in utero.

Prenatal growth and development occurs relatively rapidly and is of short duration in the rat. It is possible that biologically significant concentrations were not maintained at critical periods in development for effects to be seen. In addition, the size of the rat head is relatively small and changes in growth and development may not be easily observed. With a larger animal more subtle changes in the cranial area may be more readily observed.

Comparison of Marihuana Dose in Rats and Humans

The pregnant rats in this experiment were exposed to marihuana smoke of varying concentrations daily throughout gestation. The smoke concentrations ranged from 0.1 mg/kg to 1.0 mg/kg. To receive a similar exposure to the 0.1 mg/kg level, a 150 pound human would need to be exposed to 6.8 mg of marihuana (1% Δ^9-THC) daily throughout pregnancy. Assuming the marihuana cigarette contained about the same amount of combustible material as a tobacco cigarette, this would represent approximately 80% of the cigarette each day. To receive a similar exposure to the 1.0 mg/kg level, a 150 pound human would need to be exposed to 68 mg of marihuana (1% Δ^9-THC) daily throughout pregnancy. Assuming weight, size, and density similar to a tobacco cigarette, this would be approximately 8 cigarettes each day.

SUMMARY AND CONCLUSIONS

A very important question is the possible effect of marihuana use during pregnancy and on offspring exposed *in utero*. To date, no specific studies have been conducted to characterize the nature of the neurocranial and maxillofacial growth and development in response to marihuana exposure.

The present study was performed to evaluate the effect of marihuana on the neurocranial and maxillofacial growth of 150 rats whose dams had been exposed to marihuana smoke for intermittent periods throughout gestation; 119 rats served as controls.

Data were collected in two categories: (1) general appearance, physical condition, and body weight of the newborn rats; and (2) roentgenographic cephalometric measurements of the neurocranial and maxillofacial areas of the offspring exposed *in utero*.

1. In general, the experimental animals did not reveal any gross distortion or malformation due to marihuana at any of the dosage levels.
2. Analysis at thirty days revealed an increase in snout length and body weight for animals exposed to the lowest dose containing 0.1 mg/kg marihuana smoke, and a decrease in snout length and body weight for animals exposed to higher doses of 0.2, 0.4, and 1.0 mg/kg marihuana smoke.
3. Analysis at sixty days after birth, however, revealed no significant differences in neurocranial and maxillofacial dimensions

or body weight between control and marihuana-exposed animals at any of the dosage levels.

Further studies are indicated to investigate possible effects due to any type of smoke during pregnancy, and also possible physiologic effects responsible for the effects noted in this investigation.

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