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Facial alterations associated with alcohol, marijuana and cocaine in 80 children assessed by photoanalysis and landmark digitization

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Facial Alterations Associated with Alcohol, Marijuana and Cocaine in 80 Children Assessed by Photo Analysis and Landmark Digitization

by

Susan Jean Astley

A dissertation submitted in partial fulfillment
of the requirements for the degree of

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Approved by _____
(Chairperson of Supervisory Committee)

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Doctoral Dissertation

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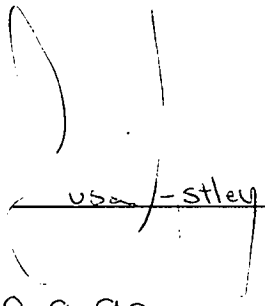
Signature 

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Abstract

Facial Alterations Associated with Alcohol, Marijuana and Cocaine in 80 Children Assessed by Photo Analysis and Landmark Digitization

by Susan Jean Astley

Chairperson of the Supervisory Committee: Professor Janet R. Daling
Department of Epidemiology

A few reports have suggested that marijuana exposure during gestation may produce a recognizable facial appearance that is similar to the face of fetal alcohol syndrome (FAS). A previous study has demonstrated that the face of FAS can be accurately recognized by dysmorphologists through systematic evaluation of facial photographs and that facial phenotype could be described through a computer analysis of facial landmarks. This method is now applied to the analysis of facial form relative to fetal marijuana exposure with additional assessment relevant to fetal alcohol and cocaine exposures.

A photo set of 80 children, aged five to seven years, was compiled. The individuals were identified from a population of 1100 mother/child pairs that had participated in a prospective study investigating the role of maternal diet, drinking and other drug use during lactation on infant growth and development. The mothers had completed a questionnaire during pregnancy and a personal interview at one month postpartum which detailed, among other things, alcohol, marijuana and cocaine use just before and/or during pregnancy. Forty of the children had mothers who reported marijuana use at least once per week during the first trimester. Forty children with no gestational exposure to marijuana were group-matched to the exposed group on maternal use of alcohol in the month prior to and during pregnancy and infant sex, race and age.

A dysmorphologist, (S.K.C.) analyzed the photos descriptively and by direct measurements. The photos were then marked for computerized digitization and analyzed morphometrically.

Neither the dysmorphic assessments nor the computer analysis could distinguish facial anomalies related to marijuana exposure. Both the dysmorphologist and the computer were able to identify children exposed to two to four ounces of alcohol per day (n = 12) as having facial features of FAS. Cocaine exposure was found to be independently associated with mild facial dysmorphic features of hypertelorism and midfacial flattening. Of 1073 mother/infant pairs that had participated in the original prospective study, the proportion of female offspring appeared to decrease with increasing maternal consumption of alcohol, cocaine and marijuana early in gestation.

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almost ten years ago. Your endless stories, your endless jokes (some good, some not so good) and Tuesday morning coffee are what really got me through this project.
The skies the limit when someone puts their trust in you. Thanks Sterling for leaving all the doors open for me.

Introduction

It has been established beyond reasonable doubt that alcohol is a teratogen in the human. Fetal exposure can result in growth deficiency, neurobehavioral dysfunction and a characteristic pattern of facial anomalies which, when observed together, are clinically referred to as Fetal Alcohol Syndrome (FAS). Children with FAS are usually the offspring of chronically alcoholic women. Not all chronically exposed children, however, are born with FAS. Some present with partial manifestations of FAS and others appear unaffected. The variation in effect is undoubtedly associated with variations in the timing, frequency and level of exposure as well as genetic susceptibility.

The impact of FAS on the individual and on society is considerable. For the child, FAS is a lifelong disability. For society, the cost of caring for these children through adulthood is substantial. In 1984, there were an estimated 7024 children born with FAS within the United States alone (Abel and Sokol, 1987). The cost for their care was estimated at 321 million dollars per year. The world-wide incidence of FAS is estimated at 1.9 per 1000 live births with levels varying substantially across different segments of the population (Abel and Sokol, 1987). FAS represents only the most severe end of a continuous spectrum of adverse effects. The number of children exhibiting the less severe manifestations of FAS is estimated to be ten times greater than the number with FAS (NCALI, 1985).

Although cautions and speculation about the dangers of alcohol use during pregnancy date back to the days of Greek and Roman mythology, many centuries elapsed before these speculations were scientifically verified. A few studies documenting the impact of alcohol exposure on fetal development were published in foreign journals in the late 50's and 60's (Heuyer et al., 1957; Uhlig, 1957; Lemoine et al., 1968), but went essentially unnoticed. In 1973, a review of eight children of alcoholic mothers with similar patterns of growth deficiency and facial anomalies was published in a broadly circulated English journal (Jones et al., 1973). The syndrome was coined FAS, the investigators were Smith and Jones, and the result was an onslaught of studies in both humans and animals to document the teratogenic impact of alcohol on man. Considerable progress has been made in our understanding of FAS over the past 17

years, but the exact mechanisms underlying the syndrome and effective methods of prevention remain unknown.

Although the adverse neurobehavioral effects resulting from fetal alcohol exposure are, by far, the most devastating aspect of FAS, the primary focus of this report is on the facial anomalies that characterize FAS and the specificity of these anomalies to alcohol.

The face of FAS is distinguished by the following cluster of anomalies:

1. short palpebral fissures (eyelid openings) relative to the intercanthal distance
2. an absent or diminished philtrum (vertical groove between the nose and upper lip)
3. a long, thin upper lip
4. a short nose relative to the midface height, and
5. a flattened maxillary (midface) region.

Although numerous clinical, epidemiologic and experimental studies have confirmed the association between this cluster of anomalies and fetal exposure to alcohol (Jones et al., 1973; Hanson et al., 1978; Streissguth et al., 1981; Clarren et al., 1987b; Sulik, 1984), recent reports have suggested that fetal marijuana exposure may also be associated with these features. The first report of this association arose from a large prospective study of 1,690 mother/infant pairs investigating the effects of maternal drinking and marijuana use on fetal growth and development (Hingson et al., 1982). Hingson reported that women who smoked marijuana during pregnancy were five times more likely than nonusers (2.0-12.7) to deliver a child with facial features resembling FAS. In contrast, the relative risk for women who averaged two or more drinks daily compared with nondrinkers was 0.6 (0.1 to 3.1) and not significant. In 1985, the association was noted again in a clinical report of five mothers who admitted to smoking marijuana daily during pregnancy while categorically denying use of alcohol and other psychoactive substances (Qazi et al., 1985). The facial anomalies observed among the children were remarkably similar to those seen in FAS. Finally, in 1987, reference was made once again to this association in a clinical assessment of alcohol related facial effects in 21 seven-year-old children, exposed gestationally to heavy quantities of alcohol and 21 controls, group-matched on age, sex and prenatal exposure

to cigarettes and marijuana. Six clinicians, all acknowledged experts in identifying children with FAS, were asked to qualitatively examine facial photographs of these children. Definite diagnoses of fetal alcohol syndrome (FAS) were only made in children with very high ethanol exposure. Most, but not all, of the children who were rated as "possible" FAS had, in fact, been exposed to alcohol. Two of the children classified as "possible" FAS were controls. Neither of these children were exposed to gestational alcohol; both were exposed to marijuana in-utero.

There are several factors that argue both for and against the plausibility of this association between fetal marijuana exposure and the presence of FAS-like facial anomalies. On the one hand, the studies that reported the association were limited in their ability to confirm its existence due to small sample sizes, weak diagnostic sensitivity and/or confounding by alcohol. On the other hand, our experience in field of teratology has shown us that syndromes often have overlapping features and are rarely the result of a single etiologic agent.

Identification of children with FAS is not an easy task. Aside from growth deficiency and neurobehavioral impairment, their most distinguishing feature is their unique, but subtle facial appearance. To the untrained eye, FAS could look remarkably similar to fetal hydantoin syndrome or may even be judged as being within the normal range of variability. The unique appearance of the FAS face is characterized by a specific cluster of anomalies and a reliable diagnosis often requires the expertise of a trained dysmorphologist. In the study performed by Hingson et al (1982), dysmorphic examinations of the infants were performed by one of the four study pediatricians working from anomaly checklists. The authors acknowledged that "identifying fetal alcohol features required subtle clinical distinctions" and the influence of alcohol exposure could not be ruled out. In the study by Clarren et al. (1987b), diagnostic reliability was high, but the sample size was restricted to observations made on two children with fetal marijuana exposure. The case review by Qazi et al. (1985) was also limited in size.

Although the limitations in the above studies might lead one to question the proposed association between marijuana and FAS-like facial anomalies, clinical and experimental studies have demonstrated repeatedly that syndromes often have overlapping patterns of

abnormalities and multiple etiologies. Facial development is a complex process that relies on a precisely timed sequence of events for normal development. The very complexity of facial morphogenesis makes this region particularly prone to abnormalities. Deviations in development can result from failure of some structure to be in the right place at the right time, resulting in a somewhat predictable and limited number of facial malformation patterns. The timing and frequency of teratogenic exposures clearly play critical roles in determining the type of damage that results as well as the severity. If distinct facial anomalies are the result of interruptions at very specific times in development, it is conceivable that any teratogen capable of disrupting a particular developmental stage could produce similar patterns of anomalies. As an example, a number of teratogenic influences, e.g., anoxia (Badtke et al., 1959), alkaloids derived from *Veratrum califirnicum* (Binns et al., 1968), various ions (Stockard, 1909) and heat shock (Ingalls, 1966) will produce cyclopia when administered over periods covering the time of neural plate and/or neural tube formation. The interplay between timing of exposure and morphologic outcome(s) can best be appreciated with a brief review of normal facial development.

In the human, recognizable facial structures develop primarily between the fourth and eighth weeks. The face develops from five facial primordia (the frontonasal prominence and the right and left maxillary and mandibular prominences) which appear around the stomodeum or primitive mouth (Moore, 1988) (Figure 0.1). These five prominences are active centers of growth in the underlying mesenchyme. The lower jaw, or mandible is the first part of the face to form. It results from merging of the medial ends of the two mandibular prominences during the fourth week.

By the end of the fourth week, nasal placodes have developed on the inferior part of the frontonasal prominence. The margins of these placodes thicken into what are called the medial and lateral nasal prominences. The placodes themselves form the nasal pits. The maxillary prominences enlarge and migrate medially and move the medial nasal prominences toward the medial plane and toward one another. The lateral nasal prominences join with the maxillary prominence by the end of the fifth week. During the fifth week, the auricles of the ears have started to develop.

During the sixth and seventh weeks, the medial nasal prominences merge with each other and with the maxillary prominences forming an intermaxillary segment This segment gives rise to the middle portion of the philtrum of the lip, the premaxilla, and the primary palate.

The lateral parts of the upper lip, most of the maxilla and the secondary palate form from the maxillary prominences. These prominences merge laterally with the mandibular prominences. The frontonasal prominence forms the forehead and the dorsum and apex of the nose. The sides (alae) of the nose are derived from the lateral nasal prominences. The nasal septum is formed from the medial nasal prominences. The maxillary prominences form most of the upper cheek regions and most of the upper lip. The mandibular prominences give rise to the lower lip, chin, and lower cheek regions. During the early fetal period, the nose is flat and the mandible is underdeveloped. As the brain enlarges, it creates a prominent forehead; the eyes move medially and the external ears rise.

Disruption of facial development is not restricted to this four week period. Structural alterations in facial form can also be induced in the third week of gestation when the embryo forms the primordial mesodermal cells that establish the five facial primordia. This period of embryonic development is referred to as the gastrulation stage. Some striking studies in a mouse model have demonstrated that two small doses of ethanol administered during this period of development result in craniofacial malformations that closely resemble FAS (Sulik, 1984), suggesting that this small window in time is a critical period for the development of FAS-like facial anomalies. Other teratogenic agents present during this time period could conceivably cause similar malformations. The apparent specificity of alcohol to the constellation of dysmorphic features known as FAS may simply be because alcohol is the teratogen to which humans expose themselves most frequently. The more frequent the exposure, the more likely the embryo will be exposed at a critical time in development. If exposure to other teratogens occurred with equal frequency, they too might result in similar facial alterations.

One final issue concerning the plausibility of the reported association between fetal marijuana exposure and the presence of FAS-like facial anomalies is the teratogenic

potential of marijuana. Marijuana is a mixture of dried leaves and bracts of the *Cannabis sativa.* Cannabis is the crude material from the plant and is made up of over 400 chemicals. Sixty-one of these are unique to cannabis and are collectively referred to as cannabinoids. The principal psychoactive component, delta-9-tetrahydrocannabinol (THC) is known to cross the placental barrier in both humans and animals (Blackard and Tennes, 1984; Harbison and Mantilla-Plata, 1972) and therefore has the potential for adversely affecting fetal development. Two thorough reviews of the teratogenic effects of marijuana have been published by Abel (Abel, 1980; Abel et al., 1986). A very brief summary of some of the positive findings is presented below.

Clear teratogenic responses to high doses of THC in mice have been documented by several investigators with the most frequently described lesions being cleft palate and exencephaly (Mantilla-Plata et al., 1975; Mantilla-Plata and Harbison, 1976; Harbison et al., 1977; Joneja, 1976). Fetal malformations have also been observed in rabbits exposed to high doses (250 to 500 mg/kg) of crude cannabis on days seven to ten (Geber and Schramm, 1969). The malformations included exencephaly, myelocele and head and spinal cord malformations. It is important to put these high dosages into perspective. In terms of human exposure, a single marijuana cigarette containing 2% THC would deliver slightly less than 10 milligrams of THC to the lungs, where most would probably be absorbed (Jones, 1980). THC rapidly leaves the blood and is stored for up to 30 days in fatty tissues. This slow elimination facilitates accumulation of the drug over time, but doses of 250 to 500 mg/kg are still far beyond what would be expected to accumulate in a human. There has been one study, however, reporting teratogenic effects at doses more comparable to human levels of exposure. Injections of as little as 4.2 mg/kg of crude marijuana extract in rats on days one through six resulted in increased rates of syndactyly, encephalocele, phocomelia and amelia (Persuad and Ellington, 1968). The results of experimental studies have varied tremendously depending on dose, species, timing and route of administration and cannabis preparation. Numerous experimental studies which have varied these parameters have failed to demonstrate teratogenic effects (Wright et al., 1976; Vardaris et al., 1976; Pace et al., 1971; Haley et al., 1975).

The teratogenic potential of marijuana has been documented in the human literature as well, but the evidence is fairly weak. In addition to the three studies cited above that

associate fetal marijuana exposure specifically with FAS-like facial anomalies, a few epidemiologic and clinical studies have provided suggestive evidence of associations between marijuana and congenital malformations, in general. In a hospital based study, 12,424 women were questioned shortly after delivery about their habits and exposures during pregnancy (Linn et al., 1983) and information about malformations was obtained retrospectively from medical records. A suggestive odds ratio of 1.36 (95% C.I. = .97 to 1.91) was observed for major malformations in offspring of marijuana smokers versus non-smokers. Although the relationship did not reach statistical significance (p = .09), of the ten independent variables in the analysis, marijuana use was the most highly predictive of a malformation. Further evidence of congenital anomalies in offspring of mothers who smoked marijuana during pregnancy appear in five clinical reports (Bogdanoff et al., 1972); Carakushanky et al., 1969; Geleherter, 1979; Hecht et al., 1968; Jacobsen and Berlin, 1972). In each of these reports, marijuana use was associated with the use of other psychoactive drugs (LSD, amphetamines and/or barbituates). Most observational studies, however, have been were unable to provide supportive evidence of an association between marijuana use and the presence of major or minor malformations (Fried et al., 1983; O'Connell and Fried, 1984; Gibson et al., 1983).

To evaluate the reported association between fetal marijuana exposure and FAS-like facial anomalies, facial photographs of 40 children with frequent exposure to marijuana in the first trimester of gestation were compared to facial photographs of 40 children with no gestational exposure to marijuana. These children were selected from a previous prospective study of 1100 mother/infant pairs investigating the effect(s) of maternal alcohol and drug use during pregnancy and lactation on infant development at one year of age. The two groups of 40 children in this study were matched on maternal alcohol consumption prior to and during pregnancy so that the effect of marijuana could be evaluated independent of alcohol. The photographs were evaluated clinically by a dysmorphologist and morphometrically by landmark analysis. This dual assessment has been used in a previous study of FAS facial morphology and has proven to be a reliable and powerful method of analysis. The methods and results of that study are presented in Chapter I.

Information on maternal use of cocaine during pregnancy was also available for this study population. In Chapter II the results of a pilot investigation of fetal cocaine exposure and associated patterns of facial alterations are presented. During the course of the study, it was noted that the proportion of female offspring decreased with increasing maternal use of alcohol, cocaine and marijuana. These alterations were also observed in the original study population of 1100 mother/infant pairs. These observations have been summarized in Chapter III.

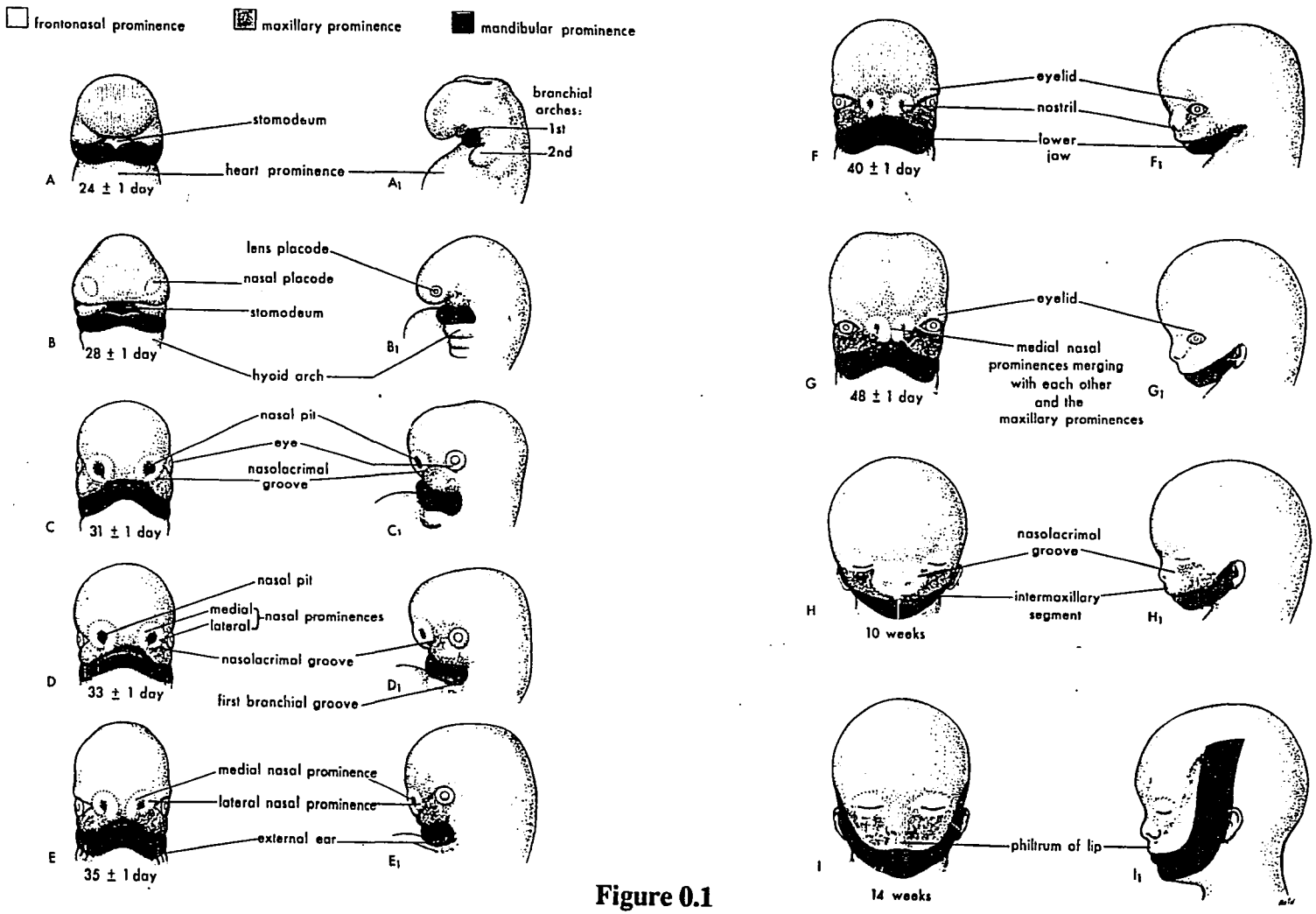


Figure 0.1

Normal development of the human face. (Moore KL, 1988)

Chapter I

Manuscript # 1

Analysis of Facial Shape in Children Gestationally Exposed to Marijuana and Alcohol.

ABSTRACT

The association between fetal marijuana and/or alcohol exposure and facial features resembling Fetal Alcohol Syndrome (FAS) was investigated in a population of 80 children. Standardized lateral and frontal facial photographs were taken of 40 children, five to seven years of age, whose mothers reported frequent use of marijuana during the first trimester of pregnancy and 40 children whose mothers reported no use of marijuana during pregnancy. The marijuana exposed and unexposed children were group-matched on preconceptional and prenatal alcohol exposure, sex, race and age at the time of assessment. The photographs were assessed clinically by a study staff dysmorphologist and morphometrically by landmark analysis. Patterns of facial anomalies, including those associated with FAS, were explored.

FAS-like facial features were not associated with prenatal marijuana exposure in this study population. No consistent patterns of facial features (unrelated to FAS) were identified among the marijuana exposed group. Maternal consumption of two or more ounces of alcohol per day in early gestation, was found to be associated with FAS-like facial features identified both clinically and morphometrically. These results were similar to findings in a previous investigation of facial effects of fetal alcohol exposure and confirmed the sensitivity of this morphometric technique to delineate facial characteristics unique to alcohol exposure.

INTRODUCTION

Delta-9-tetrahydrocannabinol (THC), the principal psychoactive component of marijuana, is known to cross the placental barrier in humans (Blackard and Tennes, 1984) and therefore has the potential for adversely affecting fetal development. Marijuana is estimated to be used by one in every ten pregnant women. A few studies have presented evidence suggesting an association between fetal marijuana exposure and facial anomalies that resemble fetal alcohol syndrome (FAS) (Hingson, et al., 1982; Qazi et al., 1985; Clarren et al., 1987b). FAS facial anomalies include a hypoplastic philtrum, thin upper lip, a short nose relative to the midface length, short palpebral fissures and a flattened maxillary region. The results of these studies suggested that features compatible with FAS may not be specific to alcohol. Small sample sizes, questionable reliability of exposure, other drug use and/or limited diagnostic sensitivity, however, have precluded drawing firm conclusions from these studies.

In this study, the association between first trimester fetal marijuana exposure and FAS-like facial anomalies was investigated in photographs of 40 children exposed frequently to marijuana during the first trimester of gestation. The exposure data were collected prospectively and the association between marijuana and alcohol exposure was effectively eliminated by group-matching. Diagnosis of facial form was assessed clinically by a dysmorphologist and morphometrically by landmark analysis. The sensitivity of the morphometric diagnostic tool has been confirmed in several studies of craniofacial malformation (Grayson et al., 1985, 1987) and has effectively delineated the FAS face in a study sample half the size of the current study population (Clarren et al., 1987b).

METHODS

Subjects

Subjects in the present study were selected from among 1100 mother/infant pairs who participated in a Seattle-based prospective study (1982-87) investigating the role of maternal diet, drinking, smoking and marijuana use during lactation on infant growth and development. There were three parts to the original prospective study. First, validity of self-reported alcohol, tobacco and other drug use and the reliability of the

maternal interview process was confirmed (Little, et al., 1984, 1986). Next, descriptive studies were conducted to contrast the dietary habits and alcohol and tobacco use of lactating and nonlactating women (Little et al., in press; Worthington-Roberts et al., 1989a; Worthington-Roberts et al., 1989b). And finally, two infant assessment studies were conducted to investigate the influence of alcohol and marijuana use during lactation on infant development at one year of age (Little, et al., 1989; Astley and Little, 1989).

The subjects' mothers in the original prospective studies were members of Group Health Cooperative of Puget Sound, a Health Maintenance Organization in Seattle, WA. All prenatal patients receiving care between May 1, 1982 and July 1, 1984 were contacted by the Cooperative in their sixth month of pregnancy regarding possible study participation. Seventy-four percent (5,298) of the prenatal patients responded positively and completed a mailed screening questionnaire detailing their alcohol and tobacco use both before and during pregnancy, some dietary information, and their plans to lactate. These patients constituted a screened pool.

A total of 1100 women were selected from the screened pool to participate in one or more of the previous prospective studies. At six weeks after delivery, a detailed personal interview was conducted in the woman's home to obtain information on diet, drinking, smoking and other drug use during pregnancy and the first month postpartum. Information on maternal demographics and reproductive history were also collected. This interview was repeated to obtain data for the third and twelfth months postpartum. Neonatal status (birth weight, gestational age, 1 and 5 minute APGAR scores, the presence or absence of congenital malformations and morbidity in the hospital) was abstracted from the infants' medical records.

Among the 1100 mothers, 61 reported using marijuana at least once a week during the first trimester of pregnancy and 933 reported no use of marijuana at any time during pregnancy. The children of these 61 mothers made up the exposed cohort in the present study. An equal number of marijuana unexposed children were selected from the group of 933 by group-matching to the exposed cohort on the following characteristics: reported maternal alcohol consumption during the month prior to conception and during pregnancy, and the infant's sex, race and birth date. Group-matching was performed by

stratifying the 933 children simultaneously by sex, age (divided into 6-month groups), race (black, nonblack) and maternal alcohol consumption (AA score) both before and during pregnancy, grouped as follows (AA < .05; .05 ≤ AA ≤ 1.9; and 2.0 ≤ AA ≤ 4.0). Matches were randomly selected from the appropriate groups with the aid of a random number chart. Letters of invitation were sent to the 61 exposed subjects regarding possible study participation. Informed consent was obtained after a full explanation of study procedures (Appendix A). When an exposed subject was successfully located and enrolled in the study, a letter of invitation was sent to the mother of a group-matched unexposed subject.

Of the 61 marijuana exposed mother/child pairs that were eligible for participation in the study, we were able to locate and enroll 40. The mothers of the 40 children that were enrolled in the study differed from the mothers of the 21 children that did not participate in that they were heavier users of marijuana, alcohol and tobacco and were less likely to have attended college. From among the 933 eligible nonexposed subjects, the mothers of 70 children were contacted in order to locate and enroll 40 group-matched subjects.

Data Collection in the Original Prospective Study

Detailed information on maternal use of alcohol, tobacco, marijuana, cocaine and other licit and illicit drugs during pregnancy was collected by personal interview at one month postpartum. Demographic characteristics and obstetric history were also collected. The interviewers were women of childbearing age, trained to obtain valid and reliable information. Maternal use of alcohol in the month prior to conception was collected in a mailed screening questionnaire completed during the sixth month of pregnancy. Use of marijuana and/or cocaine in the month prior to pregnancy was not recorded in this study. Validity of self-reported drug use was confirmed in an earlier pilot investigation of 108 randomly selected postpartum women. Self-reported drug use was compared to laboratory tests of drug levels present in body fluids. The proportion of questionable self-reports ranged from 0 to 3% depending on the drug (Little et al., 1984).

Maternal use of marijuana and cocaine was recorded in terms of how often the substance was used (days per week) and how many "joints" or "snorts" were taken per day when the substance was being used. This information was recorded for each trimester. Alcohol consumption was categorized into beer, wine and liquor and was recorded in terms of frequency of use (days per week), modal quantity, and maximum quantity per drinking occasion in the month prior to conception and during each trimester. These measures were converted to average daily ounces of absolute alcohol ingested per day (AA score) (Jessor et al., 1968).

Assessment of Facial Photographs

1. Facial Photographs

Facial form was assessed from standardized frontal and lateral facial photographs. The children were between five and seven years of age at the time of the photograph. The two year span in ages reflects the two year period in which the mothers of these children entered the previous prospective study at six months gestation. Rather than prolong data collection over a two year period to obtain photographs of all children at one age, the photographs were taken over a three month period of time and the children in the exposed and unexposed groups were simply group-matched on age. An informed consent with a full explanation of study procedures was provided for the parent or guardian and an Assent form was provided for the child. A placard with the child's study number and a 2-cm rule was included in each photograph to provide a measure of scale. The children were asked to hold a comfortable pose with their mouths closed while the photographer positioned herself to obtain frontal and lateral pictures with no detectable rotation. The photography session took approximately twenty minutes.

A set of 5x7 and 2x3 inch, black and white prints were made of each frontal and lateral view. The photographs within each set were printed to scale, within one millimeter accuracy, so that distance measures taken directly from the photographs would be comparable across all the subjects. The larger prints were used for digitization of facial landmarks and measuring distances between landmarks while the smaller prints facilitated the clinical evaluation, permitting scanning of the entire group of photographs at one time.

2. Clinical Assessment of the Photographs

The purposes of the clinical evaluation were first to evaluate the photographs for the presence of FAS-like facial features and second, to determine if any other patterns of minor anomalies were present. Facial features associated with FAS include short palpebral fissures, absent or diminished philtrum, thin upper lip, short nose relative to the midface height and flattening of the maxillary region. The clinical evaluation provided a qualitative assessment that complimented the purely quantitative approach of the computerized morphometric assessment. It also provided direction for the exploratory computerized morphometric evaluations of other facial patterns unrelated to FAS.

The photographs of all eighty children were examined and scored by the study staff dysmorphologist (S.K.C.), who has had broad experience in identifying children with FAS and other recognizable patterns of malformations. The assessments were performed without knowledge of exposure history. The measures recorded from the 5x7 photographs of each child are listed in Table I.1.

Based on the measures in Table I.1 and the dysmorphologist's overall impression of the child's face, each child was classified into one of the following five groups:

1. No unusual features
2. Unusual features, but not related to fetal alcohol syndrome
3. Possible FAS-like face
4. Probable FAS-like face
5. Definite FAS-like face

In the second phase of the clinical evaluation, the photographs were evaluated for the presence of facial patterns, unrelated to FAS.

3. Computerized Morphometric Assessment of the Photographs using Landmark Analysis

A set of 23 facial landmarks were located and marked on the 5x7 photographs of each child (Figure I.1, Table I.2). These are the same landmarks that were used in the previous study by Clarren (et al., 1987b). The relative location of the frontal landmarks 1 through 10 and the lateral landmarks 11 through 23 were entered into a database by placing each photograph on a computer digitizing tablet (MacTablet) and marking the location of each landmark with a digitizing stylus.

The analysis of facial shape, developed by Bookstein (1982, 1983, 1984, 1986), was carried out by way of triangles defined by sets of three landmarks: the mean shapes of these triangles were compared between the exposed and unexposed groups. For example, if a short midface is the facial characteristic of interest, then the triangle resulting from the three facial landmarks 1, 4, and 12 might describe that characteristic (Figure I.2). The triangle is standardized by arbitrarily selecting one edge, for example 1-4, as the baseline and assigning it a standard length of one unit on a Cartesian (x,y) coordinate system. Thus, as illustrated in Figure I.2, if landmarks 1 and 4 are assigned the Cartesian coordinates (0,0) and (1,0) respectively, the shape of the triangle is described by the third landmark (12), the midpoint of the upper vermilion border. Landmark 12 represents the x and y "shape coordinates" of the triangle. Although the shape coordinates appear to be describing a single landmark, they are, in fact, representing the shape of the triangle as a whole. The shape coordinates for the triangles of each exposed and unexposed subject are displayed in a scatter plot and the mean shape coordinates for each group are derived from the average (or centroid) of the scatter of shape coordinates within each group. A hypothetical example is presented in Figure I.3. In this example, the mean location of landmark 12' for the exposed group is closer to the baseline 1-4 than the mean location of landmark 12 for the unexposed group. Hotelling's T-square statistic would be used to determine if this observed difference in mean shape between the two groups of triangles is significant. The squared distance between the two centroids, divided by the square of their pooled standard error in that direction is distributed as Hotelling's T^2 on two degrees of freedom for the numerator. This test statistic is nearly invariant to the choice of

baseline as long as the variation in landmark locations is small relative to the distances between the baseline landmarks (Sampson et al., 1986, Bookstein, 1986).

Upon identifying which triangle(s) are significantly different between groups, the next step in the analysis is to describe how the triangles are different. Any shape change between two triangles has a direction of greatest change and a direction of least change. These directions are at 90^{o} to one another and lie in some orientation upon the triangle. To determine the magnitude and direction of shape change in the mean triangle(s), a tensor analysis is performed. Tensor analysis of landmark data has been described in detail in several publications by Bookstein (1982, 1983, 1984a). A brief description of the analysis is presented below, continuing with the hypothetical example of triangle 1-4-12 presented in Figures I.1 - I.3. A linear (or, homogeneous) deformation transforms triangle 1-4-12 into another (1'4'12'), and transforms a circle inscribed in the first triangle into an ellipse in the second (Figure I.4). In this example, we will say that triangle 1-4-12 represents the mean "normal" triangle with baseline 1-4 and mean shape coordinate 12 derived from a hypothetical group of unexposed subjects and triangle 1'4'12' represents the mean "deformed" triangle with mean shape coordinate 12' derived from a group of exposed subjects. The principal axes (or tensors) of the ellipse, rescaled and oriented homologously in triangle 1-4-12, lie along the directions in which that triangle is most stretched and most compressed by the transformation. The axes of the ellipse are the directions of greatest and least ratio of change of size. The axes in triangle 1'4'12' (referred to as the principal directions) coincide with the maximum and minimum diameters of the ellipse. To orient the axes homologously in triangle 1-4-12, the axes must bisect the sides of that triangle in a manner that is proportionally equivalent to the bisection of the sides of triangle 1'4'12' by the principal axes of the ellipse. The greatest magnitude of stretching and compression that took place in the transformation lie along the directions of the principal axes and is referred to as the principal dilatations. A dilatation is a ratio of lengths: any length in a deformed triangle divided by the corresponding length in a normal triangle. A dilatation is a measure of shape change. The magnitude of the maximum dilatation (or expansion) is the ratio of the length of the longest diameter of the ellipse divided by the corresponding length in the "normal" triangle which is the diameter of the circle. The magnitude of the minimum dilatation (or contraction) is the ratio of the smallest diameter of the ellipse divided by the diameter of the circle. For example, in Figure I.4,

the "deformed" triangle 1'4'12' representing the mean shape of the exposed group resulted from a 10% horizontal expansion and a 20% vertical contraction of the "normal" triangle 1-4-12 representing the mean shape of the unexposed group.

Tensor analyses performed on individual triangles are limited to the extent that the change in shape only applies to the area within the triangle and the actual description of shape change in the triangle can only be expressed as a displacement of a mean shape coordinate from a designated baseline, which may or may not have biological relevance. A tensor analysis on a single triangle is also restricted in the sense that the deformation of one triangle into another is described simply as a linear transformation of one set of three mean landmark locations into another set of three mean landmark locations. In other words, if the "true" deformation of the face within the area of a triangle is best described by a curvilinear change in shape, the tensor analysis on the single triangle can only describe the linear component of that shape change. In essence, a description of shape change in a single triangle provides information for just one piece of the puzzle that makes up the face. Tensor analyses can, however, be performed on groups of adjoining triangles using the method of biorthogonal grids developed by Bookstein (1978; et al., 1985; 1986).

A biorthogonal grid is a grid-like pattern of solid and dashed lines that map out the directions of greatest expansion and contraction of facial form across an area depicted by four or more facial landmarks (Figure I.5). In a biorthogonal grid, the piece-wise linear transformations depicted in individual triangles are presented in a composite picture as smoothed integrations of one linear transformation into another. The computation is based on a smooth nonlinear mapping - a thin plate spline - taking one set of landmarks into another. The intersection between each pair of lines in the grid pattern is at 90^{o}, depicting the directions of greatest contraction and expansion at that location. A biorthogonal grid provides a description of change in facial form that is independent of triangulation. It does not necessarily represent a real process of deformation - it provides a description of shape difference as deformation.

An interesting question to ask of a tensor-biometric analysis is whether shape changes across all possible triangles can be described by a simple linear (or, homogeneous) model of deformation. The simplest model is a linear transformation across all

triangles. Under the assumption of multivariate normality, the adequacy of fitting a simple linear model is tested using a variant of Hotelling's T^2 statistic (Rao, 1973). If the shape changes across all possible triangles can be described by a simple linear model, then the directions of greatest expansion and contraction mapped within the boundaries of the landmarks can be described by a single pair of tensor axes and the greatest changes in shape will be reflected in ratios of distance measures at 90^o to one another and parallel to these tensor axes. If the linear component does not provide an adequate fit to the data, a second quadratic component of shape change is computed to represent the simplest form of deviation from linearity, just as a parabola represents the simplest deviation from linearity in ordinary bivariate regression. If a linear global model explained all of the variation in shape across all triangles, the biorthogonal grid depicting that shape change would be an exact rectangular grid (Bookstein and Sampson, 1990).

Statistical Analysis

In a previous study (Clarren et al., 1987b), the mean facial shape coordinates associated with triangles 1-4-12, 22-14-19 and 19-23-14 were found to be significantly associated with the FAS facial phenotype. In the present study, we looked specifically at these triangles to determine if they differentiated the marijuana or the alcohol exposed groups from the unexposed groups. Other triangles, with shapes no so clearly related to FAS, were also evaluated.

The independent and interactive effects of fetal marijuana and alcohol exposure on the x and y coordinate values describing facial shape were assessed by multivariate analysis of variance (Johnson and Wichern, 1982). Within the context of MANOVA, Hotelling's T^2 statistic was used to explicitly test for differences in mean shape between the marijuana and alcohol exposed and unexposed groups. Use of Hotelling's T^2 is based on the assumption that the shape coordinates of each group follow a two-dimensional (or bivariate) normal distribution. The scatter of shape coordinates for both groups should be roughly elliptical in shape and of similar dispersion. The mean shape coordinates associated with other triangles were also assessed following the procedures described above. Due to the exploratory nature of that assessment, formal tests of hypotheses were not emphasized.

Differences in maternal characteristics and infant outcomes between the marijuana and alcohol exposed and unexposed groups were evaluated by chi-square and the t-test, where appropriate. Multiple regression analysis was used to evaluate associations between marijuana and alcohol exposure on birth outcome measures and distance measures recorded from the photographs.

RESULTS

Study Population and Neonatal Outcomes

Of the 61 marijuana exposed children that were eligible for participation in this study, we were able to locate and enroll 40. These subjects had been last contacted five to seven years ago when they participated in the original prospective study. Of the 21 mother/child pairs that were not enrolled, nine could not be located, two moved out of state, nine were contacted-but did not want to participate in the study and one infant had died of SIDS. These 21 mothers differed from the 40 mothers of the children that participated, in that they were more likely to have attended college and reported using less marijuana and alcohol during pregnancy.

Forty children with no reported exposure to marijuana were effectively group-matched to the marijuana-exposed group on preconceptional and prenatal alcohol exposure, sex, race and birth date. Women who reported use of marijuana were, however, more likely to be younger, to be in a lower income bracket and were less likely to be married or to have attended college (Table I.3). Letters of invitation were sent to a total of 70 mothers in order to locate and enroll 40 group-matched unexposed children. Of the 30 subjects that were not enrolled, 24 could not be located, one moved out of state and five expressed no interest in participating in the study.

Among the 40 marijuana users, 15 reported using marijuana one to two times a week, 11 reported using it three to four times a week and 14 reported using it every day (Table I.4). Fifteen of the women reported smoking marijuana two to five times a day on the days when they used marijuana. The highest reported use of marijuana by one woman was ten times a day, every day throughout pregnancy to alleviate nausea. The women

who used marijuana were also more likely to use cocaine and smoke cigarettes during pregnancy.

Estimated alcohol exposure (reported maternal consumption in the month prior to conception and during pregnancy) in the 80 children is presented in Table I.5. Twelve mothers (15%) reported drinking two to four ounces of alcohol per day in the month prior to conception. Ninety-eight percent of the women reported drinking less than one half ounce of alcohol per day during pregnancy. Alcohol exposure was higher in pregnancies that resulted in male offspring.

Neonatal status (gestational age, birth weight, body length, head circumference, and 1 and 5 minute APGAR scores) was nearly identical between the marijuana exposed and unexposed groups (Table I.6).

Clinical Assessment of the Photographs

The relationship between maternal use of marijuana in the first trimester of pregnancy and the clinical identification of FAS-like facial characteristics among the children is presented in Table I.7. The likelihood of identifying FAS-like facial features among the marijuana exposed and unexposed groups were essentially the same. Children exposed to first trimester marijuana were not more likely to be classified by the dysmorphologist as having FAS-like facial features (as assessed by chi-square). Two children in the marijuana exposed group were classified as probable FAS; the mother's of these children reported consuming one or two ounces of alcohol per day in the month prior to conception. One child in the marijuana unexposed group was classified as probable FAS; the mother had a low AA score in the month prior to pregnancy (0.2 ounces per day), but did report two episodes of binge drinking during pregnancy (consumption of five or more drinks per occasion). The lack of association between marijuana and FAS-like facial features was not attributable to differences in sex, race or age between the two groups. Evaluation of the photographs for facial patterns unrelated to FAS, identified a few children with isolated, unusual features, but failed to find any consistent patterns that differentiated the marijuana exposed from the unexposed.

In contrast, when the 80 children were stratified according to their mother's reported use of alcohol in the month prior to conception, the presence of thin upper lips and somewhat flat philtrums increased in frequency (although not significantly so) with increasing fetal exposure to alcohol (Table I.8). It should be noted that maternal use of alcohol in this study population was relatively low compared to the levels of alcohol consumption that have previously been associated with FAS facial features in children. FAS facial alterations are typically associated with maternal consumption of at least four ounces of alcohol per day (Hanson et al., 1978; Clarren et al., 1987b); only one child in the current study was exposed to this level of maternal consumption. No child was classified by the dysmorphologist (S.K.C.) as having a definite FAS-like face. The proportion of children classified as having a "probable" FAS-like face increased with increasing maternal consumption of alcohol, but the increase was subtle and could have occurred by chance. Marijuana and cocaine use was equally distributed among the women who reported consuming less than two ounces of alcohol per day prior to pregnancy and the women who reported consuming two or more ounces per day.

Computerized Morphometric Assessment of the Photographs

In the present study, triangles 1-4-12, 22-14-19 and 23-14-19 (previously confirmed to be associated with the FAS facial phenotype) did not differentiate, to a statistically significant degree, the children exposed to marijuana during the first trimester from the children with no reported exposure to marijuana during gestation. This lack of association persisted even when the exposed group was restricted to those exposed every day. In addition, we were unable to identify any triangles or patterns of minor anomalies, not previously related to FAS, that differentiated the marijuana exposed group from the unexposed group. The apparent lack of association between fetal marijuana exposure and facial dysmorphology in this study was not attributable to differences in sex, race or age between the exposed and unexposed groups.

Shape changes in two of the three triangles previously associated with fetal alcohol exposure and the FAS facial phenotype (Clarren et al., 1987b) were significantly associated with maternal consumption of alcohol in the month prior to conception, among the male children in this study. It should be noted that in the present study population, alcohol exposure was higher among the males than the females. The sex of

the child was also strongly correlated with facial size in this study population. As a result, it was necessary to perform the computerized morphometric assessment separately on the males and females. Upon doing so, the mean shape coordinates associated with triangles 22-14-19 and 19-23-14 were found to significantly differentiate male children exposed to maternal consumption levels of two ounces of alcohol per day (n = 10) in the month prior to conception (T-square = 6.65, p = .048 and T-square = 6.33, p = .054 respectively, with p = 2 and n = (10-1)+(38-1) = 46 degrees of freedom for both tests) (Figure I.6) from males exposed to lower levels of alcohol (n = 38). The direction of deformation suggested that males with higher exposure to alcohol had relatively shorter midfaces. This finding was evident in the measures of midface height (distance midway between the inner canthi and the lower edge of the top lip) recorded directly from the photographs (Table I.9). The size of the study population was too small to stratify the alcohol results by marijuana exposure and perform formal tests of significance. Visual evaluation of the digitized plots, however, confirmed that the effect of alcohol in this study population was <u>not</u> influenced by marijuana exposure.

Triangle 23-21-22 also significantly differentiated the males whose mothers consumed two to four ounces of alcohol per day in the month prior to conception (n = 10) from the males whose mothers consumed less than two ounces per day (n = 38) (T-square = 10.02, p = 0.01) (Figure I.7). The direction of deformation in the triangles suggested that higher alcohol exposure was associated with retrognathia.

Only two of the 32 mothers of female children reported consuming two or more ounces of alcohol per day in the month prior to conception. Although shape changes in triangles 22-14-19, 19-23-14 and 23-21-22 were not apparent, the sample size was far too small to draw conclusions.

Triangle 22-14-17 (Figure I.8) also differentiated the males exposed to two ounces of alcohol per day in the month prior to conception (n = 10) from the males exposed to less than two ounces of alcohol per day (n = 38) (T-square = 7.92, p = 0.028). The direction of deformation in this triangle suggested that relative nose lengths were shorter among the males exposed to higher levels of alcohol. This triangle did not differentiate between the females with comparable exposures. This finding was corroborated in the distance measures taken directly from the photographs of all eighty

children. When nose lengths measured directly from the photographs was regressed on ounces of alcohol consumed per day in the month prior to conception, a significant inverse relationship was noted (Table I.9). The sex of the child also influenced nose length with males having longer noses than females. An interaction term between sex and alcohol entered the equation, suggesting that the effect of alcohol on nose length differed among the males and the females, which is consistent with the results of the digitized analysis of triangle 22-14-17.

A biorthogonal grid was constructed from lateral landmarks 14 through 23 to summarize the deformational contrasts between the 10 males with exposure to maternal consumption of two or more ounces of alcohol per day with the 38 males with less exposure (Figure I.9a). A significant linear shape change across all ten landmarks was confirmed (F-statistic on 2 and 31 degrees of freedom = 4.96, p = 0.013). The change in mean shape, as depicted by the biorthogonal grid, describes a shortening of the midface region, especially along the upper midline and retrusion of the chin, relative to the baseline 14-23. In addition, an F statistic for nonlinearity was computed (F = 1.08 on 14 and 33 degrees of freedom; p = .40) confirming that the transformation across all triangles was adequately described by a simple (linear) global model. In other words, the directions of expansion and contraction were relatively consistent across all triangles mapped within the boundaries of these ten landmarks. The linear component of this model explained 50% of the shape change between the two groups.

The shifts in the ten mean landmarks between the exposed and unexposed groups were parallel and for the most part had magnitudes proportional to their mean distances from the baseline (14-23) as would be expected if the overall shape change between groups was linear (or homogeneous). Under a linear (or homogeneous) transformation, each landmark shifts by a multiple of one single vector. The multiple for each landmark is proportional to the distance of the landmark from the baseline (Bookstein and Sampson, 1990).

DISCUSSION

In the current study, fetal marijuana exposure was <u>not</u> found to be associated, to a statistically significant extent, with facial features compatible with fetal alcohol

syndrome. A few epidemiologic and clinical studies, however, have presented evidence suggesting an association between prenatal marijuana exposure and the presence of congenital malformations (Hecht et al. 1968; Carakushanky et al. 1969; Bogdanoff et al. 1972; Jacobson and Berlin, 1972; Geleherter, 1980; Linn et al., 1983) or more specifically, features compatible with fetal alcohol syndrome (Qazi et al., 1985; Clarren, et al., 1987b). The inconclusive nature of these reports can be attributed to one or more of the following factors: weak diagnostic sensitivity, questionable reliability of exposure, inadequate sample size and/or strongly correlated covariates.

Only one study, to our knowledge has reported a significant association between FAS-like facial features and maternal use of marijuana during pregnancy. In this prospective cohort study of 1,690 mother/infant pairs, Hingson, et al. (1982) reported that women who smoked marijuana during pregnancy were five times more likely than nonusers (2.0 - 12) to deliver a child with FAS-like features ($p = .001$). The infants were examined at two to three days of age by one of four pediatricians working from anomaly checklists. The authors noted that marijuana use in their study population was strongly correlated with alcohol use. It is not clear, however, whether the five-fold increased risk associated with marijuana reflected the risk level after alcohol was entered into the logistic regression equation. They do note that children of women who averaged two or more drinks daily were <u>not</u> more likely to have FAS-like features when compared to children of women who reported no use of alcohol. Their reported lack of association between FAS-like features and maternal consumption of two to four drinks per day is consistent with most studies that have assessed FAS-like features using anomaly checklists. FAS-like features are typically associated with maternal consumption levels of four or more drinks per day (Clarren et al., 1987b). Combining the group that reported consuming two to three drinks a day with the group that reported consuming four or more drinks per day may have weakened the association with FAS-like features. In our digitized analysis of facial form, male children whose mothers reported consuming four or more drinks per day (two or more ounces of alcohol) prior to conception were significantly different from those exposed to lower levels. When the women who reported consuming two drinks per day (one ounce of alcohol) were combined with those who consumed four drinks per day, the differences between the two exposure groups (< 2 compared to ≥ 2 drinks) were no longer significant.

In the current study, maternal consumption of as little as two ounces of alcohol per day, very early in gestation, was associated with FAS-like facial features. In a previous study (Clarren et al., 1987b), facial effects of fetal alcohol teratogenesis were associated with maternal use of four or more ounces of alcohol per day during pregnancy. The increased sensitivity of the current diagnosis is most likely attributable to the improved alignment of these facial photographs allowing for more precise comparisons of facial form. From a clinical standpoint, the FAS-like facial characteristics observed among these moderately exposed children were more subtle and appeared less frequently than are typically observed among children with higher levels of exposure. Perhaps the frequency of occurrence and severity of facial alterations resulting from ethanol exposure are dose related. It has recently been postulated that the facial features associated with FAS lie along a continuum of midline malformations leading up to the most severe expression, holoprosencephaly (Sulik, 1984a).

The deformations depicted by triangles (22-14-19), (22-14-17), (23-14-19) and (23-21-22), and the distance measures recorded directly from the photographs suggested that the children with higher alcohol exposure had shorter noses and were retrognathic. Both of these features are consistent with the FAS facial phenotype. In the current study, the triangular deformations and the distance measures taken directly from the photographs also confirmed that midface length was shorter among the children with higher alcohol exposure. In the literature, the midface is often described as being long relative to the nose. In this study, because all photographs incorporated a measure of scale, we were able to record real measures of size across all photographs. It appeared that both nose length and midface height decreased with increasing alcohol exposure. In the males, the mean nose lengths decreased from 3.53 cm to 3.42 cm to 3.25 cm as maternal alcohol consumption increased from (0 to 0.9) to (1 to 1.9) to (2 to 4) ounces per day. Correspondingly, mean midface length decreased from 5.51 cm to 5.44 cm to 5.21 cm within each group.

Although triangles 22-14-19 and 23-14-19 were predictive of alcohol exposure in both the current study and the previous study by Clarren et al., (1987b), it should be noted that the description of shape change for these triangles, as depicted by the directions of the principal axes, differed. Direct comparisons, however, between the "affected" groups in the two studies cannot be made because of differences in race, sex and level

of alcohol exposure. In the previous study, the group of eight children, for which significant shape changes were detected, were exposed to 4 or more ounces of alcohol per day, were 75% black and consisted of both males and females. In contrast, the group of ten children in the present investigation, for which significant shape changes were found, were exposed to only 2 to 4 ounces of alcohol per day, were all males, and only 10% were black. The current study confirmed that the shapes of triangles 22-14-19 and 23-14-19 were significantly different between blacks and whites, independent of alcohol exposure. The directions of the principal axes, attributable to race in the current study, match those attributed to alcohol and race in the previous study. The current study confirmed that these two triangles do identify significant contrasts between alcohol exposed and unexposed groups, independent of race and a permutation test in the previous study confirmed that there was a significant shape change in these two triangles attributable to ethanol exposure, after statistically controlling for racial differences. To determine if the direction of deformation attributable to alcohol seen in this predominantly white population is the same among a black population, a study sample of exposed and unexposed blacks will need to be assessed.

FAS-like facial features were not associated with our measures of maternal use of alcohol during pregnancy. Reported use of alcohol during pregnancy was very low in this study population and was similar in the marijuana exposed and unexposed groups. Previous studies have suggested that reported use of alcohol in the month prior to conception is a better estimate of the mother's true alcohol consumption during the first six to eight weeks of pregnancy, prior to confirmation of pregnancy. It is during this time period that facial development is most susceptible to teratogens (Pratt, 1981; Slavkin, 1979).

Two limitations in this study that could contribute to a false-negative association between marijuana and FAS-like facial features is the reliability of reported drug use and lack of information on use of marijuana in the month prior to conception. Both of these limitations may have resulted in an underestimation of marijuana exposure. In the original prospective study, laboratory drug testing was not performed on all study participants. Instead, validity of self-reported drug use was investigated in a pilot study that preceded the original study. Self-reported drug use was compared to laboratory tests of drug levels present in body fluids in a random sample of 108 postpartum

women. The proportion of questionable self-reports ranged from 0 to 3% depending on the drug (Little et al., 1986). These results are encouraging, and probably suggest that if drug use has been under-reported in this study population, it is unlikely to account for the complete lack of association found between marijuana exposure and FAS-like facial features. Addressing the second limitation, use of marijuana in the first trimester was used to estimate exposure during the first few weeks of gestation when facial development is most vulnerable to teratogenesis. Prepregnancy levels of maternal marijuana use may have provided more accurate estimates of fetal exposure in the first six to eight weeks when women are often unaware of their pregnancies. Although prepregnancy use of marijuana will remain unknown in this study population, a study by Fried et al., (1985) did find that of three "soft drugs" used by pregnant women (alcohol, nicotine and marijuana), marijuana use by heavy users was the least reduced between prepregnancy and the first trimester.

In summary, children with frequent exposure to marijuana during the first trimester were not more likely to have FAS-like facial features when compared to an unexposed group. In addition, we were unable to identify any pattern of facial features (unrelated to FAS) that differentiated the marijuana exposed group from the unexposed group. Several methodological aspects of this study lend credence to these negative study results. The most sensitive period for the induction of facial malformations lies between the second and twelfth weeks of embryonic development (Slavkin, 1979). The population we studied received relatively heavy exposure to marijuana during this vulnerable time period. Information on marijuana, alcohol, tobacco and other drug use during pregnancy was collected prospectively in personal interviews conducted at one month postpartum, and the correlation between maternal alcohol and marijuana use during pregnancy was effectively eliminated by group-matching. Most importantly, however is the fact that maternal consumption of alcohol in the month prior to conception was associated with features compatible with FAS in this study population, lending credence to the sensitivity of our diagnostic approach, despite the relatively small sample size. This level of affect is lower than ever previously reported, but was also of marginal significance.

Table I.1

Clinical measures recorded from the 5x7 photographs of each child.

1. Midface height(cm) (vertical midline distance from entrocanthion to the lower edge of the upper lip)
2. Nose length (cm) (vertical midline distance from the entrocanthion to the subnasale)
3. Nose length / midface height
4. Intercanthal distance (cm)
5. Right and left palpebral fissure lengths (cm)
6. Intercanthal distance / Mean (right and left) palpebral fissure length
7. Philtrum
   - not flat
   - somewhat flat
   - definitely flat
8. Midface contour
   - not flat
   - somewhat flat
   - definitely flat
9. Upper Lip
   - not thin
   - somewhat thin
   - definitely thin
10. Ptosis (present - absent)

Table I.2

Definition of the facial landmarks (Clarren et al., 1987b)

Landmark	Common name	Definition
		FRONTAL VIEW
1 , 4		Intersection of the eyebrow curve and a vertical line through the exocanthion.
2 , 3		Intersection of the eyebrow curve and a vertical line through the midpoint of the palpebral fissure.
5 , 8	Exocanthion	Lateral intersection of upper and lower eyelids.
6 , 7	Entrocanthion	Medial intersection of upper and lower eyelids.
9 , 10		Most lateral points on alar curvature.
11 ,13	Cheilon	Lateral intersection of upper and vermilion.
12		Midpoint of upper vermilion border.
		LATERAL VIEW
14	Nasion	Point of maximum curvature over nasal bridge.
15	Exocanthion	Lateral intersection of upper and lower eyelids.
16	Pronasale	Point of maximum curvature over nasal tip.
17	Subnasale	Intersection of columela and philtrum.
18		Point of maximum curvature of soft tissue fold from zygoma.
19		Border of upper vermilion and philtrum.
20	Cheilon	Lateral intersection of upper and lower vermilion.
21		Border of lower vermilion and lower lip.
22	Gnathion	Point of maximum curvature of chin.
23		External auditory opening.

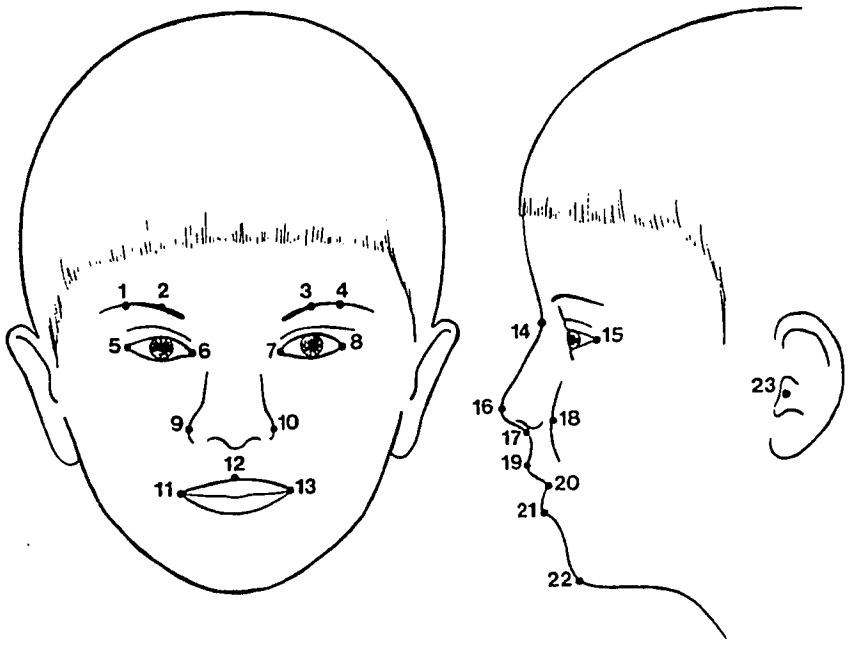


Figure I.1

Twenty-three facial landmarks were identified on the frontal and lateral photographs of each child. The coordinates associated with each landmark were entered into a database by computer digitization. The landmark definitions are presented in Table I.2 (Clarren et al., 1987b).

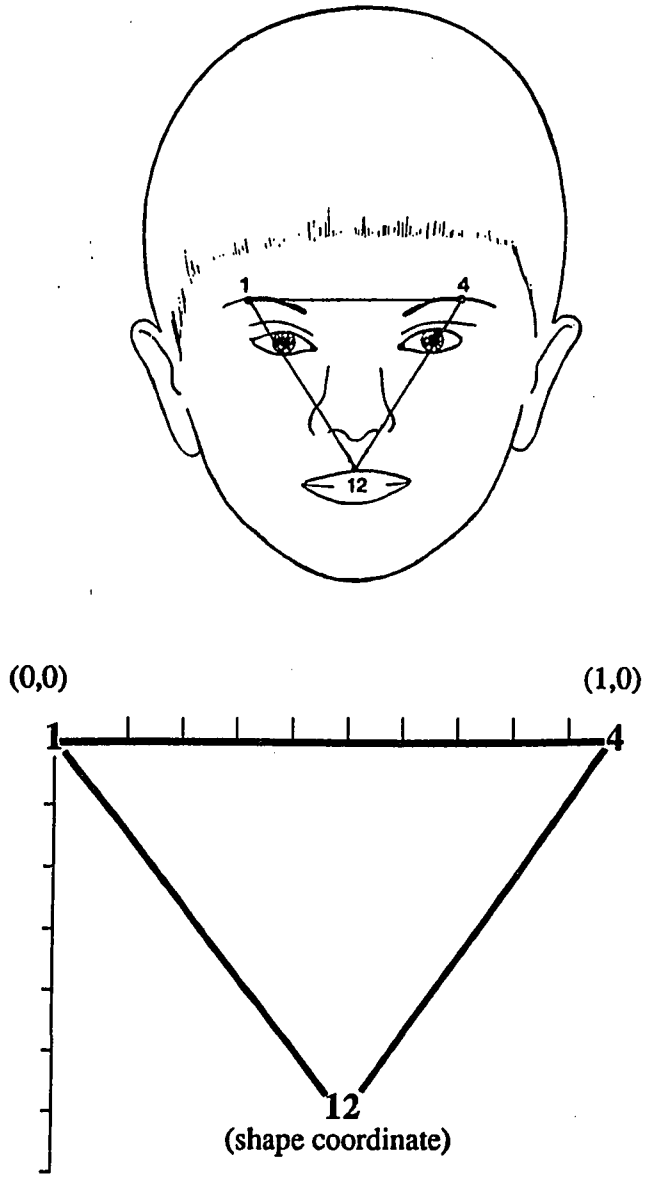


Figure I.2

The triangle formed by the three facial landmarks 1-4-12 is standardized on a Cartesian (x,y) coordinate system. Landmark (12) represents the shape coordinate for this triangle.

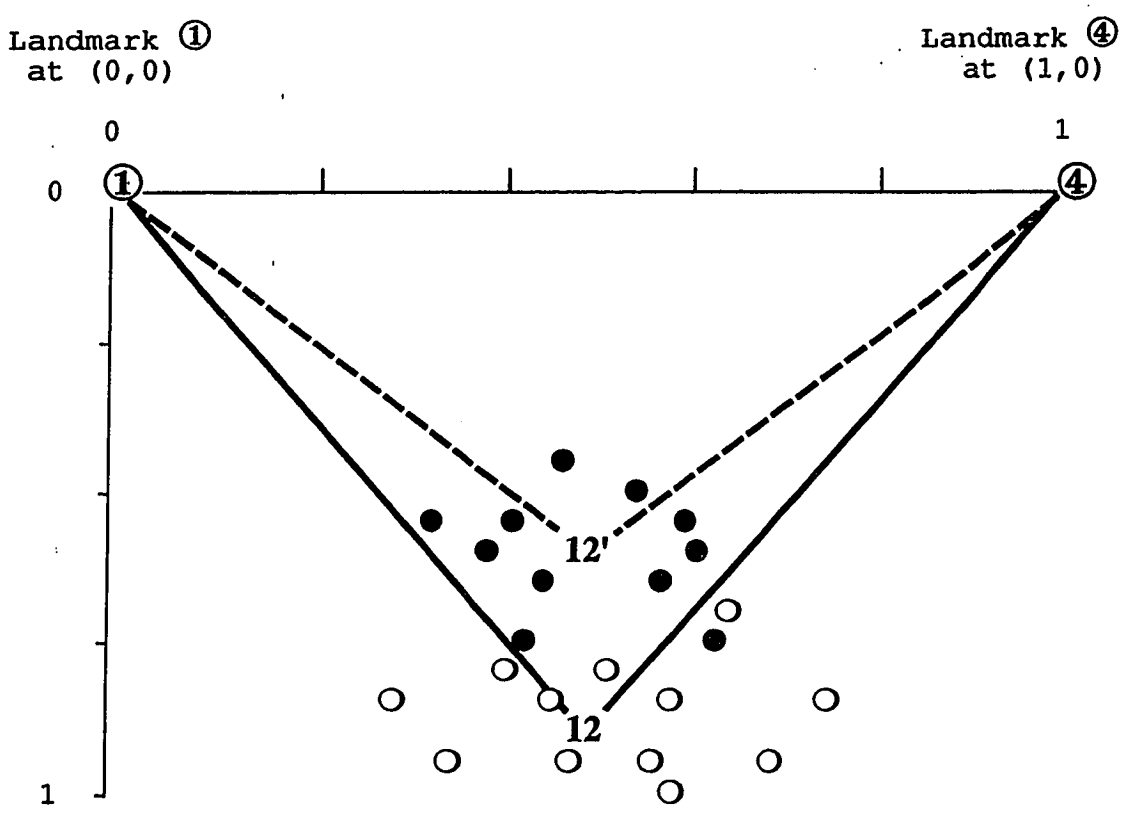


Figure I.3

The shape coordinates (landmark 12) of the exposed "●" and unexposed "○" subjects are displayed as a scatter plot in this hypothetical example. The scatter plot graphically illustrates the position of the midpoint of each child's upper vermilion border (landmark 12) relative to the position of landmarks 1 and 4 on the eyebrows. The mean shape coordinates for each group are represented by the average (or centroid) of the scatter of shape coordinates within each group. The locations of the mean shape coordinates for the exposed and unexposed groups are represented by **12'** and **12** respectively. The observed difference in shape between these two mean triangles would be assessed using Hotelling's T^2 statistic.

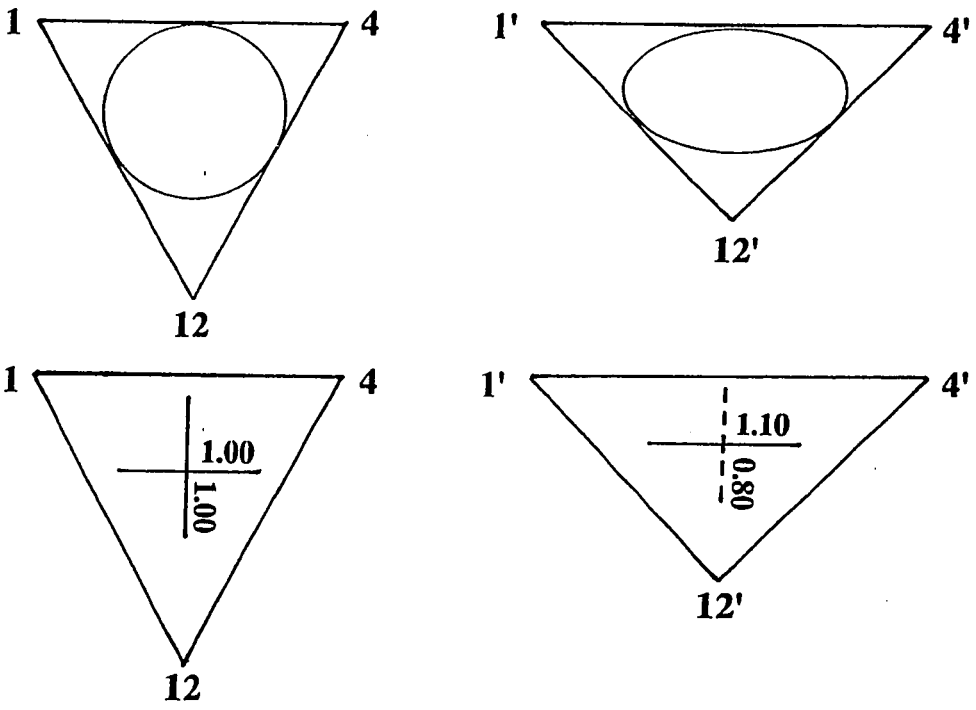


Figure I.4

The transformation of one triangle into another transforms a circle inscribed in the first triangle into an ellipse in the second. The axes (or tensors) of the ellipse, rescaled and oriented homologously in triangle (1-4-12) lie along the directions in which that triangle is most stretched and most compressed by the transformation. The magnitude of the stretching and compression is reflected in the length of the axes. In this hypothetical example, the "exposed" triangle 1'4'12' resulted from a 10% horizontal expansion and a 20% vertical contraction of the "unexposed" triangle 1-4-12.

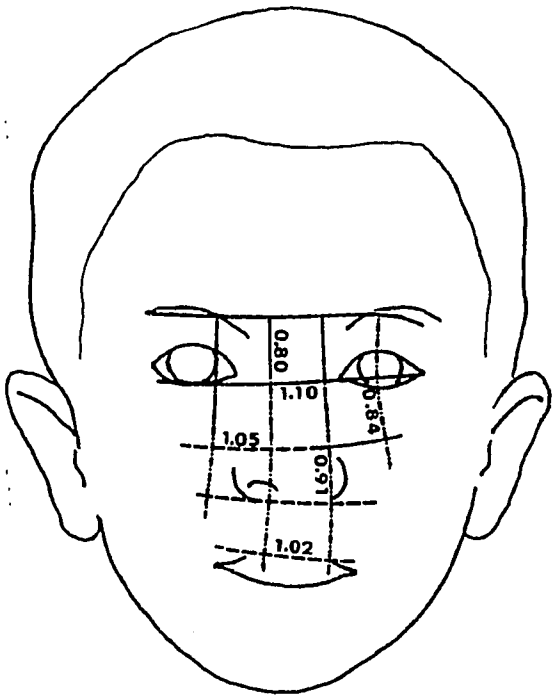


Figure I.5

Hypothetical example of a biorthogonal grid depicting the difference in mean shape between an exposed and unexposed group. The curves reflect the direction of relative extension (solid and dashed lines) and contraction (dotted and dash-dotted lines) of the facial area depicted by landmarks 1 through 13. Refer to the text for a more detailed explanation.

Table I.3

Selected characteristics of women who did and did not use marijuana during the first trimester of pregnancy.

Characteristic	Marijuana	
	Unexposed (N = 40)	Exposed (N = 40)
	N or mean (range)	
Child's race (parent's race)		
caucasian-caucasian	37	35
caucasian-oriental	0	1
caucasian-chicano	1	0
caucasian-native American	0	1
caucasian-other	1	0
black-black	1	3
Child's age at the time of the photograph (years)	6.3 (4.9-7.5)	6.3 (5.0-7.4)
Marital status		
married	37	31
single	2	5
separated	1	4
Yearly income *		
< $ 10,000	1	9
$ 10,000 to $ 24,999	10	14
> $25,000	27	14
unknown	2	3
Mother's age at child's birth †	30.7 (22-48)	26.9 (17-35)
Number of abortions †	0.2 (0-2)	0.7 (0-3)
Number of pregnancies	2.5 (1-6)	2.6 (1-11)
Mother's education *		
did not finish high school	0	6
finished high school only	11	16
attended college	29	18

* Significance levels for difference within categories between exposed and unexposed were calculated with χ^2 (without Yates correction): $p < .01$.

† Significance levels for difference in means between exposed and unexposed calculated by t-test: $p < .01$.

Table I.4

Other drug use among the women who did and did not use marijuana during the first trimester of pregnancy.

Characteristic	Marijuana	
	Unexposed (N = 40)	Exposed (N = 40)
	N or mean (range)	
Trimesters when marijuana was used		
1st only	0	11
1st and 2nd only	0	3
1st and 3rd only	0	2
all three	0	24
Freq of marijuana use		
1-2 times a week	0	15
3-4 times a week	0	11
every day	0	14
Number of joints smoked per occasion		
1	0	25
2-5	0	14
10	0	1
Trimesters when cocaine was used *		
never	38	29
1st only	2	7
1st and 2nd only	0	1
1st and 3rd only	0	1
1st, 2nd and 3rd	0	1
3rd only	0	1
Frequency of cocaine use *		
never	38	29
< once a month	2	8
once a month	0	1
2-3 times a month	0	2
Number of times cocaine was used * per occasion during pregnancy		
never	38	29
1-3	2	9
4-6	0	2
Smoked cigarettes during pregnancy	12	26

Table I.4 (continued)

Other drug use among the women who did and did not use marijuana during the first trimester of pregnancy.

Characteristic	Marijuana	
	Unexposed (N = 40)	Exposed (N = 40)
	N or mean (range)	
Number of cigarettes smoked per day †		
none	28	14
1-10	6	11
11-20	6	11
21-40	0	4
Average mg of nicotine per day among smokers	6 (0-22)	11 (0-40)
Ave. ounces of alcohol per day the month before pregnancy (AA score)	0.7 (0-2.0)	0.8 (.01-4.0)
Ave. ounces of alcohol per day during pregnancy (AA score)	0.1 (0-1.0)	0.1 (0-1.0)

* Significance levels for differences within the collapsed categories (ever versus never) between exposed and unexposed was calculated using χ^2 (without Yates correction):
($p < .01$).

† Significance level for difference within categories between exposed and unexposed was calculated using χ^2 (without Yates correction): ($p = .009$).

Table I.5

Gestational exposure to alcohol among the male and female children estimated by maternal consumption of alcohol prior to and during pregnancy.

AA score (ounces absolute alcohol / day)	Females (N = 32)		Males (N = 48)	
Month before conception				
0 to 0.9	18	(56%)	24	(50%)
1.0	12	(38%)	14	(29%)
2.0	1	(3%)	10	(21%)
4.0	1	(3%)	0	(0%)
During pregnancy				
0 to 0.5	32	(100%)	46	(96%)
1.0	0	(0%)	2	(4%)

Table I.6

Birth outcomes among the infants whose mothers did and did not use marijuana during the first trimester of pregnancy.

Infant Characteristics	Marijuana	
	Unexposed (N = 40)	Exposed (N = 40)
	N or mean (range)	
Child's sex		
female	*15	17
Gestational age in weeks		
< 36	0	2
36 to 42	39	36
> 42	1	1
unknown	0	1
Birth weight in grams		
< 2500	0	2
2500 to 4000	33	32
> 4000	7	5
unknown	0	1
Gestational age in weeks	40.0 (36-43)	39.3 (32-43)
Body length (cm)	51.1 (46-54)	50.2 (45-56)
Head circumference (cm)	34.7 (31-39)	34.2 (31-38)
APGAR score (1 minute)		
< 7	4	4
7 to 10	36	35
unknown	0	1
APGAR score (5 minute)		
< 7	0	0
7 to 10	40	39
unknown	0	1

* group-matched to the exposed group.

Table I.7

Facial characteristics of children
and maternal use of marijuana during the first trimester.

Child's Facial Characteristics	Marijuana	
	Unexposed (N = 40)	Exposed (N = 40)
	(n)	(n)
Midface		
not flat	28	26
somewhat flat	8	13
definitely flat	4	1
Philtrum		
not flat	36	34
somewhat flat	4	6
definitely flat	0	0
Upper lip		
not thin	31	30
somewhat thin	6	5
definitely thin	3	5
Classification of overall facial appearance by dysmorphologist		
No unusual features	34	33
Unusual features, but not FAS-like face	4	4
Possible FAS-like face	1	1
Probable FAS-like face	1	2
Definite FAS-like face	0	0

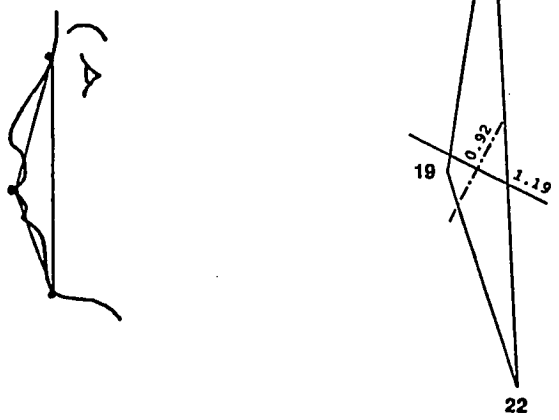
No significant differences between marijuana exposed and unexposed groups as assessed by Chi-square.

Table I.8

Facial characteristics of children
and early gestational exposure to alcohol.

Child's Facial Characteristics 12)	Maternal preconceptional use of alcohol (absolute ounces of alcohol per day)		
	0 to 0.9 (N = 42)	1 to 1.9 (N = 26)	2 to 4 (N =
	(%)	(%)	(%)
Midface			
not flat	67	73	58
somewhat flat	28	23	25
definitely flat	5	4	17
Philtrum			
not flat	93	81	83
somewhat flat	7	19	17
definitely flat	0	0	0
Upper lip			
not thin	81	73	68
somewhat thin	12	15	16
definitely thin	7	12	16
Classification of overall facial appearance by dysmorphologist			
No unusual features	91	80	67
Unusual features, but not FAS-like face	5	12	25
Possible FAS-like face	2	4	0
Probable FAS-like face	2	4	8
Definite FAS-like face	0	0	0

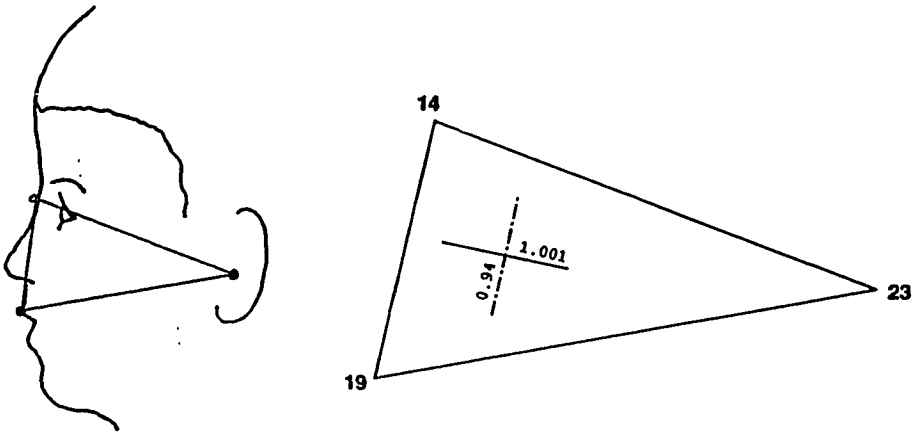
No significant differences between marijuana exposed and unexposed groups as assessed by Chi-square.



Triangle 22-14-19

Among all 48 males:
AAMB ≥ 2.0 (n = 10)

T-square = 6.65
p = 0.048



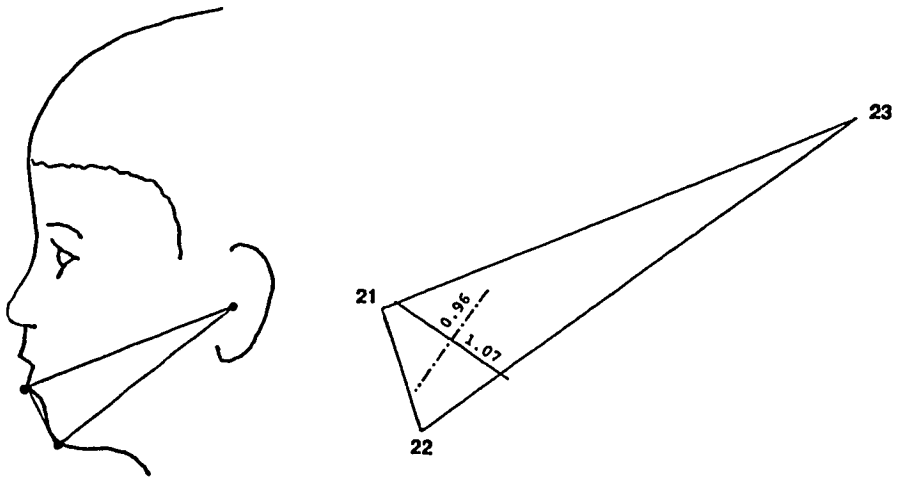
Triangle 23-14-19

Among all 48 males:
AAMB ≥ 2.0 (n = 10)

T-square = 6.33
p = 0.054

Figure I.6

Triangles associated with fetal alcohol exposure in the males.



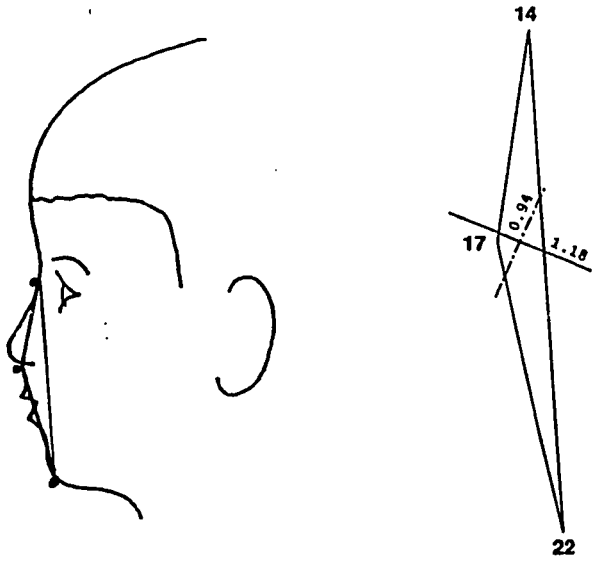
Among all 48 males:
AAMB $\geq$ 2.0 (n = 10)

T-square = 10.02
p = 0.012

Figure I.7

Retrognathia associated with prenatal alcohol exposure in males.

Triangle 23-21-22



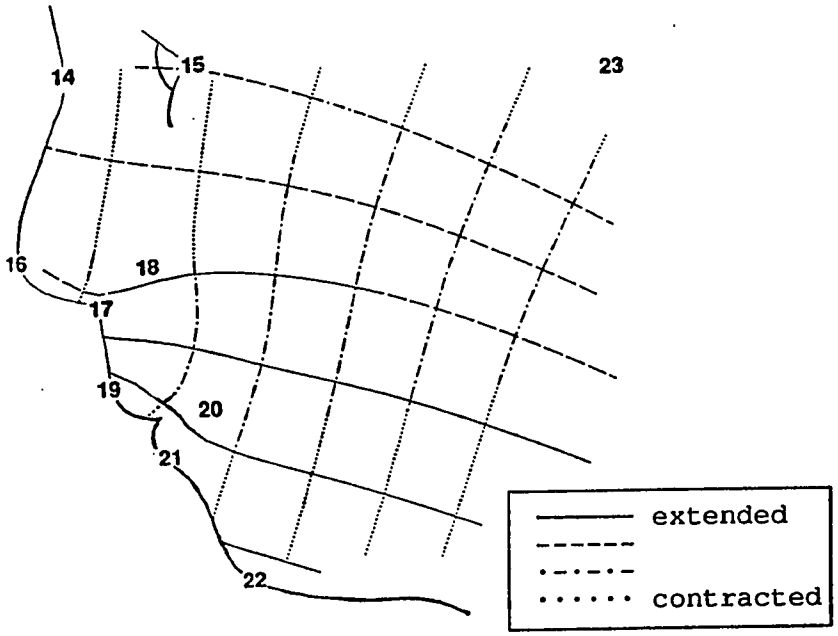
Among all 48 males:
AAMB $\geq$ 2.0 (n = 10)

T-square = 7.92
p = 0.028

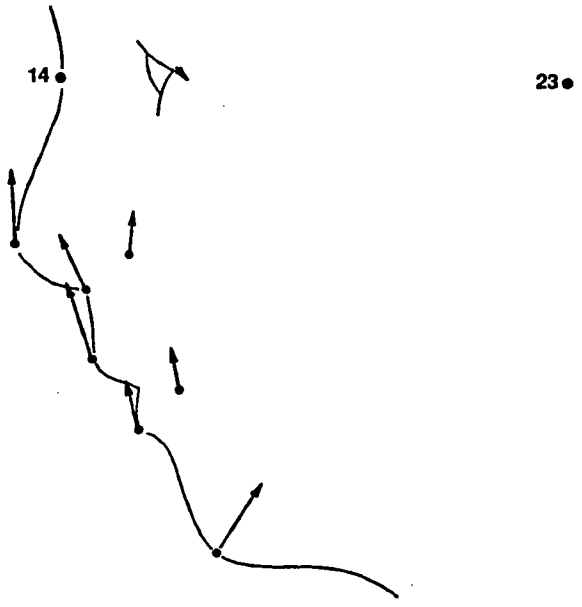
Figure I.8

Short nose length associated with prenatal alcohol exposure in males.

Triangle 22-14-17



a). Biorthogonal grid depicting the difference in mean shape between the 10 males exposed to maternal consumption of two or more ounces of alcohol per day, early in pregnancy to the 48 males exposed to lower levels of maternal alcohol consumption.



b.) Observed mean displacements of the ten lateral landmarks relative to baseline 23-14.

Figure I.9
Facial shape change in males with fetal alcohol exposure.

Table I.9

Regression of nose length and midface length
measured directly from the photographs of all 80 children
on maternal consumption of alcohol in the month prior to conception and sex.

MIDFACE LENGTH

Variable	b	S.E of b	Beta	F	Sig. F
*AAMB	-.09	.05	-.18	3.0	.085
**sex	-.25	.08	-.36	11.7	.001
(constant)	5.5	.07		6418.4	.0000

$r^2 = .15$ F = 6.7 p = .002 N = 80

NOSE LENGTH

Variable	b	S.E of b	Beta	F	Sig. F
*AAMB	-.07	.02	-.43	8.5	.005
**sex	-.09	.04	-.41	7.1	.009
sexAAMB	.08	.04	.41	5.4	.023
(constant)	1.69	.03		4131.4	.0000

$r^2 = .12$ F = 3.4 p = .022 N = 80

* AAMB: absolute ounces of alcohol per day in the month prior to conception.
** sex, male = 0, female = 1

Chapter II

Manuscript # 2

A Pilot Study of Facial Shape in Children Gestationally Exposed to Cocaine.

ABSTRACT

An association between fetal cocaine exposure and facial dysmorphology was conducted as a pilot project in a population of 80 children. Facial form was assessed from standardized lateral and frontal facial photographs of 13 children between four and seven years of age, whose mothers' reported use of cocaine during pregnancy and 67 children whose mothers' reported no use of cocaine during pregnancy. Patterns of facial anomalies were assessed clinically by a dysmorphologist and morphometrically by landmark analysis.

Fetal cocaine exposure was associated with increased intercanthal distance and increased midface retrusion. Fetal cocaine exposure was also associated with decreased birth weight and shortened gestation. Evidence supporting the credibility of these morphometric and fetal growth outcomes lies in the correspondence between the timing of exposure and the critical period of development for each outcome. First trimester cocaine exposure was associated with facial dysmorphology and third trimester exposure was associated with decreased infant birth weight. These findings, although statistically significant, reflect the outcomes on a very small number of children and will require further investigations with larger study populations for confirmation.

INTRODUCTION

The use of cocaine has increased dramatically in the United States over the past decade. Among young adults, 18 to 25 years of age, the lifetime prevalence of cocaine use has increased from 9% in 1972 to 28% in 1982 (Abelson & Miller, 1985). A recent survey among America's high school students found that 15.2% of the graduating class of 1987 had tried cocaine (Johnston et al., in preparation). The use of cocaine has not only increased, but has shifted demographically. In the late 70's and early 80's, the "typical" users were white, middle to upper-class, employed males between the ages of 25 and 35. Cocaine use has subsequently spread to a much broader cross-section of the population. It's use has escalated among woman, minority groups, lower income groups and adolescents. A major contributor to this spreading use has been the increasing availability of cocaine at reduced prices. On average, a gram of cocaine is now cheaper than an ounce of marijuana in many places across the United States (Washton and Gold, 1987)

Cocaine use among pregnant women has been reported to be as high as 18% in inner city populations (Zuckerman et al., 1989). Cocaine has been confirmed to cross the placental barrier in humans (Chasnoff and Schnoll, 1987) and has been associated with increased rates of preterm delivery, spontaneous abortion, abruptio placentae, and childhood neurobehavioral deficits (Acker et al., 1983; Bingol et al, 1986, 1987; Chasnoff et al., 1986, 1988, 1989; Oro & Dixon, 1987; MacGregor et al., 1987).

Scientific reports addressing the teratogenicity of cocaine is scant and the results are mixed. In mice, abnormalities in soft tissue and skeletal structures, increased resorptions and altered sex ratio were noted after single exposures to 60 mg/kg SC between gestational days six and twelve (Mahalik et al., 1980). Fantel and Macphail (1982), however, found no evidence of abnormalities in mice exposed repeatedly to 60 mg/kg IP between gestational days seven and sixteen. Higher doses (75 mg/kg) were lethal and lower doses (50 mg/kg) resulted in increased resorption. In humans, fetal cocaine exposure has been associated with urinary tract defects (Chavez et al., 1989), increased rate of congenital anomalies (Little BB et al., 1989) and major and minor craniofacial anomalies (Bingol et al., 1987). An equal number of studies, however, have reported no evidence of congenital anomalies associated with fetal cocaine

exposure (Hadeed and Siegel, 1989; MacGregor et al., 1987; Chasnoff et al., 1986; Madden et al., 1986).

The current report presents the results of a pilot investigation of fetal cocaine exposure and facial dysmorphology in humans.

METHODS

This pilot data was derived from a facial morphology study that was designed to investigate the relationship between first trimester marijuana exposure and facial anomalies. Facial form was assessed from standardized lateral and frontal photographs in forty children between four and seven years of age, whose mothers' reported frequent use of marijuana during the first trimester of pregnancy and forty children whose mothers reported no use of marijuana during pregnancy. The results of that investigation have been reported elsewhere (see Chapter I). In the course of that study, patterns of facial anomalies other than those associated with FAS and marijuana were also explored and the association between a unique facial pattern and fetal exposure to cocaine was evaluated.

Source of Subjects

The 80 subjects who participated in the facial morphology study were selected from among 1100 mother/infant pairs who had previously participated in a Seattle-based prospective study (1982-87) investigating the role of maternal diet, drinking, smoking and marijuana use during lactation on infant growth and development (Little, et al., 1984, 1986, 1989; Worthington-Roberts et al., 1989a, 1989b; Astley & Little, 1989).

Subjects in the original prospective study were members of Group Health Cooperative of Puget Sound, a health maintenance organization in Seattle, WA. All prenatal patients receiving care between May 1, 1982 and July 1, 1984 were contacted by the Cooperative in their sixth month of pregnancy regarding possible study participation. Seventy-four percent (5,298) of the prenatal patients responded and completed a mailed screening questionnaire detailing their alcohol and tobacco use both before and during

pregnancy, some dietary information, and their plans to lactate. These patients constituted a screened pool.

A total of 1100 women were selected from the screened pool to participate in the original prospective study. At six weeks after delivery, a detailed personal interview was conducted in the woman's home to obtain information on diet, drinking, smoking and other drug use during pregnancy and the first month postpartum. Information on maternal demographics and reproductive history were also collected. This interview was repeated to obtain data for the third and twelfth months postpartum. Neonatal status
(birth weight, gestational age, 1 and 5 minute APGAR scores, the presence or absence of congenital malformations and morbidity in the hospital) was abstracted from the infants' medical records.

Among the 1100 mothers, 61 reported using marijuana at least once a week during the first trimester of pregnancy and 933 reported no use of marijuana at any time during pregnancy. Forty of the 61 children exposed to marijuana were located and enrolled in the marijuana follow-up study. An equal number of marijuana unexposed children were selected from the group of 933 by group-matching to the exposed cohort on the following characteristics: reported maternal alcohol consumption during the month prior to conception and during pregnancy and the infant's sex, race and birth date. An informed consent with a full explanation of study procedures was provided. Among the 80 children that participated in the facial morphology study, 13 were exposed to cocaine during gestation. These 13 exposed children and the remaining 67 with no reported exposure to cocaine during gestation, make up the study population in the current pilot investigation.

Assessment of Facial Photographs

1. Facial Photographs

Standardized frontal and lateral facial photographs were taken of the children when they were between five and seven years of age. The two year span in ages reflects the two year period in which the mothers of these children entered the previous prospective

study at six months gestation. Rather than prolong data collection over a two year period to obtain photographs of all children at one age, the photographs were taken over a three month period of time and the children in the exposed and unexposed groups were simply group-matched on age. A placard with the child's study number and a 2-cm rule was included in each photograph to provide a measure of scale. The children were asked to hold a comfortable pose with their mouths closed while the photographer positioned herself to obtain frontal and lateral pictures with no detectable rotation.

A set of 5X7 inch, black and white prints were made of each frontal and lateral view. The photographs were printed to scale (50% life size) so that distance measures taken directly from the photographs would be comparable across all the subjects.

2. Clinical Assessment of the Photographs

The photographs of all children were initially evaluated clinically by the staff dysmorphologist (S.K.C.) to determine if unique patterns of facial characteristics were present. This evaluation was performed without knowledge of the exposure histories. The clinical evaluation provided a qualitative assessment that complimented the purely quantitative approach of the digitized morphometric assessment. During the clinical evaluation, the following distance and ratio measures were recorded directly from the photographs of each child.

1. Midface height (cm) (vertical midline distance from entrocanthion to the lower edge of the upper lip)
2. Nose length (cm) (vertical midline distance from the entrocanthion to the subnasale)
3. Nose length / midface height
4. Intercanthal distance (cm)
5. Right and left palpebral fissure lengths (cm)
6. Intercanthal distance / mean (right and left) palpebral fissure length

3. Computerized Morphometric Assessment of the Photographs using Landmark Analysis

A set of 23 facial landmarks were located and marked on the 5x7 photographs of each child (Figure II.1, Table II.2). These are the same landmarks that were used in the previous study by Clarren (et al., 1987b). The relative location of the frontal landmarks 1 through 10 and the lateral landmarks 11 through 23 were entered into a database by placing each photograph on a computer digitizing tablet (MacTablet) and marking the location of each landmark with a digitizing stylus.

The analysis of facial shape, developed by Bookstein (1982, 1983, 1984, 1986), was carried out by way of triangles defined by sets of three landmarks: the mean shapes of these triangles were compared between the exposed and unexposed groups. For example, if a short midface is the facial characteristic of interest, then the triangle resulting from the three facial landmarks 1, 4, and 12 might describe that characteristic (Figure II.2). The triangle is standardized by arbitrarily selecting one edge, for example 1-4, as the baseline and assigning it a standard length of one unit on a Cartesian (x,y) coordinate system. Thus, as illustrated in Figure II.2, if landmarks 1 and 4 are assigned the Cartesian coordinates (0,0) and (1,0) respectively, the shape of the triangle is described by the third landmark (12), the midpoint of the upper vermilion border. Landmark 12 represents the x and y "shape coordinates" of the triangle. Although the shape coordinates appear to be describing a single landmark, they are, in fact, representing the shape of the triangle as a whole. The shape coordinates for the triangles of each exposed and unexposed subject are displayed in a scatter plot and the mean shape coordinates for each group are derived from the average (or centroid) of the scatter of shape coordinates within each group. A hypothetical example is presented in Figure II.3. In this example, the mean location of landmark 12' for the exposed group is closer to the baseline 1-4 than the mean location of landmark 12 for the unexposed group. Hotelling's T-square statistic would be used to determine if this observed difference in mean shape between the two groups of triangles is significant. This test statistic is nearly invariant to the choice of baseline as long as the variation in landmark locations is small relative to the distances between the baseline landmarks.

Upon identifying which triangle(s) are significantly different between groups, the next step in the analysis is to describe how the triangles are different. Any shape change between two triangles has a direction of greatest change and a direction of least change. These directions are at 90^{o} to one another and lie in some orientation upon the triangle. To determine the magnitude and direction of shape change in the mean triangle(s), a tensor analysis is performed. Tensor analysis of landmark data has been described in detail in several publications by Bookstein (1982, 1983, 1984a). A brief description of the analysis is presented below, continuing with the hypothetical example of triangle 1-4-12 presented in Figures II.1 - II.3. The deformation of the triangle 1-4-12 into another (1'4'12') transforms a circle inscribed in the first triangle into an ellipse in the second (Figure II.4). In this example, we will say that triangle 1-4-12 represents the mean "normal" triangle with baseline 1-4 and mean shape coordinate 12 derived from a hypothetical group of unexposed subjects and triangle 1'4'12' represents the mean "deformed" triangle with mean shape coordinate 12' derived from a group of exposed subjects. The principal axes (or tensors) of the ellipse, rescaled and oriented homologously in triangle 1-4-12, lie along the directions in which that triangle is most stretched and most compressed by the transformation. The rotation of the axes in triangle 1'4'12' (referred to as the principal directions) coincide with the maximum and minimum diameters of the ellipse. To orient the axes homologously in triangle 1-4-12, the axes must bisect the sides of that triangle in a manner that is proportionally equivalent to the bisection of the sides of triangle 1'4'12' by the principal axes of the ellipse. The greatest magnitude of stretching and compression that took place in the transformation lie along the directions of the principal axes and is referred to as the principal dilatations. A dilatation is a ratio of lengths; any length in a deformed triangle divided by the corresponding length in a normal triangle. A dilatation is a measure of shape change. The magnitude of the maximum dilatation (or expansion) is the ratio of the length of the longest diameter of the ellipse divided by the corresponding length in the "normal" triangle which is the diameter of the circle. The magnitude of the minimum dilatation (or contraction) is the ratio of the smallest diameter of the ellipse divided by the diameter of the circle. For example, in Figure II.4, the "deformed" triangle 1'4'12' representing the mean shape of the exposed group resulted from a 10% horizontal expansion and a 20% vertical contraction of the "normal" triangle 1-4-12 representing the mean shape of the unexposed group.

This morphometric tool could certainly be used to evaluate all possible triangles resulting from the 23 landmarks. Such an exploratory approach, however, runs the risk of identifying significant differences between groups that are attributable to chance alone due to multiple comparisons. Instead, the number of triangles evaluated was restricted to the number of unique facial characteristics that were identified in the clinical evaluation.

Statistical Analysis

The independent and interactive effects of fetal cocaine, alcohol and marijuana exposure on the x and y coordinate values describing facial shape were assessed by multivariate analysis of variance (Johnson and Wichern, 1982). Within the context of MANOVA, Hotelling's T^2 statistic was used to explicitly test for differences in mean shape between the cocaine exposed and unexposed groups.

This morphometric tool could certainly be used to evaluate all possible triangles resulting from the 23 landmarks. Such an exploratory approach, however, runs the risk of identifying significant differences between groups that are attributable to chance alone due to multiple comparisons. Instead, the number of triangles evaluated was restricted to the number of unique facial characteristics that were identified in the clinical evaluation.

Differences in maternal characteristics and infant outcomes between the cocaine exposed and unexposed groups were evaluated by χ^2 and the T-test, where appropriate. Multiple regression analysis was used to evaluate associations between cocaine and other drug exposure on birth outcome measures and distance measures recorded from the photographs.

RESULTS

Study Population

Overall, this study population was comprised of predominantly white, middle to upper income, college educated women. The women who reported use of cocaine in this

study population (n = 13) tended to be younger, less educated and in a lower income bracket than the women who reported no use of cocaine during pregnancy (Table II.2).

Of the 13 women who reported using cocaine during pregnancy, 12 used it in the first trimester, whereas only 2 and 3 used it in the second and third trimesters respectively. The one women who reported not using cocaine in the first trimester reported using it only once in the third trimester. Women who used cocaine were more likely to have had a male child (Table II.3). Males were also more likely to have been exposed to higher levels of cocaine.

The women who reported using cocaine were also more likely to have used marijuana, and to have smoked cigarettes (Table II.4). Even though a substantially higher proportion of the cocaine users smoked cigarettes, the nicotine exposure among the smokers in each group was comparable because eight of the thirteen women in the cocaine group reported using nicotine-free cigarettes. Reported use of alcohol in the month prior to conception and during pregnancy was slightly higher among the cocaine users, but the difference was not significant.

Neonatal Status

There was very little difference in infant body length, head circumference and APGAR scores at birth between the cocaine exposed and unexposed infants (Table II.5). Maternal weight gain and the number of previous spontaneous abortions and stillbirths were comparable between the two groups. Exposure to cocaine in the third trimester was significantly associated with decreased birth weight and gestational age, but it should be noted that these results are based on only three male, exposed infants and other variables could potentially explain this contrast. The mean birth weight of the infants with and without third trimester cocaine exposure was 2880 grams (N = 3; S.D = 468) and 3521 grams (N = 76; S.D = 485) respectively (t = 2.25, p = .028). The mean gestational age among the infants with and without third trimester cocaine exposure was 37 weeks (N = 3, S.D. = 2.6) and 39.7 weeks (N = 76, S.D. = 1.6) respectively (t = 2.85, p = .006). Maternal smoking, expressed as the number of cigarettes smoked per day during pregnancy, was not significantly associated with reduced birth weight, birth length or head circumference.

Clinical and Digitized Morphometric Assessment of the Photographs

Midfacial retrusion/hypoplasia (defined as a relative posterior positioning of the zygoma and soft tissues relative to the nose and ear) and increased intercanthal distance were significantly associated with first trimester exposure to cocaine among the male children in this study. Cocaine exposure differed between the male and female children in this study population. Ten of the 13 exposed children were male and in general the male children had also received higher exposures. Since the sex of a child has a strong influence on facial size, it was necessary to perform the digitized morphometric assessment separately on the males and females. Upon doing so, the mean shape coordinates associated with triangles 23-14-19, 23-14-17 and 23-14-22, all reflecting midface flattening or retrusion, were found to significantly differentiate male children exposed to cocaine during the first trimester (n = 9) from the male children with no exposure to cocaine during pregnancy (n = 38) (T^2 = 6.44, p = .053; T^2 = 8.78, p = .019; T^2 = 7.91, p = .028 respectively) (Figure II.5). These effects were not attributable to differences in alcohol or marijuana exposure. No similar effect was observed in the three exposed females, all of whom were exposed to cocaine only once in the first trimester.

Exposure to three or more days of cocaine throughout gestation (n = 4) was associated with increased intercanthal distance relative to palpebral fissure length in the males when compared to the 44 males with a single exposure to cocaine or no exposure at all (T^2 = 11.6, p = .0065) (Figure II.6). This effect was not attributable to differences in alcohol or marijuana exposure. The intercanthal distances among the three females exposed to cocaine once in the first trimester were comparable to the intercanthal distances among the 29 unexposed females. These finding were corroborated in the distance measures taken directly from the photographs (Table II.6). The mean intercanthal distance among the four males exposed to cocaine three or more times throughout gestation (3.2 cm) was significantly longer than the mean distance among the 44 males exposed to lower levels of cocaine (2.8 cm) (T-test, T = 2.36, p = .03). Among the unexposed children (n = 67), males had significantly shorter intercanthal distances than the females (T-test, T = -2.7, p = .009). No significant differences were noted between the unexposed groups and the groups with only one exposure.

DISCUSSION

Cocaine use in the United States has escalated to near epidemic proportions. The increased availability of cocaine at reduced cost, coupled with the still widely held, but incorrect, belief that cocaine is a harmless drug has helped to build a strong market.

Individual consumption of cocaine varies widely. In the United States, the weekly consumption for individuals seeking treatment typically ranged from 9 to 30 grams (W.H.O., 1987). A recreational user is more likely to consume one to three grams per week. In 1983, the street cost of cocaine ranged from $75 to $150 per gram (Washton and Gold, 1987).

The availability of cocaine has resulted in increased use across all segments of the population, including pregnant women. In a sample of 679 women consecutively recruited from a Boston City Hospital prenatal clinic, self-report or urine assay results determined that 18% of the women used cocaine during pregnancy (Frank et al., 1988). These women represented the predominantly nonwhite, lower income, unmarried population served at Boston City Hospital. In contrast, among our screened sample of predominantly white, middle income, college educated women attending a Seattle prenatal clinic (n = 1100), three percent reported use of cocaine during pregnancy.

The changes in facial form observed among the cocaine exposed children in our study population were subtle. For all practical purposes, they would have gone unnoticed. It is important to note, however, that these facial changes were associated with relatively low levels of cocaine use in a population of women who would otherwise be classified as low risk for adverse perinatal outcomes. The facial effects, in and of themselves, are of little clinical relevance. The important factor is that patterns of facial anomalies are often valuable clinical markers for impaired behavior and intellectual function.

Two studies, to our knowledge, have presented evidence of craniofacial malformations associated with fetal exposure to cocaine. In a prospective study conducted in two large inner city hospitals in East Harlem and the South Bronx between 1984 and 1985, three groups of women and their offspring were compared at parturition (Bingol et al., 1987).

Group one included 50 women who used cocaine only, group two included 110 women who were polydrug users and group three included 3,340 women who were drug free. Sixty percent of group one women administered cocaine intranasally, averaging 20 to 50 mg/administration, approximately 1 to 5 g/week; 30% inhaled it in the form of free base, roughly 100 mg/"hit", averaging 1 to 1.5 g/day; and 10% used it intravenously, usually 10 to 30 mg per injection. Cranial defects were present only in the children in group one. One infant had parietal bone defects without herniation of meninges and two infants had delayed ossification centers of the calvarium. The cranial defects noted in that study were consistent with a report of significant delay in skull ossification occurring in CF-1 mice exposed to single injections of 60 mg/kg on days 11 and 12 of gestation (Mahalik et al., 1980). Additionally, the occurrence of cataracts in one child, with a single exposure to cocaine in the first trimester, is noteworthy in light of the reported increased rate of malformed or missing lenses observed in CF-1 mice exposed to single doses of 60 mg/kg cocaine on the seventh day of gestation (Mahalik et al., 1980).

Although the exact mechanism of cocaine teratogenicity is unknown, cocaine is known to interfere with dopamine and norepinephrine re-uptake resulting in placental vasoconstriction and subsequent fetal hypoxia (Richie and Greene, 1980; Moore et al., 1986; Woods et al., 1987). Fetal hypoxia has been shown to cause increased rates of craniofacial anomalies in rabbits and mice (Murakami and Kameyama, 1963; Badtke et al., 1959) and may be a mechanism leading to increased rates of anomalies in cocaine exposed infants.

In summary, fetal cocaine exposure in this population was associated with increased intercanthal distance and increased midface retrusion. These findings, although statistically significant, reflect the outcomes on a very small number of children, in a study that was not originally designed to look at cocaine effects. The results should be interpreted cautiously. One cannot infer from the results presented here that fetal cocaine exposure adversely affects craniofacial development. Larger, prospectively designed studies will be required to substantiate these preliminary findings. The fact that cocaine exposure was also associated with decreased birth weight and gestational age provides further support that cocaine was exerting an adverse affect on this study population. Additional evidence supporting the credibility of these findings lies in the

correspondence between the timing of the exposure and the critical period of development for each outcome. First trimester cocaine exposure was associated with facial dysmorphology and third trimester exposure was associated with decreased infant birth weight.

In light of the prevalence of cocaine use among pregnant women, these preliminary findings strongly support the need for further investigation.

Table II.1

Definition of the facial landmarks (Clarren et al., 1987b).

Landmark	Common name	Definition
		FRONTAL VIEW
1, 4		Intersection of the eyebrow curve and a vertical line through the exocanthion.
2, 3		Intersection of the eyebrow curve and a vertical line through the midpoint of the palpebral fissure.
5, 8	Exocanthion	Lateral intersection of upper and lower eyelids.
6, 7	Entrocanthion	Medial intersection of upper and lower eyelids.
9, 10		Most lateral points on alar curvature.
11, 13	Cheilon	Lateral intersection of upper and vermilion.
12		Midpoint of upper vermilion border.
		LATERAL VIEW
14	Nasion	Point of maximum curvature over nasal bridge.
15	Exocanthion	Lateral intersection of upper and lower eyelids.
16	Pronasale	Point of maximum curvature over nasal tip.
17	Subnasale	Intersection of columela and philtrum.
18		Point of maximum curvature of soft tissue fold from zygoma.
19		Border of upper vermilion and philtrum.
20	Cheilon	Lateral intersection of upper and lower vermilion.
21		Border of lower vermilion and lower lip.
22	Gnathion	Point of maximum curvature of chin.
23		External auditory opening.

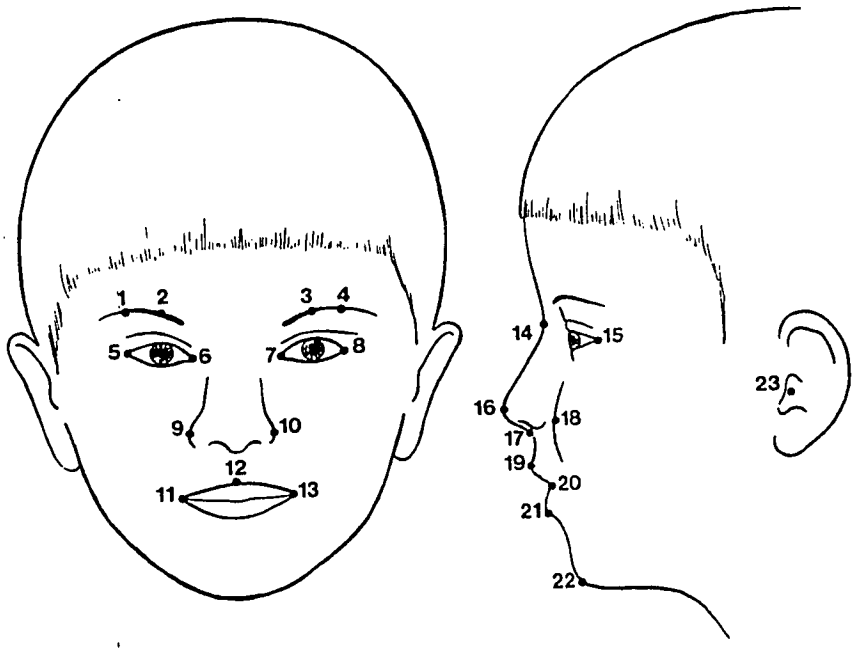


Figure II.1

Twenty-three facial landmarks were identified on the frontal and lateral photographs of each child (Clarren et al., 1987b). The coordinates associated with each landmark were entered into a database by computer digitization. The landmark definitions are presented in Table II.1.

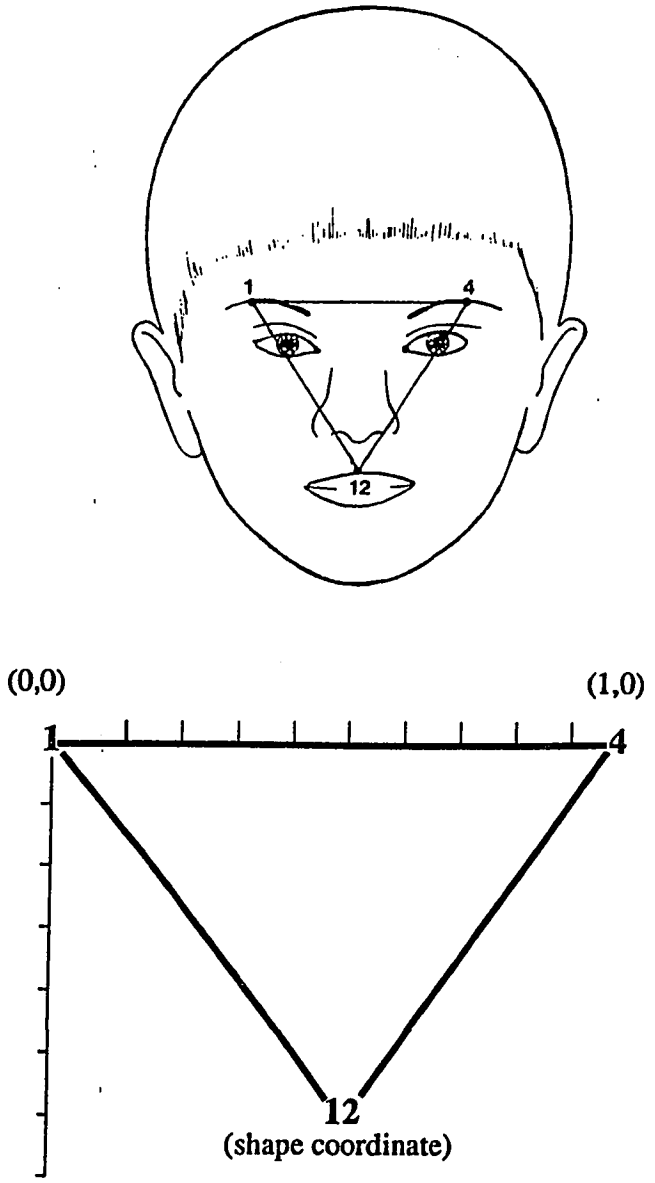


Figure II.2

The triangle formed by the three facial landmarks 1-4-12 is standardized on a Cartesian (x,y) coordinate system. Landmark (12) represents the shape coordinate for this triangle.

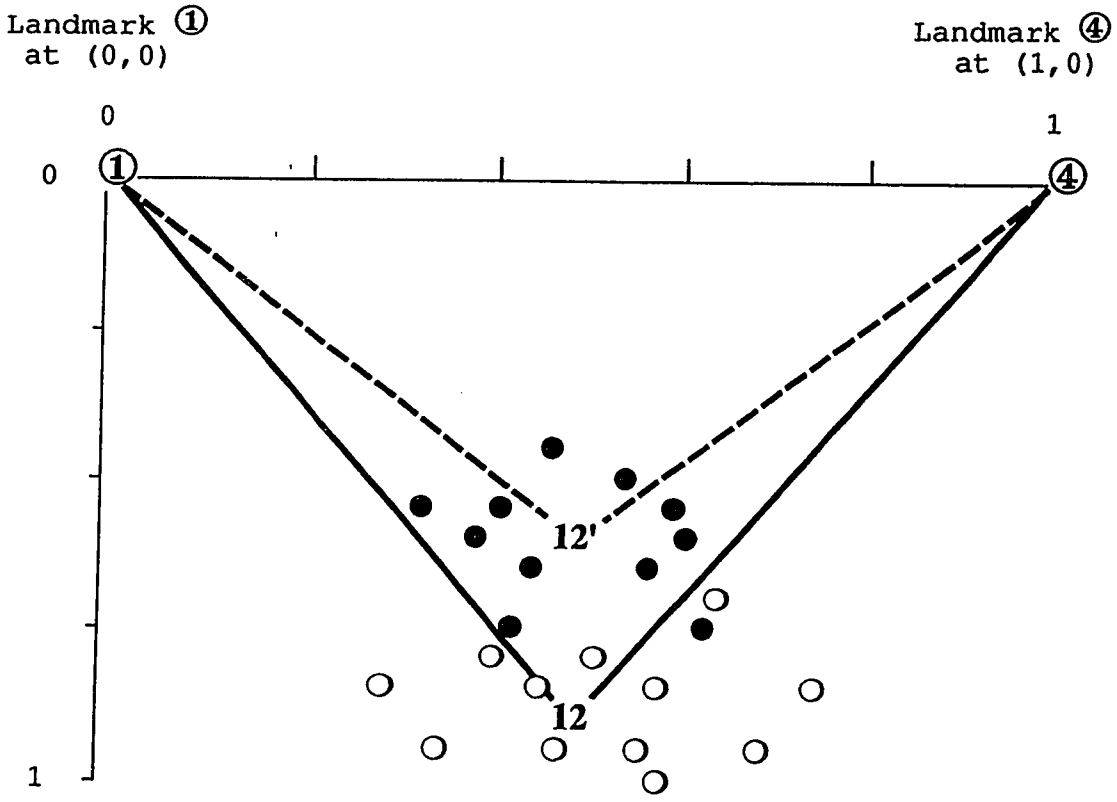


Figure II.3

The shape coordinates (landmark 12) of the exposed "●" and unexposed "○" subjects are displayed as a scatter plot in this hypothetical example. The scatter plot graphically illustrates the position of the midpoint of each child's upper vermilion border (landmark 12) relative to the position of landmarks 1 and 4 on the eyebrows. The mean shape coordinates for each group are represented by the average (or centroid) of the scatter of shape coordinates within each group. The locations of the mean shape coordinates for the exposed and unexposed groups are represented by **12'** and **12** respectively. The observed difference in shape between these two mean triangles would be assessed using Hotelling's T^2 statistic.

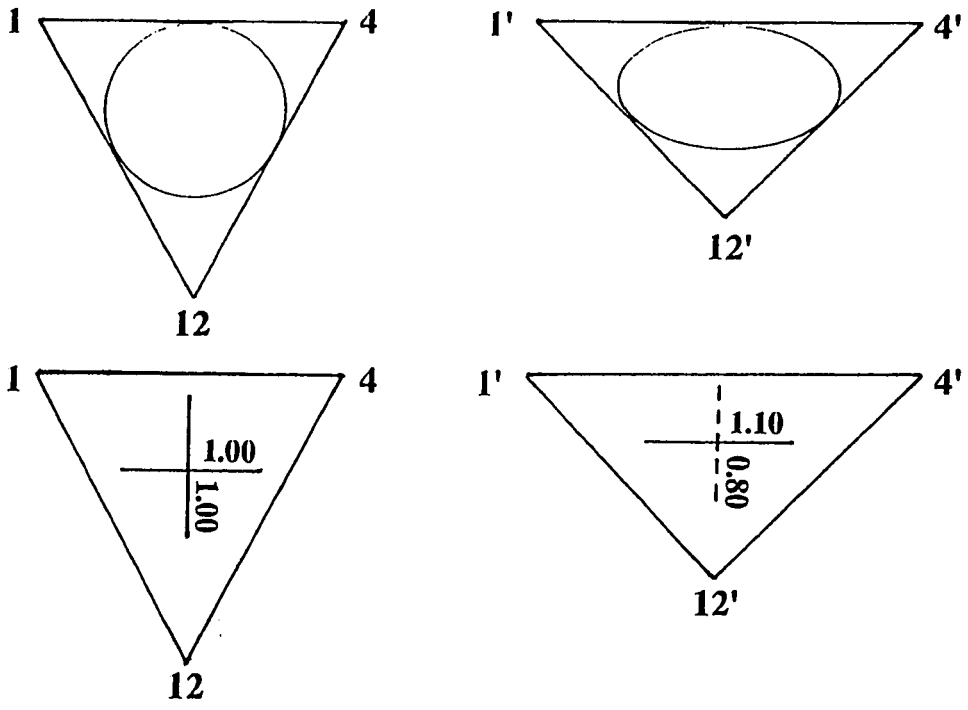


Figure II.4

The transformation of one triangle into another transforms a circle inscribed in the first triangle into an ellipse in the second. The axes (or tensors) of the ellipse, rescaled and oriented homologously in triangle (1-4-12) lie along the directions in which that triangle is most stretched and most compressed by the transformation. The magnitude of the stretching and compression is reflected in the length of the axes. The "exposed" triangle 1'4'12' resulted from a 10% horizontal expansion and a 20% vertical contraction of "unexposed" triangle 1-4-12.

Table II.2

Selected characteristics of women who did and did not use cocaine during pregnancy.

Characteristic	Cocaine	
	Unexposed (N = 67)	Exposed (N = 13)
	% or mean (range)	
Child's race (parent's race)		
caucasian-caucasian	90	92
caucasian-oriental	1.5	0
caucasian-chicano	1.5	0
caucasian-native American	1.5	0
caucasian-other	1.5	0
black-black	4	8
Child's age at the time of the photograph (years)	6.3 (4.9-7.5)	6.5 (5.4-7.5)
Marital status		
married	84	85
single	8	15
separated	8	0
Yearly income		
< $ 10,000	11	23
$ 10,000 to $ 24,999	30	31
> $25,000	53	38
unknown	6	8
Mother's age at child's birth	29.1 (19-38)	27.2 (17-34)
Number of abortions	0.4 (0-3)	0.7 (0-2)
Number of pregnancies	2.5 (1-11)	2.6 (1-5)
Number of live births	1.8 (1-6)	1.7 (1-4)
Mother's education		
did not complete high school	6	15
completed high school only	33	39
attended college	61	46

NO significant differences between exposed and unexposed groups.

Table II.3

Cocaine exposure among the female and male offspring.

Cocaine: total days of maternal use during pregnancy	Females (N = 32)		Males * (N = 48)	
	N	(%)	N	(%)
0	29	(91%)	38	(79%)
1	†3	(9%)	‡6	(13%)
3	0	(0%)	3	(6%)
27	0	(0%)	1	(2%)

* The male-female ratio among the cocaine exposed group (10 out of 13, 77%) is significantly different from an expected 50% (T-test: T = 2.21, p =.047).

† Received exposure in the first trimester only.

‡ One child was exposed only in the third trimester and five were exposed only in the first trimester.

Table II.4

Other drug use among the women who did and did not use cocaine during pregnancy.

Characteristic	Cocaine	
	Unexposed (N = 67)	Exposed (N = 13)
	% or mean (range)	
Trimesters when cocaine was used		
never	100	0
1st only	0	69
1st and 2nd only	0	8
1st and 3rd only	0	8
1st, 2nd and 3rd	0	8
3rd only	0	8
Frequency of cocaine use		
never	100	0
< once a month	0	77
once a month	0	8
2-3 times a month	0	15
Number of times cocaine was used per occasion during pregnancy		
never	100	0
1-3	0	85
4-6	0	15
Trimesters when marijuana was used		
never *	57	15
1st only	12	23
1st and 2nd only	5	0
1st and 3rd only	1	8
all three	25	54
Freq of marijuana use		
never	57	15
1-2 times a week	17	31
3-4 times a week	13	15
every day	13	39
Number of joints smoked per occasion		
0	57	15
1	28	46
2-5	15	31
10	0	8

Table II.4 (continued)

Other drug use among the women who did and did not use cocaine during pregnancy.

Characteristic	Cocaine	
	Unexposed (N = 67)	Exposed (N = 13)
	% or mean (range)	
Smoked cigarettes during pregnancy †	40	92
Number of cigarettes smoked per day		
none	61	8
1-10	15	54
11-20	22	15
21-40	2	23
Average mg of nicotine per day among smokers	9 (0-30)	9 (0-40)
Ave. ounces of alcohol per day the month before pregnancy (AA score)		
< 1	54	46
1 to 2	45	54
> 2	1	0
Ave. ounces of alcohol per day during pregnancy (AA score)		
< 0.5	99	92
1.0	1	8

* No use of marijuana versus use of marijuana: $\chi^2 = 5.9$ (2 df), p = .02.

† Smoked versus did not smoke: $\chi^2 = 9.8$ (2 df), p = .002.

Table II.5

Birth outcomes among the infants whose mothers did and did not report using cocaine during pregnancy.

Pregnancy Outcomes	Cocaine	
	Unexposed (N = 67)	Exposed (N = 13)
	% or mean (range)	
Child's sex		
female	43	23
Gestational age in weeks		
< 36	3	8
36 to 42	93	92
> 42	3	0
unknown	1	0
Gestational age in weeks	39.7 (32-43)	39.2 (35-42)
Birth weight in grams		
< 2500	1.5	8
2500 to 4000	79	77
> 4000	18	15
unknown	1.5	0
Birth weight in grams (36 to 42 weeks)	3523 (2800-4870) n = 63	3507 (3100-4280) n = 12
Body length at birth (cm)	50.8 (45-56)	50.0 (48-54)
Head circumference (cm)	34.5 (31-39)	34.2 (33-36)
APGAR score (1 minute)		
< 7	10	15
7 to 10	87	85
unknown	3	0
APGAR score (5 minute)		
< 7	0	0
7 to 10	97	100
unknown	3	0

Table II.5 (continued)

Birth outcomes among the infants whose mothers did and did not report using cocaine during pregnancy.

Pregnancy Outcomes	Cocaine	
	Unexposed (N = 67)	Exposed (N = 13)
	% or mean (range)	
Maternal weight gain (lbs)	36 (10-60)	40 (30-69)
Spontaneous fetal deaths		
trimester one	0.2 (0-4)	0.2 (1-2)
trimester two	0.03 (0-1)	0
Stillbirths	0.03 (0-1)	0

Table II.6

Maternal cocaine use during pregnancy
and palpebral fissure length (cm), intercanthal distances (cm),
and intercanthal distance / mean (right and left) fissure length
among male and female offspring

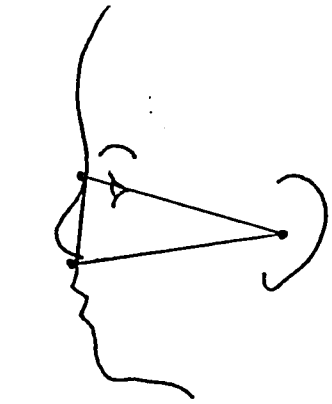
Cocaine Use	*Palpebral Fissure lengths (cm)		Intercanthal distance (cm)		Intercanthal/ *Palpebral Fissure	
	mean	(SD)	mean	(SD)	mean	(SD)
Males (n = 48)						
No Exposure (n = 38)	2.46	(.17)	†2.86	(.25)	1.17	(.10)
1 day (N = 6)	2.50	(.25)	2.89	(.26)	1.16	(.15)
3 to 27 days (n = 4)	2.37	(.11)	‡3.17	(.39)	§1.33	(.15)
Females (n = 32)						
No Exposure (n = 29)	2.45	(.17)	3.02	(.23)	1.24	(.13)
1 day (N = 3)	2.53	(.21)	2.96	(.21)	1.17	(.16)

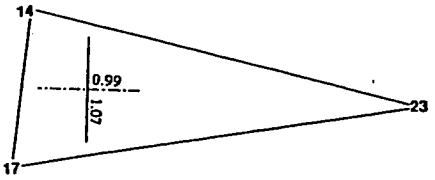
* Represents the mean length of the right and left palpebral fissures.

† compared to the 29 unexposed females (t-test: t = -2.7, p = .009)

‡ compared to the mean of the 44 males with less exposure (t-test: t = 2.4, p = .03)

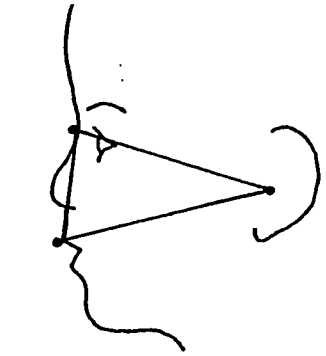
§ compared to the mean of the 44 males with less exposure (t-test: t = 2.8, p = .007)

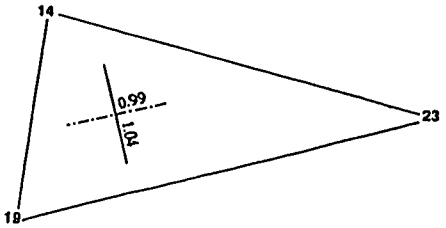




Triangle 23-14-17

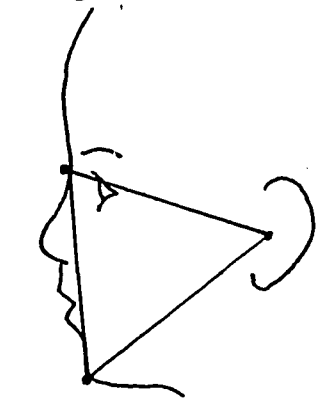
T-square = 8.78
p = .019

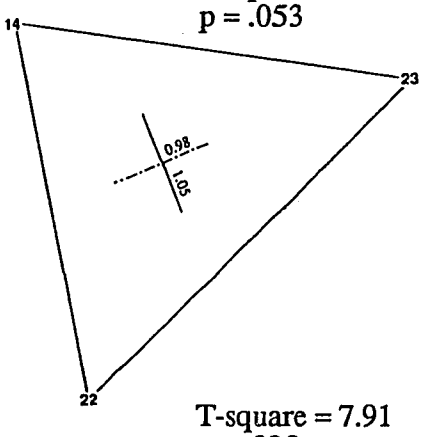




Triangle 23-14-19

T-square = 6.44
p = .053





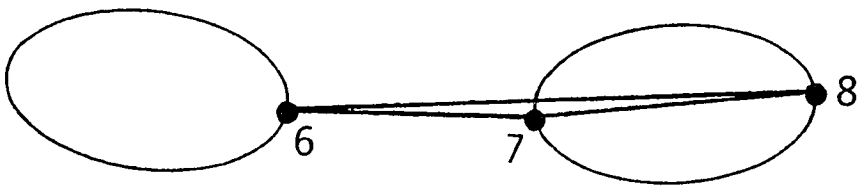
Triangle 23-14-22

T-square = 7.91
p = .028

Figure II.5

Midface retrusion associated with first trimester fetal cocaine exposure among males.

Males with 1 to 9 days of first trimester cocaine exposure (n = 9) are contrasted with males with no cocaine exposure (n = 38)



Triangle 6-8-7

T-square = 11.6
p = .0065

Figure II.6

Increased intercanthal distance in males associated with maternal use of cocaine throughout pregnancy.

Males with 3 or more days of gestational exposure (n = 4) are contrasted to males with 0 or 1 day of gestational exposure (n = 44) to cocaine.

Chapter III

Manuscript # 3

Maternal Alcohol, Cocaine and Marijuana Use During Pregnancy and Sex Ratios Among Live-born Offspring

ABSTRACT

The sex ratio of live-born offspring associated with maternal use of alcohol, marijuana and cocaine early in pregnancy was investigated in a study population of 1073 mother-infant pairs. The investigation used data that was obtained in a Seattle-based, prospective study (1982-87) investigating the role of maternal diet, drinking, smoking and marijuana use during lactation on infant growth and development at one year. The mothers of the children in this study were predominantly white, middle income, college educated women between 15 and 45 years of age. Of the 1073 women, 935 reported use of alcohol in the month prior to conception and 163 and 37 reported use of marijuana and cocaine respectively during pregnancy.

Among women who reported no use of marijuana or cocaine, the proportion of female offspring decreased from 51% among 126 women who consumed no alcohol to 49% among 717 women who consumed 0.01 to 1.9 ounces of alcohol per day to 38% among 44 women who consumed 2 to 5 ounces per day in the month prior to conception. Daily use of marijuana early in pregnancy among women who consumed 2 to 5 ounces of alcohol prior to pregnancy resulted in a significant dose related decrease in the proportion of female offspring born. Use of cocaine during pregnancy also resulted in a marked decrease in the proportion of female offspring born, independent of alcohol consumption. These findings are supported by some, but not all reports of altered offspring sex ratios associated with in-utero exposure to these three drugs.

INTRODUCTION

Experimental studies have shown that in-utero exposure to alcohol, marijuana and cocaine is associated with increased fetotoxicity (Hutchings et al., 1989; Mahalik et al., 1980; Church et al., 1988; Clarren et al., 1987a). The studies by Hutchings et al., (1989) and Mahalik et al., (1980) have also observed altered sex ratios among fetuses and live born offspring, suggesting that there may be a selective lethal effect on the conceptus. Altered sex ratios associated with maternal use of alcohol or marijuana have also been documented in the human literature (Tennes et al., 1985; Iosub et al., 1981; Qazi and Masakawa, 1976; Fried, 1982). In the present report, the association between maternal use of alcohol, marijuana and cocaine during pregnancy and the sex ratio of live-born offspring was investigated.

METHODS

Subjects

The research presented here uses data that was obtained in a Seattle-based, prospective study (1982-87) investigating the role of maternal diet, drinking, smoking and marijuana use during lactation on infant growth and development. There were three parts to the prospective study. First, validity of self-reported alcohol, tobacco and other drug use and the reliability of the maternal interview process was confirmed (Little, et al., 1984, 1986). Next, descriptive studies were conducted to contrast the dietary habits and alcohol and tobacco use of lactating and non-lactating women (Little et al., in press; Worthington-Roberts et al., 1989a, 1989b). And finally, two infant assessment studies were conducted that explored the relationship between alcohol and marijuana use during lactation and infant development at one year of age (Little et al., 1989; Astley & Little, 1989).

All subjects were members of Group Health Cooperative of Puget Sound, a health maintenance organization in Seattle, WA. All prenatal patients receiving care between May 1, 1982 and July 1, 1984 were contacted by the Cooperative in their sixth month of pregnancy regarding possible study participation. Seventy-four percent (5,298) of the prenatal patients responded and completed a mailed screening questionnaire detailing

their alcohol and tobacco use both before and during pregnancy, some dietary information, and their plans to lactate. These patients constituted a screened pool.

A total of 1100 women were selected from the screened pool to participate in one or more or the substudies listed above. Selection for each substudy was based on maternal nutritional factors and/or substance use during pregnancy and lactation. All 1100 births were liveborn, singleton births.

Of the 1100 mother-infant pairs, the sex of the child was recorded for 1073 of the pairs. These 1073 mother-infant pairs make up the study population for the current report.

Data Collection

Detailed information on maternal use of alcohol, tobacco, marijuana, cocaine and other licit and illicit drugs during pregnancy was collected by personal interview at one month postpartum. Demographic characteristics and obstetric history were also collected. The interviewers were women of childbearing age, trained to obtain valid and reliable information. Maternal use of alcohol in the month prior to conception was collected in a mailed screening questionnaire completed during the sixth month of pregnancy. Validity of self-reported drug use was confirmed in an earlier pilot investigation of 108 randomly selected postpartum women. Self-reported drug use was compared to laboratory tests of drug levels present in body fluids. The proportion of questionable self-reports ranged from 0 to 3% depending on the drug (Little et al., 1984).

Maternal use of marijuana and cocaine was recorded in terms of how often the substance was used in each trimester. Alcohol consumption was categorized into beer, wine and liquor and was recorded in terms of frequency of use, modal quantity, and maximum quantity per drinking occasion. These measures were converted to average daily ounces of absolute alcohol ingested per day (AA score) (Jessor et al., 1968).

Statistical Analysis

The study population was stratified on maternal use of marijuana, alcohol and cocaine. χ^2 and Fisher's exact test were used to evaluate the frequency of female offspring within each exposure category.

RESULTS

The study population was comprised of predominantly white, middle income, college educated women. Their ages ranged from 15 to 45 with a mean of 28 years (Table III.1).

Of the 1073 mothers, 935 (87%) reported use of alcohol in the month prior to conception (Table III.2). Seven percent reported consuming two or more ounces of alcohol per day during that period. Sixty percent of the women reported use of alcohol during pregnancy. The highest level of consumption during pregnancy was one to two ounces per day reported by twelve women. Fifteen percent of the women reported use of marijuana during pregnancy and three percent of the women reported use of cocaine. Use of alcohol in the month prior to conception and use of marijuana and cocaine during pregnancy were significantly correlated. The Pearson correlation coefficients were: alcohol-marijuana (.20, $p<.001$), alcohol-cocaine (.15, $p<.001$) and marijuana-cocaine (.32, $p<.001$).

When the study population was stratified separately on maternal use of alcohol, marijuana and cocaine, the proportion of viable females decreased, although not significantly, with increasing maternal use of each substance (Table III.3). Among the 888 subjects with no reported use of marijuana or cocaine, the proportion of females continued to decrease, although not significantly so, with increasing exposure to alcohol in the month prior to conception. When the study population was stratified simultaneously on marijuana and alcohol use, maternal use of marijuana during pregnancy did not appear to influence the offspring sex ratio among women who reported no or very low levels of alcohol in the month prior to conception (Table III.4). The proportion of female offspring did decrease with increasing maternal use of marijuana among women who consumed one or more ounces of alcohol in the month

prior to conception. When the population was stratified simultaneously on alcohol and cocaine use, cocaine appeared to be associated with a decreased proportion of female offspring independent of maternal alcohol consumption in the month prior to conception (Table III.5). Maternal use of both alcohol and cocaine appeared to have a stronger influence than use of either substance alone. The number of women who reported using cocaine during pregnancy, however, was relatively small (n = 37) limiting the conclusions that can be drawn about the independent effect of cocaine in this study sample. Maternal use of all three drugs was associated with a decreased proportion of female offspring when compared to a group that reported no use of alcohol, marijuana or cocaine (Table III.3). The proportion of female offspring (38%) was comparable to the proportion among the group that used only alcohol (37%).

DISCUSSION

The results of this investigation suggest that maternal consumption of two or more ounces of alcohol per day very early in gestation may be associated with a decrease in the proportion of viable female offspring born in this study population. There are no studies, to our knowledge, with which to directly compare this finding. Despite the rather large number of studies that have collected detailed information on maternal consumption of alcohol during pregnancy, few have reported offspring sex ratios resulting from those pregnancies. In a medical-record based prospective study of 12,127 pregnancies, Sokol et al. (1980) reported a ratio of 45% female offspring among a group of 204 women with alcohol abuse recorded as an antepartum risk factor. A ratio of 50% females was observed among 11,923 women with no record of alcohol abuse. Although actual levels of maternal alcohol consumption were not available in that study, the proportion of female offspring born to the alcohol-abuse group was lower, although not significantly so. There is also one other publication that noted a differential effect of alcohol exposure on male and female conceptuses. It does not refer specifically to embryo lethality, but rather to a less severe manifestation of alcohol damage - decreased birth weight. The study was a hospital-based prospective study of 1491 mother/infant pairs in Finland investigating the effects of maternal tobacco and alcohol use during pregnancy on infant birth weight (Kariniemi and Rosti, 1988). Alcohol consumption was associated with a significant decrease in the birth weight of female fetuses (90 g): whereas, it had no measurable affect on the birth weight of male

fetuses. Exact levels of maternal alcohol consumption were not recorded, but the authors noted that those mothers who admitted alcohol consumption, claimed it to be moderate. No one was suspected of being a heavy drinker and there was not a single case of FAS diagnosed among the babies.

Women in the current study population who consumed alcohol were also more likely to use cocaine and/or marijuana. When the independent affect of alcohol consumption on offspring sex ratios was examined, the proportion of female offspring did decrease with increasing exposure to alcohol (Table III.3b), but the association was not statistically significant. In contrast, a significant reduction in the proportion of female offspring was observed among nonhuman primates exposed weekly to ethanol in utero (Clarren, personal communication). A significant dose-related decrease in live-born female offspring occurred with increasing maternal alcohol exposure (0 to 1.8 g/kg) starting in the first week of gestation (Table III.6). Weekly doses of 2.5 to 4.1 g/kg were 100% fetotoxic when administration began in the first week. When administration was delayed until after the 30th day of gestation, both the conception rate (52%) and the proportion of live born females (43%; 3 out of 7) were comparable to the controls.

Daily use of marijuana also appeared to be associated with a decreased proportion of female offspring in the current study population (Tables III.3d and III.4). Tennes et al., (1985) noted a similar observation among 756 mother-infant pairs participating in a prospective study of fetal marijuana exposure. Women who reported smoking marijuana three times a week or more throughout pregnancy (n = 31) gave birth to 39% females as compared with 50% females among nonusers (n = 498). Tennes reported an even stronger association between paternal use of marijuana and reduced numbers of female offspring, suggesting an effect at the level of spermatogenesis. Decreased sperm motility and abnormal sperm morphology have been associated with heavy marijuana use among males (Hembree et al., 1979). There is undoubtedly a strong correlation between paternal and maternal use of marijuana, which would make it difficult to isolate the independent contributions of each on the birth outcome.

Use of marijuana was strongly correlated with use of alcohol in both the current study and the study by Tennes and colleagues (1985). When the current study population was stratified on maternal use of alcohol and marijuana, use of marijuana alone did not

appear to influence the sex ratio (Table III.4). Heavy use of marijuana among women who reported consuming two to five ounces of alcohol in the month prior to conception, however, was associated with a significant decrease in the proportion of female offspring. This observation is based on a very small number of subjects and will require further study on larger populations to confirm. It is interesting to note, however, that Abel (1985) reported a substantial increase in fetotoxicity associated with combined exposure to marijuana and alcohol in rats. Increased fetotoxicity was not associated with exposure to alcohol or marijuana alone. Offspring sex ratios were not reported.

Although marijuana exposure alone was not associated with significantly reduced female-to-male offspring ratios in the current study, in utero exposure to marijuana alone has been associated with reduced proportions of female offspring in several animal studies. Hutchings et al., (1989) reported a significant dose-related decrease in the sex ratio of live female-to-male offspring in rats exposed daily to 15 or 50 mg/kg tetrahydrocannabinol (THC) from the eighth day of gestation. The proportion of female offspring was 46% and 41% respectively compared to nontreated controls (53%). Higher exposure levels in the rats may account for the contrast between our study results. A similar result was reported by Morgan et al., (1988) among rats exposed to 15 or 50 mg/kg THC from gestational days 2 through 22. The proportion of female offspring was 45% and 39% respectively compared to 59% among pair-fed controls. The authors noted that the altered sex ratios resulted from a selective lethal effect on female embryos. A reduction in the proportion of viable female fetuses was also reported by Sofia et al. (1979) among rabbits exposed to 30 mg/kg THC daily, from gestational days 7 through 19. Forty percent of the offspring were female compared to 53% among the controls.

Cocaine use during pregnancy was also associated with a rather marked decrease in the proportion of female offspring in this study (Table III.3c). The association appeared to be independent of maternal use of alcohol in the month prior to conception (Table III.5). These observations again are based on a small number of women who reported use of cocaine. Evaluations of larger study populations are necessary to confirm these observations.

Clinical and epidemiologic reports of offspring sex ratios associated with maternal exposure to cocaine during pregnancy are mixed. Oro et al., (1987) reported 35% female offspring among a group of 46 women who used cocaine and/or amphetamine during pregnancy. Forty-four percent of the 45 drug free control offspring were female. Cocaine exposure was confirmed at the time of birth by maternal and infant toxicology screens. Although the reported reduction in the proportion of females is consistent with our results, the exposed group included women that used cocaine and/or amphetamine. The independent effect of cocaine cannot be ascertained from the reported data. MacGregor et al., (1987) reported 46% females among 70 women that used cocaine during pregnancy compared to 53% females among 70 controls with no known exposure to drugs during pregnancy. Sixty-six percent of the women that used cocaine also used other drugs such as marijuana, opiates, amphetamines and LSD - again making it impossible to ascertain from the reported data, the independent influence of cocaine. Hadeed & Siegel (1989) and Cherukuri et al. (1988) reported no significant differences in the proportion of female offspring in study populations of 56 and 55 women respectively who used cocaine. The exposed group in Hadeed & Siegel's study was restricted to women who screened positive for cocaine only with no history of alcohol abuse. The exposed group in the study by Cherukuri et al. (1988) included women who used both cocaine and alcohol. In all of the studies cited above, information on early pregnancy use of cocaine was not reported. It is during this period that the conceptus is most susceptible to the lethal effects of a drug.

In the animal literature, Hutchings et al (1989) reported no differences in offspring sex ratios among rats exposed to 30 or 60 mg/kg cocaine daily from gestational day 8 through 22. In contrast, Mahalik et al., (1980) reported a significant increase in male offspring in one of six groups of rats exposed to single injections of 60 mg/kg cocaine.

Overall, it is difficult to make meaningful comparisons between the results of the present study and the studies cited above because of the lack of comparability in exposure classification. The most notable alterations in sex ratios in our data appeared to be associated with the groups that reported high levels of multiple drug use early in pregnancy. Most of the studies cited above did not collect or report "levels" of exposure within their study populations; the study populations were simply dichotomized as "exposed" or "unexposed". If our data had been simply collapsed into

"exposed" and "unexposed" groups, most of the variations in offspring sex ratios would not have been apparent.

In summary, the results of this investigation suggest that the female conceptus may be more susceptible than the male to the toxic effects of high alcohol or cocaine exposure early in gestation. The results are less clear with respect of maternal marijuana use, but there is evidence that the combined use of alcohol and marijuana may be more deleterious than use of alcohol alone. Although these results are supported, in part, by a few reports in both the animal and human literature, there are an equal number of reports that either appear to conflict with our findings or simply have not reported offspring sex ratios. Confirmation of these results may require little more than evaluating offspring sex ratios in the large prospective studies that have already been completed. Many of these studies have collected reliable estimates of maternal alcohol and drug use prior to and during pregnancy and have sufficiently large study populations to effectively separate the effects of alcohol from the potential effects of other drugs.

Table III.1

Selected maternal characteristics among 1073 women.

Characteristics	N or mean	% (range)
Maternal race		
Caucasian	994	93 %
other	79	7 %
Marital status		
married	948	88 %
not married	125	12 %
Yearly income		
< $ 10,000	72	7 %
$ 10,000 to $ 24,999	410	38 %
> $25,000	562	52 %
unknown	29	3 %
Mother's education		
did not complete high school	54	5 %
completed high school	311	29 %
attended college	708	66 %
Mother's age at child's birth	28.4	(15-45)
Previous abortions	0.4	(0-4)
Previous pregnancies	2.4	(1-11)
Previous live births	1.8	(1-8)

Table III.2

Maternal drug among 1073 women.

Maternal Drug Use	N	(%)
Trimesters when marijuana was used		
never	909	(85)
1st trimester	125	(12)
2nd and/or 3rd trimester only	39	(3)
Frequency of marijuana use		
never	909	(84)
<1 to 3 times per month	95	(9)
1 to 5 times a week	51	(5)
every day	17	(2)
unknown	1	(<1)
Trimesters when cocaine was used		
never	1036	(97)
1st trimester	35	(3)
2nd and/or 3rd trimester only	2	(<1)
Frequency of cocaine use		
never	1036	(97)
< once a month	31	(3)
1 to 3 times a month	6	(<1)
Alcohol use in the month prior to conception (ave. ounces of absolute alcohol per day)		
never	132	(13)
0.01 to 1.9	862	(80)
2.0 to 5.0	73	(7)
unknown	6	(<1)
Alcohol use during pregnancy (ave. ounces of absolute alcohol per day)		
never	428	(40)
0.01 to 0.9	630	(59)
1.0 to 2.0	12	(1)
unknown	3	(<1)

Table III.3

Maternal use of alcohol, cocaine and marijuana during pregnancy and corresponding offspring sex ratios.

(a) **Alcohol use in the month prior to conception**

(ounces of absolute ethanol per day)

	0 to 0.9		1 to 1.9		2.0 to 5.0	
Females *	383	**(50%)**	113	**(51%)**	27	**(37%)**
Males	390		107		46	

* $\chi^2 = 4.8$ (2 df), $p = .09$ Total N = 1066

(b) **Alcohol use in the month prior to conception (no marijuana or cocaine use during pregnancy)**

	0 to 0.9		0.01 to 1.9		2.0 to 5.0	
Females **	336	**(49%)**	83	**(52%)**	17	**(38%)**
Males	347		77		28	

** $\chi^2 = 2.8$ (2 df), $p = .24$ Total N = 888

(c) **Cocaine use during pregnancy**

	never		< once a month to 3 times a month	
Females †	516	**(50%)**	11	**(30%)**
Males	520		26	

† $\chi^2 = 5.8$ (1 df), $p = .03$ Total N = 1073

Table III.3 (continued)
Maternal use of alcohol, cocaine and marijuana during pregnancy and corresponding offspring sex ratios.

(d) Marijuana use during pregnancy

	0 to 3 times a month		1 to 5 times a week		every day	
Females ‡	486	**(49%)**	22	**(43%)**	6	**(35%)**
Males	496		29		11	

‡ $\chi^2 = 2.1$ (2 df), $p = .35$ Total N = 1050

(e) Use of alcohol, cocaine and marijuana during pregnancy

	NO alcohol, marijuana or cocaine		Heavy use ¶ of alcohol, marijuana and cocaine	
Females §	67	**(51%)**	40	**(37%)**
Males	65		70	

§ $\chi^2 = 4.7$ (1 df), $p = .03$ Total N = 242

¶ 2 to 5 ounces of alcohol per day in the month prior to conception, daily use of marijuana during pregnancy and use of any quantity of cocaine during pregnancy.

Table III.4

Proportion of live-born female offspring associated with maternal use of alcohol and marijuana during pregnancy (N = 1066).

	Marijuana use during pregnancy			
AA score *	0 to 1/month (N = 976)	3-4/month 2-5/week (N = 73)	every day (N = 17)	
0 to 0.9 (N = 773) †	**50 %** (n = 725)	**46 %** (n = 39)	**67 %** (n = 9)	$\chi^2 = 1.2$ p = 0.54
1 to 1.9 (N = 220)	**51 %** (n = 194)	**64 %** (n = 22)	**0 %** (n = 4)	$\chi^2 = 5.6$ p = 0.06
2 to 5 (N = 73)	**44 %** (n = 57)	**17 %** (n = 12)	**0 %** (n = 4)	$\chi^2 = 5.3$ p = 0.05
	$\chi^2 = 0.9$ p = 0.63	$\chi^2 = 6.9$ p = 0.03	$\chi^2 = 8.2$ p = 0.02	

* Average ounces of absolute alcohol per day in the month prior to conception.

† All sample sizes in parentheses reflect the total number of males and females.

Table III.5

Proportion of live-born female offspring associated with maternal use of alcohol and cocaine during pregnancy (N = 1067).

	Frequency of cocaine use during pregnancy		
AA score *	never (N = 1030)	1 to 3 times/month (N = 37)	
0 to 0.9 (N = 774) †	**50 %** (n = 755)	**37 %** (n = 19)	$\chi^2 = 1.3$ p = 0.26
1 to 1.9 (N = 220)	**53 %** (n = 209)	**18 %** (n = 11)	$\chi^2 = 5.1$ p = 0.02
2 to 5 (N = 73)	**38 %** (n = 66)	**28 %** (n = 7)	$\chi^2 = 0.2$ p = 0.63
	$\chi^2 = 4.7$ p = 0.09	$\chi^2 = 1.2$ p = 0.56	

* Average ounces of absolute ethanol per day in the month prior to conception.

† All sample sizes in parentheses reflect the total number of males and females.

Table III.6

Sex ratio among 32 full-term offspring of nonhuman primates (*Macaca nemestrina*) exposed to ethanol weekly throughout gestation.

Maternal Dose g/kg EtOH	N	Male Offspring	Female Offspring	% Females *
0	7	3	4	57
0.3	7	4	3	43
0.6	6	4	2	33
1.2	6	4	2	33
1.8	6	5	1	17

* Linear regression analysis: $F = 21.3$, $p = .02$, $r^2 = .88$

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Appendix A
Consent and Assent Forms

CHILDREN'S HOSPITAL AND MEDICAL CENTER,
GROUP HEALTH COOPERATIVE AND
UNIVERSITY OF WASHINGTON

CONSENT FORM

Facial Development Study

Sterling K. Clarren, M.D., Professor
Department of Pediatrics 526-2206
Principal Investigator

Ruth E. Little, Sc.D., Associate Professor
Department of Epidemiology, University of Michigan
Study Consultant

STUDY OFFICE: 526-2206

PURPOSE OF THIS STUDY

Several years ago you helped us do a study of how a mother's diet, drinking, smoking and drug use habits during pregnancy and lactation influence the well being of her developing child. We would now like to look at facial development that is measurable when children are between four and seven years old. We would like to see if maternal habits during the first trimester of pregnancy influence infant facial development. Maternal variables which were reported on the prepartum screening form and the one-month postpartum interview from the previous study will be used in this follow-up study. The maternal variables that we would like to assess include nutrition, alcohol and caffeine consumption, tobacco and marijuana use and use of prescription and non-prescription drugs. Your participation will help us further identify the things that give children the best chance to grow and develop well.

PROCEDURE

Sterling Clarren, M.D. is the Principal Investigator for this study. Dr. Clarren was a member of the research staff on the previous study. He is a pediatrician at Children's Hospital and Medical Center in Seattle and is a faculty member at the University of Washington. Ruth E. Little, Sc.D. will participate as a research consultant. We will be taking two facial photographs of your child, a frontal view and a side view. Your presence is welcome and encouraged throughout the photo session. The session will take approximately 45 minutes and will take place at Children's Hospital and Medical Center in Seattle.

RISK, STRESS OR DISCOMFORTS

The risks associated with having a photograph taken, as you well know, are extremely small. There will be no stress or discomfort for you or your child in this study.

COMPENSATION

As a token of our appreciation, you will receive $25 upon completion of the photographic session.

PROTECTION OF YOUR PRIVACY

We realize that the taking of photographs are personal, and we want to reassure you that you or your child's name will never be attached to any of the photographs or records connected with this study. We will always use your study code number. This number and not the name of you or your child will be attached to the photographs and records. The key to the identity of these numbers will be kept in a locked file. You or your child's name will never be used in connection with any of the study results. Only study staff members will have access to the identity of the participants. The photographs will not be published. They will be retained for an indefinite period of time by Dr. Clarren. None of the data in your research records will be made available to your GHC physicians unless specifically requested by you. If you wish to withdraw from the study, the photos and negatives will be destroyed at your request. Please call the research office (526-2206) about this research or call the GHC Center for Health Studies (448-2932) or Children's Hospital (526-2023) if you would like more information regarding your rights as a research subject.

You are free to withdraw from the study at any time without jeopardizing your care at Group Health or without loss of other benefits to which you are otherwise entitled.

_____ _____

Sterling K. Clarren, M.D.
Principal Investigator

Date

PARENT/LEGAL GUARDIAN'S STATEMENT

The facial development study described above has been explained to me and I voluntarily consent to have my child participate. I have had the opportunity to ask questions. I understand that future questions I may have about this research or about subject's rights will be answered by one of the investigators listed above. I understand that CHMC does not have no-fault insurance coverage and that CHMC will not agree voluntarily to provide compensation for injuries that my child may suffer as a result of participating in this research project. Medical treatment provided as part of this protocol, and treatment for any physical injury or other adverse effects will be covered by GHC to the extent of your GHC coverage as long as you remain an enrollee of GHC. If you are no longer enrolled at GHC, this care will not be covered by GHC.

Date	SIGNATURE OF CHILD
Date	PARENT/LEGAL GUARDIAN

Copies to: Subject/parent
Investigator's file

CHILDREN'S HOSPITAL AND MEDICAL CENTER,
GROUP HEALTH COOPERATIVE
AND
UNIVERSITY OF WASHINGTON

ASSENT FORM

Facial Development Study

Sterling K. Clarren, M.D., Professor
Department of Pediatrics 526-2206
Principal Investigator

Ruth E. Little, Sc.D., Associate Professor
Department of Epidemiology, University of Michigan
Study Consultant

STUDY OFFICE: 526-2206

ORAL EXPLANATION FOR THE CHILD

We would like to take two pictures of your face. We will have you sit in a chair and put your chin on a chin-rest that looks like this. This will help you sit still when we take your picture. You will need to sit in the chair for about five minutes each time we take a picture.

Do you have any questions? Is this OK with you?

If you change your mind and don't want us to take your picture, tell me and we will stop.

_____ _____

Date SIGNATURE OF CHILD

_____ _____

Date PARENT/LEGAL GUARDIAN

CURRICULUM VITAE Susan Jean Astley

DATE OF BIRTH

November 17, 1955

PLACE OF BIRTH

Chicago Heights, Illinois USA

EDUCATION

B.S.	University of Illinois Champagne, Illinois	1977	Biology
M.S.	Oregon State University Corvallis, Oregon	1979	Physiology Radiology
Ph.D.	University of Washington Seattle, Washington	1988	Epidemiology

POSITIONS HELD

1976-77	Teaching Assistant, Phycology, Cornell University, Shoals Marine Laboratory, Appledore Island, Maine
1977-78	Teaching Assistant, Phycology, Oregon State University Marine Science Center, Newport, Oregon
1978-79	Teaching Assistant, Biology, Department of Radiology, Oregon State University, Corvallis, Oregon
1979-80	Research Assistant, Marine Toxicology, Department of Radiology, Oregon State University, Corvallis, Oregon
1980-81	Environmental Consultant, Northwest Environmental Consultants, Seattle, Washington
1981-82	Technician, Regional Primate Research Center, University of Washington, Seattle, Washington
1982-86	Research Technologist, Fetal Alcohol Syndrome, Department of Pediatrics, University of Washington, Seattle, Washington
1987-88	Research Assistant, Maternal and Child Health, Department of Epidemiology, University of Washington, Seattle, Washington
1987-	Research Scientist, Fetal Alcohol Syndrome, Department of Pediatrics, University of Washington, Seattle, Washington
1988-	Research Assistant, Study Design Consultant, Department of Epidemiology, University of Washington and Children's Hospital and Medical Center, Seattle, Washington
1989-	Visiting Scientist, Institute of Experimental Pathology and Therapy, Gora Trapetziva Sukhumi, USSR. US-USSR Cooperative Exchange Program in Primatology. Joint research program with Dr. Boris Lapin (Director) for an epidemiologic study of lymphoma in baboons.

PUBLICATIONS

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