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Maternal Factors in Developmental Toxicity

John M. DeSesso

Chemical and Biological Systems, Civil Systems Division, The MITRE Corporation, McLean, Virginia, and Department of Anatomy and Cell Biology, Georgetown University, Schools of Medicine and Dentistry, Washington, DC

The maternal organism provides the developing embryo with its physical environment, nutrients, and a mechanism for eliminating metabolic wastes. Since the physiological state of the pregnant female affects her ability to provide those requirements for the developing embryo, it is not surprising that there are maternal factors that can affect the wellbeing of the embryo. Extremes of maternal age in both humans and animals have been implicated in growth retardation, as well as autosomal trisomies. The influence of maternal size on fetal size is more pronounced among larger species with longer gestation periods such as humans and domestic animals. A clear relationship between the parity of the mother and potential developmental toxicity in humans has not been established due to the confounding influences of maternal age. Among laboratory rodents, however, it appears that offspring of multiparous animals are at increased risk of developmental toxicity. A variety of infectious agents, particularly viruses, have either been demonstrated or implicated as causes of developmental toxicity. In addition, hyperthermia is a possible confounding factor inherent with maternal infection. Although under experimental conditions hyperthermia is teratogenic in laboratory animals, a causative role for transient hyperthermia, which occurs during febrile states concomitant with infections, cannot be clearly established. Chronic maternal vascular disease states including essential hypertension, heart disease, or diabetes mellitus are likely to contribute to uteroplacental insufficiency and developmental toxicity. Poor maternal nutrition among humans contributes to growth retardation, but not to malformations. The production of "abnormal" maternal antibodies, such as are present in Rh incompatibility, can cause fetal wastage. An important maternal factor in humans is uteroplacental insufficiency, which can occur in normal states like twinning, as well as in abnormal conditions including reduced placental size, chronic maternal hypoxia, or uterine ischemia. Although all these maternal factors can contribute to developmental toxicity, they do not necessarily occur as isolated events. Some developmental toxicants exert deleterious effects within both the embryo and the maternal system.

Key words: age, size, parity, infection, vascular disease, nutrition, uteroplacental insufficiency, hyperthermia, antibodies

Address reprint requests to Dr. John M. DeSesso, The MITRE Corporation, 7525 Colshire Drive, McLean, VA 22102.

INTRODUCTION

During the period of prenatal development, the developing mammalian organism lies within the uterine cavity of the pregnant female. Thus, the maternal organism is the immediate physical environment of the developing mammal. In addition to providing the physical environment of the developing organism, the maternal system, by way of the placenta, supplies the developing mammal with nutrients, electrolytes, vitamins, and oxygen, as well as a mechanism for elimination of metabolic byproducts and wastes. The intimate apposition of maternal to fetal tissues within the placenta also provides a conduit whereby xenobiotic compounds that have been absorbed, and possibly biotransformed, by the maternal system can enter the developing organism. It stands to reason, then, that the physiological state of the pregnant female is of considerable importance to the developing mammal.

Although it has been recognized that there are both embryonic and non-embryonic factors that affect developmental toxicity [1], many recent experiments in the area of potential causes or mechanisms of congenital malformations have focused on events that occur within the developing embryo proper. The majority of teratology experiments have explored such problems as the identification of agents that cause developmental toxicity; the characterization of the role that is played by the stage of embryonic development at the time a teratogen is applied; the role that embryonic cell death may play in the genesis of malformation; or how interference with such processes as morphogenetic movements or tissue interactions affect the outcome development. The efficiency of maternal-embryonic exchange via the placenta has been recognized as being potentially important in developmental toxicity [2,3]. However, except for experiments examining the capability of the placenta to modulate the transfer of substances to and from the developing organism, the role of the placenta in developmental toxicity remains relatively unexplored.

Since the pregnant animal is the physical environment of the embryo, it seems implicit that her physiological state will affect the well-being of the developing organism. In addition, since the physiological state of adult animals can be modulated by a variety of factors, including age, health status, nutrition, stress, or life style, these "maternal factors" may affect successful development of the conceptus. The purpose of the present paper is to discuss, briefly, those factors that appear to exert their primary influence on the maternal system; but have been associated with developmentally toxic effects on the offspring. The paper will attempt to separate those factors that are exclusively maternal factors from those that appear to affect directly not only the mother but also the placenta and embryo. The discussion will include inferences both from the clinical literature on humans and from pertinent experiments performed on animals.

MATERNAL AGE

The association of advanced maternal age with the adverse outcome of pregnancies has been recognized since Shuttleworth [4] noted the increased incidence of mongoloid (Down syndrome) children among mothers of advanced age. His findings were confirmed and extended by Bleyer [5] and Penrose [6,7]. These latter studies provided statistical evidence that the incidence of Down syndrome is more prevalent

among offspring of females that are age 40 years or older than among those of younger mothers. More recently, Hedberg et al. [8] and Hay and Barbano [9] have suggested that females who are 35 years or older (especially for their first pregnancy) should be considered to be at greater risk for a variety of adverse outcomes of pregnancy, including the presence of congenital malformations. At the other extreme of maternal age, very young mothers (less than 17 years) are likely to give birth to small-for-date infants (born after 37 weeks of gestation and weighing less than 2,500 grams) [10] and infants at risk of perinatal mortality [11]. Additionally, the incidence of a few congenital malformations such as anencephaly have been suggested to be higher among both young mothers and those who are older than 40 years of age [12,13].

It is not clear exactly what role maternal age may have in producing congenitally malformed infants. In the case of infants with chromosomal abnormalities, especially the trisomy type defects such as Down syndrome, non-disjunction during oogenesis has been indicted by most investigators as the causative event. The reasoning behind that indictment is that non-disjunction of autosomes increases with advancing maternal age. It has been proposed, among other hypotheses, that changes in the maternal endocrine system that occur as a normal process of aging may cause a delay in the release of the developing ovum from the Graafian follicle ("over-ripeness") [14]. An alternative hypothesis, suggested by Matsunaga [15], is that the frequency of non-disjunction is similar in all ages of animals; however, the uteri of post-mature animals lose the ability to recognize and reject the implantation of trisomic zygotes.

Among laboratory animals, the reproductive capacity (i.e., the number of concepti per litter) decreases with advancing maternal age at a rate characteristic for each species [16]. In an attempt to separate the effects of aging on the maternal reproductive system from intrinsic deficiencies of the oocytes, several investigators have performed reciprocal transfers of fertilized zygotes prior to implantation between the uteri of young and old maternal animals. To do this, either young or old female animals were mated with fertile males and zygotes were flushed from the uteri within 72 hours after the estimated time for ovulation and transferred to a recipient pseudo-pregnant female. Experiments in golden hamsters [17] and rabbits [18] demonstrated that zygotes from young animals did not survive well in aged uteri. However, the transfer of zygotes from older mothers to young uteri did not survive well either. In both of the latter experiments, the investigators did not cull abnormal zygotes from their experiments. Talbert and Krohn [19] performed similar experiments in mice. However, to control for the defects that may exist within aged oocytes, all morphologically abnormal zygotes at the time of transfer were discarded from the study. Nearly three times the number of abnormal zygotes were recovered from older females than from younger females. Among normal zygotes, they found that zygotes from young mice did not survive well in aged uteri, while zygotes from aged mice survived well in the young uteri. Since abnormal zygotes were eliminated from their study, it appears that the intrinsic effects of aging on the oocytes were largely eliminated. In summary, it appears that maternal aging does adversely affect the ability of laboratory animals to maintain pregnancy.

MATERNAL SIZE

The influence of maternal height and weight on the ultimate size of the fetus at term have been recognized in both humans and animals. In retrospective epidemiolog-

ical studies that examined a combined total of more than 28,000 human births, North [10] and Donnelly et al. [11] reported that shorter and lighter mothers were likely to give birth to low birthweight or small-for-date infants.

In domestic animals, the size of the mother has been demonstrated to play a predominant role in the size of the fetus. This was clearly demonstrated by the experiments of Walton and Hammond [20] who produced reciprocal crosses via artificial insemination between Shetland ponies and Shire horses. Although the foals of both crosses possessed similar genetic contents, the foals delivered of the larger (Shire) mares were three times the size of the foals delivered from the Shetland mares. Indeed, in a review of potential maternal influences on fetal size, Hafez [21] concluded that the size of the dam appears to be of widespread importance in determining the size of the fetus in a variety of species. He noted that this maternal effect is particularly pronounced among the larger species that also have longer gestation periods.

PARITY

Among humans, a specific relationship between parity of the mother and developmental toxicity has not been clearly established. Some studies have suggested that certain types of congenital malformation (e.g., hypospadias) occur more often among young, primiparous women ("handicapping of the firstborn") [12,13,22,23], whereas other studies looking for similar malformations [24] have failed to demonstrate such a relationship. Both Donnelly et al. [11] and North [10] demonstrated via retrospective epidemiological studies that primiparous females from lower socioeconomic strata bore a higher incidence of small-for-date infants than other women. The effects of the confounding influence of maternal youth could not be removed from the analysis.

Several investigators [18,25] have suggested that the decline in reproductive capacity of older laboratory animals is caused by the aging of the uterus that accompanies the bearing of multiple litters. Wexler [26] reported that the uterine arteries of multiparous rats displayed a predilection for degenerative changes including destruction of elastic tissue and proliferation of endothelial cells during the periods of pregnancy and lactation, respectively. Simultaneously, the uterine veins became dilated and appeared thin-walled and flaccid. The changes in both arterial and venous structures appeared during the third pregnancy and progressed with repeated pregnancies. Larson and Foote [27] demonstrated that the uterine blood flow in aged multiparous rabbits was significantly reduced compared to young, nulliparous, or uniparous females. These findings suggest that multiparous animals are at increased risk for developmental toxicity when challenged by developmentally toxic agents.

MATERNAL INFECTION

The presence of infectious agents in the maternal organism during pregnancy can lead to both fetal wastage and congenital malformations through a variety of mechanisms. The susceptibility of the human embryo to the untoward effects of infectious agents was discovered by Gregg [28] who observed that mothers who had been infected with rubella virus during the first trimester of pregnancy gave birth to fetuses that displayed a variety of defects related to the central nervous system

including cataracts and deafness. Since that time, intense searches have disclosed relatively few other infectious organisms that cause gross congenital malformations in humans, although a variety of these organisms have been associated with other aspects of developmental toxicity.

The infectious organisms that have received the most attention have been the viruses. To date, the infectious agents that are well-documented etiologic factors in human congenital malformations include rubella virus [28], cytomegalovirus [29,30], and the protozoan *Toxoplasma gondii* [31]. *Treponema pallidum* (syphilis) can cause congenital deafness, hydrocephaly, mental retardation, and internal organ changes such as diffuse fibrosis of the lungs and liver [32]. The herpes simplex type II virus has been implicated in developmental toxicity because it has a potential association with malformations of the central nervous system, including microcephaly, microphthalmia, retinal dysplasia, and mental retardation [33].

Maternal infections with other viruses (including measles, chicken pox, ECHO, Coxsackie group B, and influenza viruses) have been described in association with the birth of malformed human infants. Although some of these viruses are experimental teratogens in animals [34,35], prospective epidemiological studies have failed to demonstrate a causal relationship between these viral infections and human developmental toxicity [36,37].

Recently the AIDS virus has been reported to have been transmitted to a fetus via the maternal fetal exchange [38], and a growth-retarded infant has been reported to have been born to a mother who was infected with AIDS [39]. It must be pointed out, however, that in the latter case, the only sign of developmental toxicity was growth retardation; the mother had received chemotherapy during her pregnancy, which could have been responsible for the observed growth retardation.

A major difficulty in determining whether or not infectious agents cause developmental toxicity has been the absence of a discrete syndrome of congenital malformations in the offspring. Many times the infants are growth-retarded or have other non-specific evidence of developmental toxicity. Even for those infectious agents that are known to cause developmental toxicity the mechanisms of action are unclear because the infection may include not only the mother, but also the placenta and embryo. In such cases, the adverse effects at each of the three sites (mother, placenta, embryo) may not be of sufficient magnitude to cause developmental toxicity, but a summation of the effects may be harmful to the offspring. In the event that transplacental infection occurs, the infectious agent may exert a direct teratogenic effect on the embryo as well as effects via the maternal system.

Potentially confounding factors include maternal anorexia and maternal antibodies. These topics will be discussed below. Another potentially confounding factor that is introduced by infectious agents is that most are pyrogenic. This means that the pregnant female experiences a transient episode of hyperthermia. Hyperthermia has been demonstrated by numerous investigators to be an experimental teratogen in mammals (see discussion below).

HYPERTHERMIA

Elevation of temperatures beyond the physiological level (37–38°C), regardless of cause, is termed hyperthermia. Hyperthermia causes teratologic effects in a wide variety of experimental animals both in vivo and in vitro [c.f. 40, 41]. Under

experimental conditions, developmental toxicity has been caused by localized heating of the uterus or by elevation of the whole body temperature of pregnant animals. Hyperthermia can induce malformations at some point in pregnancy in nearly all animal species tested. Usually the offspring exhibit some type of neural tube malformation if exposed during the critical period for central nervous system development. Based on the experimental data and several case reports in humans, Miller et al. [42] have suggested that hyperthermia may be the etiologic factor in the genesis of certain cases of anencephaly among humans. Corroboration of this hypothesis via epidemiological studies has not been forthcoming [c.f.43]. Nevertheless, maternal hyperthermia can be regarded as a confounding factor in the developmental toxicity of other kinds of agents such as viruses [c.f. 44] or non-ionizing radiation [45–47].

CHRONIC NON-INFECTIOUS DISEASE

Several chronic maternal disease states cause developmental toxicity by interfering with appropriate growth of the fetus and, in some cases, by causing structural malformations. In humans, many of these conditions relate to the cardiovascular system. Among them are essential hypertension, diabetes mellitus, heart disease, chronic renal insufficiency, and severe anemias. In all of these human conditions, there are confounding factors that are due to the use of pharmacologic agents for treatment of the maternal disease state. In addition, these maternal conditions are frequently associated with disturbances in the nutritive and respiratory functions of the placenta during later gestation.

The pre-existing condition of diabetes mellitus in pregnant humans has been recognized as a cause of fetal macrosomia (abnormally large infants) and perinatal death [48,49]. In addition, diabetic women are at greater risk of bearing structurally malformed offspring [50]. A variety of malformations has been reported including those of the cardiovascular, skeletal, and central nervous systems [52,52]. It is not clear by what mechanism the diabetic state causes or induces developmental toxicity. Maternal hypoglycemia, caused by elevated insulin levels in the mother, can adversely affect prenatal development [53]. Insulin can adversely affect embryonic development of avian embryos. Although the potential for direct action of insulin on mammalian embryos is real (c.f. reviews by Landauer [53] and Kimmel [54], the placenta is impermeable to maternal insulin during organogenesis [55], eliminating the direct effects of exogenous maternal insulin as a possible mechanism. The demonstration that other factors associated with maternal diabetes, such as altered maternal serum glucose levels and ketosis, cause developmental toxicity in in vitro investigations [56,57] has not provided the basis for a simple mechanism to explain the developmental toxicity of maternal diabetes.

Essential hypertension, which antedates pregnancy, as well as chronic renal insufficiency, regardless of cause, have been linked with developmental toxicity. Chronic renal insufficiency can be caused by numerous conditions including systemic lupus erythematosus, chronic glomerulonephritis, and chronic pyelonephritis. All three of these diseases are believed to be disorders of the immune system in which maternal antibodies and complement attack maternal renal tissues, ultimately leading to the destruction of portions of the kidney and compromise of renal function. In conjunction with central hypertension, this contributes to utero-placental insuffi-

ciency, which may be the ultimate cause of the developmental toxicity and will be discussed below [58].

Several other chronic maternal disease states contribute to developmental toxicity, such as conditions of phenylketonuria, hyper- and hypothyroidism, and virilizing tumors. However, the prevalence of these conditions is extremely low [59].

MATERNAL NUTRITION

A variety of nutritional deficiencies and excesses have been shown to be teratogenic in experimental animals [c.f. 60,61]. At present, only endemic cretinism (due to disturbance of maternal iodine metabolism), maternal zinc deficiency [62], and possibly folate deficiency [63,64] have been implicated in human gross malformations.

Nevertheless, disturbances in maternal nutrition can affect the rate of fetal growth during the last one-sixth of gestation. Changes of this sort are particularly noticeable in animals such as rodents, whose litter weights are large relative to the maternal body weight [58].

In humans, significantly reduced birth weights have been recorded during times of famine. Gruenwald et al. [65] reported that the mean fetal weights for Japanese mothers between 1945 and 1946, which was a period of severe food deprivation in Japan, were approximately 10% lower than the mean birth weights between 1963 and 1964, which was a period of prosperity. These authors presented data suggesting that the difference in the weights of the fetuses between those two periods of time were the result of poor growth during the last 6 to 9 weeks of gestation. Furthermore, this decrease in fetal growth was ascribed to a reduction in maternal caloric intake rather than deprivation of a particular, required nutrient. The condition of a reduced caloric intake has been termed undernutrition as opposed to malnutrition, which is used to describe a poor quality diet which may lack certain specific and needed nutrients.

When well-nourished pregnant ewes are subjected to undernutrition during the last 40–45 days of gestation, the fetal growth rate decreases by 45% within 3 days [66]. Although undernutrition was associated with small-for-date fetuses at term, congenital malformations were not observed in any lambs, including those whose mothers were starved beginning at gestational day 35. In contrast to sheep, both rodents and lagamorphs have borne offspring that are not only small-for-date but also malformed. This has been demonstrated subsequent to dietary restriction in mice [67,68], rats [69]; and rabbits [70,71]. Therefore, it appears that the polytocous rodents and lagamorphs with their short gestational periods are more susceptible to the effects of maternal undernutrition than larger animals and humans.

MATERNAL ANTIBODIES

The passage into the fetus of any maternal antibodies against embryonic/fetal tissues could be a source of developmental toxicity. Although maternal antibodies enter the fetus throughout gestation, by and large, the embryo appears to be indifferent to the maternal immune reponses and can be considered to be an immunologically privileged site [72]. Maternal antibodies of the IgG(7S) class gamma immunoglobulins are actively transported across the placenta by means of receptor-mediated endocytosis [3]. The transfer of these maternal antibodies is especially important

during the last few weeks of gestation at which time passive immunity against various infectious diseases is conferred on the fetus.

There are some conditions in which maternal antibodies do cause developmental toxicity. For instance, Rh incompatibility between the mother and fetus is the cause of erythroblastosis fetalis or hemolytic disease of the newborn [73]. This disease is related to the presence of antigens on the cell surface of fetal erythrocytes that are not found on the erythrocytes of the mother. Fetal erythrocytes that invade the maternal bloodstream during pregnancy will sensitize the mother and will elicit the production of antibodies against the red blood cells of the fetus. Subsequent transfer of these antibodies to the fetus is the etiology of the disease.

Brent and associates have reported that experimentally derived antibodies against rat kidney [74] and visceral yolk sac [75] are teratogenic. They have identified the responsible agent as IgG [76], the source of which was due to neither maternal immunologic nor metabolic derangements [77]. They further demonstrated that the site of action was neither in the maternal nor fetal systems but rather in the developing yolk sac and chorioplacenta [78,79]. The actual mechanism of developmental toxicity appears to act through deprivation of nutrient transport across the placenta during critical stages of development.

NUMBER AND POSITION OF FETUSES

The number and position of fetuses within the bicornuate uterus of polytocous laboratory animals may exert deleterious effects on the outcome of pregnancy. Although identical conditions do not exist in humans, twinning or other multiple births can cause growth retardation or some other compromise of one fetus at the expense of another due to placental fusions and competition for nutrients. In several laboratory species, the placental and fetal weights at term have been reported to vary as a function of the relative position of the conceptus within the uterine horn. For instance, in mice [80] and in rabbits [81,82], the placental and fetal weights were inversely proportional to the number of concepti per uterine horn. For mice, McLaren and Michie [83,84] reported that the heaviest fetuses were at either the ovarian or vaginal end of the uterine horn and the lightest fetuses were in the middle of the uterine horn. In New Zealand White rabbits [81] and in Dutch belted rabbits [85]; the heaviest fetuses were reported to be near the ovarian end of the uterine horn and the smallest fetuses tended to be near the cervical end of the uterine horn. In rats, the heaviest fetuses were in the middle of the uterine horn [86,87]. These observations suggest that the position of a conceptus within a uterine horn does affect the ability of a conceptus to obtain and compete for nutrients. The nature of the effect, however, appears to be species-dependent since the largest fetuses are not in the same locations among species. These observations also suggest that in laboratory animals, some embryos will be better prepared than others to respond to challenges of developmentally toxic agents.

MATERNAL STRESS

Since stress, by its very definition, is a disturbance in the normal physiology of the maternal organism, it seems highly likely that maternal stress could adversely affect the development of the embryo. In humans, stress can be induced by a variety

of causes and is manifested in numerous ways on numerous target organs. The same is true for pregnant laboratory animals, which can be subjected to the stresses of transportation [88], transient food and water deficiencies [89,90], and restraint [90]. Each of the preceding conditions has been indicted as the cause of developmental toxicity. Other suspected causes of stress-induced developmental toxicity in laboratory animals include noisy caging conditions and poor ambient lighting. It is apparent that the term "maternal stress" is a catchall for a variety of conditions, each of which may or may not have the capacity to adversely affect the development of offspring. Maternal stress has been under active investigation over the past few years and is the topic of another paper in this symposium.

UTEROPLACENTAL INSUFFICIENCY

Circulation of maternal blood through the trophoblastic lacunae of the developing placenta is established by the end of the second week of gestation. As the conceptus continues to grow, its well-being becomes increasingly dependent upon adequate placental perfusion by and physiological exchange with the maternal blood. The embryo's supply of most nutrients and oxygen is limited more by the rate of maternal blood flow through the placenta than by the rate of diffusion [3]. Any condition, regardless of its cause, that chronically reduces the rate of blood flow through the uterus can cause fetal hypoxia and intrauterine fetal starvation.

The fact that placental size correlates more closely with fetal weight than does gestational age [91] suggests a close relationship between fetal growth and the functional capacity of the placenta. Since both the fetus and its placenta are derived from a common zygote, Gruenwald [92] suggested the existence of a common mechanism that regulates the growth.

In experimental animals it has been demonstrated that small placentae or placentae that are experimentally reduced in size will affect the birth weight of fetuses. Mellor [66] showed that survival of fetal lambs was jeopardized more by small placentae than by severe underfeeding of pregnant ewes. In rhesus monkeys, Hill and associates [93–95] demonstrated that isolation of the smaller placentae of the bidiscoid hemochorioplacentae through ligation of all interplacental blood vessels resulted in growth-retarded infants at term.

Chronic maternal hypoxia could be expected to exert deleterious effects on the developing fetus. Chronic maternal hypoxia exists in pregnant women who live at high altitudes in areas such as Denver, Colorado or the mountains of Peru. Data concerning the birth weights of infants born at various heights above sea level have been reviewed by Gruenwald [92]. Those data indicate that infants born at high altitudes, even after the mothers have adapted to such altitudes, exhibit reduced birth weights when compared to infants born at altitudes closer to sea level.

Reduced uterine blood flow causes a reduction in the rate of uteroplacental circulation. Chronic reduction in uteroplacental circulation can not only cause moderate hypoxia, but also can compromise fetal nutrition. Experiments by Wigglesworth [96,97] demonstrated that unilateral ligation of the anastomotic uterine channel close to the origin of the uterine branch of the hypogastric artery with resultant restriction in uteroplacental blood flow during the latter part of gestation causes growth-retarded

fetuses.* In the contralateral uterine horn, the anastomotic channel remained intact and served as a control. At term, the surviving fetuses in the ligated horn exhibited a progressive decrease in fetal body weights that appeared to be inversely related to the distance of the fetus from the ligature. The fetuses closest to the ligature had the greatest likelihood of resorption or, if they survived, had the lowest birth weights. Subsequent investigations to determine the mechanism which underlies this phenomenon [98] revealed that ligation of the uterine artery caused only a temporary reduction in uterine blood flow, but that temporary reduction was sufficient to permanently check placental and fetal growth. The reduced blood flow through the uterus for the remainder of gestation was found to be adequate for the reduced amount of embryonic and placental tissue. Regardless of the mechanism, reduction in uterine blood flow in the latter part of gestation is developmentally toxic in that it causes reduced fetal body weights and greater likelihood of fetal death.

Uterine ischemia during organogenesis has been induced by temporarily clamping the uterine vasculature for periods of time from 30 minutes to 2 hours. Uterine ischemia is both teratogenic and lethal to developing embryos in rodents [99–101]. The early studies on uterine ischemia were carried out on anesthetized, unconscious rats. The results of those studies varied not only with the duration of clamping, but also with the period of gestation during which the procedure was performed. It was further demonstrated by George et al. [102] that the hypothermia incumbent with the state of anesthesia greatly affected the results of the experiments. Later experiments performed by Bruce [100] utilized a surgically implanted vascular occlusion cuff which remained in situ around the uterine vasculature and was connected via catheters to an outside source of air pressure. Upon recovery of the pregnant rat from the surgical procedures, the investigator could induce temporary uterine ischemia in the conscious rat for variable periods of time throughout the remaining portion of gestation. Data from these experiments indicated that uterine ischemia early in gestation causes fetal malformations as well as decreased fetal weights at term.

Experimental administration of vasoactive substances, such as epinephrine, norepinephrine, or vasopressin, causes severe uterine vasoconstriction in both humans and experimental animals [103,104]. A variety of maternally administered vasoactive compounds has been associated with reduced fetal weights, malformations, and death [105–107]. Greiss and coworkers [108,109] demonstrated that stimulation of sympathetic nerves to the uterus also produces marked uterine vasoconstriction and could be expected to result in similar fetal effects.

DISCUSSION

Although the preceding has identified several maternal physiological and disease states that can affect the developing embryo (summarized in Table I), it must be recalled that the maternal-fetal unit is a dynamic physiological system. There may be conditions or agents that can alter not just one portion of the system but both. For instance, vasoactive substances which can constrict the vasculature of the maternal organism could cross the placenta and, if the appropriate receptors are present, exert similar effects on the developing embryo. Thus, under some conditions, it may be

*The bicornuate uterus of rodents receives its blood supply from an anastomotic channel fed by the uterine branch of the hypogastric artery and by the ovarian branch of the abdominal aorta. From this anastomotic channel, located along the mesometrial border of the uterus, arteries radiate into the placentae of the implantation sites.

TABLE I. Maternal Factors Associated With Developmental Toxicity

Age (>40 yr; <17 yr)	Maternal vascular disease
Size (weight and height)	Essential hypertension
Parity	Heart disease
Maternal infection	Diabetes mellitus
Rubella virus	Maternal nutrition
Cytomegalovirus	Under nutrition
Toxoplasmosis	Malnutrition
Syphilis	Maternal antibodies
Herpes simplex virus	Number and position of fetuses
Varicella virus (?)	Maternal stress
Cocksackie B virus (?)	Uteroplacental insufficiency
ECHO virus (?)	Size of placenta
Chicken pox (?)	Placental infarcts
AIDS virus (?)	Chronic maternal hypoxia
Hyperthermia	Uterine ischemia

impossible to determine if an agent affects the maternal system to the exclusion of the embryonic system or vice versa.

In addition, the maternal system changes dynamically throughout pregnancy. For instance, Buelke-Sam et al. [110] demonstrated that blood flow to the reproductive organs in the pregnant rat changes dramatically during the course of pregnancy. This means that the susceptibility of the maternal system, similar to the embryo, is not static during the course of gestation. This concept is similar to the principle of teratogenesis that states that the susceptibility of an embryo to a developmental toxicant is at least partially dependent on the development stage at the time of exposure [111].

Complexities arise when one tries to determine the site of action for particular agents on the developing materno-embryonic system. Since the maternal and embryonic systems are so closely intertwined, it is not unreasonable to expect that developmental toxicants may affect both systems. For example, hydroxyurea, a potent teratogen that causes rapid deleterious effects in embryos [112–114], induces even more rapid alterations in the maternal system. In vivo microscopy of living rabbit embryos in situ after a subcutaneous maternal injection of a teratogenic dose of hydroxyurea elicited cardiovascular alterations of the embryos within 3 to 9 minutes of treatment [115]. These rapid changes led Millicovsky and DeSesso [116] to postulate that a mechanism other than transfer of hydroxyurea across the placenta was the cause of the effects. They determined that uterine ischemia induced by complete clamping of all uterine vessels caused similar cardiovascular alterations within the embryos in a similar short time frame. In addition, they determined that ligation of the embryonic umbilical cord and the vitelline blood vessels protected embryos from these cardiovascular alterations, presumably by preventing embryonic uptake of maternally released vasoactive substances.

In a final series of experiments, Millicovsky et al. [117] determined by the technique of radioactive microspheres that maternal adminstration of hydroxyurea caused a preferential decrease of 77% in uterine blood flow and a concomitant increase in uterine vascular resistance of 400% over base line which lasted for nearly 10 minutes. These conditions resulted in a virtual shutdown of uterine vascular flow and a transient uterine ischemia, leading to the postulate that uterine ischemia contributed to the developmental toxicity of the compound. Utilizing a similar technique,

Buelke-Sam et al. [118] demonstrated marked reduction in the uterine blood flow of pregnant rats following a developmentally toxic dose of mirex.

It is becoming apparent, then, that individual toxic agents can adversely affect embryonic development through actions initiated at multiple sites within the materno-embryonic unit. In the case of developmentally toxic viruses, primary effects can occur through infection of the mother, placenta, embryo, or any combination thereof. Even agents like hydroxyurea, that have pronounced deleterious effects on the embryo proper, can cause toxic effects within the maternal orgnism. The toxic effects within the mother can contribute to or exacerbate the toxicity within the embryo by compromising the ability of the maternal embryonic system to support an effective compensatory response to challenge by developmentally toxic agents. Thus, although the role of maternal factors in both normal and abnormal development is not often conspicuous, these factors are intimately involved with the well-being of the offspring and deserve closer scrutiny as we attempt to understand the mechanisms underlying developmental toxicity.

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