THE COMBINED EFFECTS OF ULTRASONIC ENERGY EXPOSURE AND PROTEIN-DEFICIENT DIET ON BOTH MATERNAL AND FETAL MICE

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Chapter I

Introduction

In recent years, increased emphasis has been placed upon the effects of maternal nutrition on the outcome of gestation, particularly the effect of maternal protein intake on fetal growth and development. Growth and development of the fetus depends on a steady supply of nutrients from the mother, and thus protein deprivation during pregnancy might be harmful to the developing organism. Experimental data from both human (Burke, et al., 1943a; Lechtig, et al., 1975) and animal studies (Kohrs, et al., 1976; Pond, et al., 1968; Zeman and Stanbrough, 1969) indicate that there is a positive relationship between maternal protein malnutrition during pregnancy and subsequent adverse effects on fetal growth and development.

It is still unclear, however, whether adequate growth and development of fetus could occur at the expense of the maternal organism during protein malnutrition. Therefore, attention has to be focused on maternal tissue changes and their relationship to fetal growth during protein malnutrition, then it is possible to assess the role of maternal tissues as a proper barrier for protecting the fetus against nutritional deficiency. At the same time, it is important to investigate whether maternal nutritional status is related to placental growth and whether the plcental growth pattern reflects the fetal condition. The human fetus is obviously not available for investigation, but the placenta is an available human reproductive tissue. If the pattern of changes in placental growth is correlated with that of fetal tissues in animals, similar changes in human placenta would give a clue on how environmental stresses imposed on the female during pregnancy influence the human fetus.

It has been shown that normal placental growth in rats proceeds in the same way as in fetal organs of the rat (Winick and Noble, 1966b). The same

general pattern of cellular growth has been reported for human placenta (Winick, et al., 1967), although the timing is somewhat different. These observations offer an important basis for resolving questions regarding the relationship between maternal nutrition and fetal growth in human.

Exposure to ultrasonic energy may represent a stress to the developing fetus (O'Brien, et al., 1972), with its rapid commercial development and increasing utilization in the clinical practice of medicine, especially in obstetrics. In obstetric examination, ultrasound provides a visual method of evaluating normal and abnormal fetuses, placental problems, and uterine disorders (Gottesfeld, 1978) so that the fetus and the mother may receive proper care. However, the increased use of ultrasonic energy dictates that a reliable assessment of its risks to human population has to be performed. It has been shown that adverse effects on fetal growth could occur as a result of exposure to ultrasound during the prenatal period in certain mouse strains (Januzik, 1976; O'Brien, 1976).

Although previous studies suggest adverse effects of either prenatal protein deprivation or ultrasonic energy exposure on fetal growth and development, the combined effects of these two stresses on either the fetus or the maternal organism have not yet been explored in either human or experimental animals. Since it is difficult if not impossible to adequately evaluate the combined effects of these two stresses on human subjects, the use of an animal model is indicated.

This investigaion was undertaken to assess the combined effects of restricted protein intake and ultrasonic energy exposure during pregnancy on the maternal and fetal mouse. In addition, it was sought to determine whether the pattern of placental growth in this animal model could serve as a guide to fetal growth, and whether maternal tissues serve as a barrier providing fetal

protection against these stresses. Results of such investigation should provide an understanding of the interaction of these two stresses on the maternal organism and the products of conception.

Chapter II

Literature Review

A. Maternal protein deficiency and outcome of gestation

Additional protein is needed during pregnancy for the expanded maternal plasma, uterus, and breasts, as well as for protein synthesis in the fetus and placenta (Williams, 1945). During the course of pregnancy, about 800 grams(g) of additional protein accumulates in the body, and most of these proteins are in the uterus and its contents (Thomson and Hytten, 1961). Therefore, some adverse effects could be expected to occur in offsprings if the female has a protein-deficient diet during pregnancy. The effects of protein deficiency in pregnancy, however, are difficult to define precisely because of metabolic relationships between energy and protein. A certain critical level of caloric intake is essential to protect protein from catabolism and to meet basic energy requirements. However, studies performed in both human and animal models have provided information to indicate that inadequate maternal protein intake during pregnancy may lead to impairment of intrauterine growth and development of fetus.

1. Human studies

Although the relationship between maternal nutrition and pregnancy outcome in human is exceedingly complex and poorly understood, there are interesting evidences of damage to the human fetus due to inadequate maternal nutrition during pregnancy. These evidences are "wartime experiments" which are based on clinical data collected during World War II when people were subjected to severe dietary restriction. The results of these experiments suggest a

positive relationship between nutrition and pregnancy outcome.

During the postwar famine in Holland (during 6 months in 1945) the major adverse effect on reproductive performance was a reduction of birth weight (Smith, 1947). The average reduction of birth weight was about 240g (8oz) and the amount of reduction represented about 9% of the weight in normal times. Antonov (1947) also performed a similar study which dealt primarily with children born during the siege of Leningrad in 1942 and showed that the severe hunger during the siege affected the condition of newborn children at that time. The stillbirth rate rose to twice the normal figure and the average weight of infants born at that time was 500 to 600g less than normal. Unfortunately, these wartime experiments were not able to differentiate the decreased reproductive performance caused by specific nutrient deficiency during pregnancy from those due to gross inadequacy of multiple nutrients and environmental and social insults.

The role of maternal nutrition during pregnancy on fetal growth has been the subject of many other human studies (Ebbs, et al., 1941; Burke, et al., 1943a and 1943b; Lechtig, et al., 1975). Many of the human studies of nutritional influences on pregnancy have been based on the correlation between maternal diet and infant weight. Burke and her colleages (1943a) studied diets consumed by 216 pregnant women, and compared the maternal nutritional status with the outcome of pregnancy. A significant increase in birth weight and length was demonstrated in this study, with each additional increment of protein in the prenatal diet. This suggested that the amount of protein in the prenatal diet was an important factor in determining length and weight of newborn infant. More recently, Lechtig and his colleages (1975) employed the method of nutritional supplementation of an experimental group of Guatemala women to see the relationship between maternal nutrition and outcome of gestation. In this

study, the effects of two types of food supplementation (protein and protein-caloric supplementations) during pregnancy on birth weight were studied in the poorest communities of Guatemala, and a significant increase in birth weight was demonstrated with caloric supplementation. These data help to clarify the interrelationship between maternal nutrition and outcome of gestation in human situation where nutrition and socioeconomic factors are interrelated. However, a human study demands painstaking work, a great deal of time, and a large number of subjects to yield statistically significant results.

2. Animal studies

It is difficult to investigate the role of nutritional status in human populations because of moral and procedural considerations, and thus animal studies become valuable tools for examining the role of maternal nutrition on outcome of gestation. Considerable evidence concerning the effects of nutritional restriction of female animals on outcome of gestation has accumulated, but results on reproductive performance have not been consistent. It appears in the literature that maternal malnutrition gives a significant adverse effect on reproductive performance in subprimate mammals (Berg, 1965; Pond, et al., 1968; Simonson, et al., 1972; Smart, et al., 1972; Tumbleson, et al., 1972; Wallace, 1948b; Zeman, 1967; Zeman and Stanbrough, 1969), but relatively little effect has been reported in primate mammals (Cheek, et al., 1976; Riopelle, et al., 1975). Since primates are more comparable to human in terms of growth rate, reproductive cycle, and placental and uterine structure (Kohrs, et al., 1976), these animals could be an appropriate model for investigating the effects of dietary modifications during human pregnancy.

Some aspects of the deleterious effects of protein deprivation during pregnancy of the rhesus monkey (Kohrs, et al., 1976) have been reported.

Reproductive efficiency was markedly reduced, fetal and infant mortality rates were markedly increased, and birth weight was significantly reduced in the infants born to protein-restricted mothers fed a diet containing only 25% as much protein as control diet. The temperature control mechanism seen in infant monkeys appeared to be seriously affected also by protein restriction during pregnancy (Kohrs, et al., 1979). The six infant monkeys born to protein-restricted mothers during pregnancy had less ability than control infants to maintain their body temperature after exposure to a hypothermic environmental stress. This might be comparable to premature, low birth-weight human infants who have a similar problem, and thus the sitution for the monkey could be considered to be close to the human.

In the other study of the effect of maternal malnutrition (protein or protein-caloric restriction) on fetal growth of rhesus monkey (Cheek, et al., 1976), however, there were no significant changes in fetal body weight and placental weight of protein-restricted group with or without caloric restriction. Moreover there were no significant changes in protein, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), cholesterol, and phospholipid contents of fetal brain of malnourished mother. Riopelle, et al. (1975) were also unable to find the effect of the reduced maternal protein intake during pregnancy on fetal growth of monkeys. They fed pregnant rhesus monkeys semisynthetic isocaloric diets which provided either 4, 2, or 1g of protein per Kg body weight per day, and noted a modest increase in fetal mortality in pregnancies of mothers fed the lowest amount of protein, but did not notice a significant effect on birth weight. These results indicated that fetal growth of the primate during gestation is somewhat protected against maternal protein or protein-caloric restriction. Accordingly, it can be said that maternal protein restriction during pregnancy have mild effects on

reproductive performance of primates.

The restriction of maternal protein intake, however, produces far greater fetal growth changes in the subprimates than in primates, due to a greater protein requirement associated with a much faster rate of fetal growth in relation to the maternal size than that of primates (Payne and Wheeler, 1967). Therefore, the metabolic stress of pregnancy imposed on the subprimate mother is much greater than that for the primate.

Various subprimates have been extensively used to examine the role of maternal nutrition in outcome of gestation, although it has been argued that the results of prenatal growth study with subprimate mammals may not be applicable to the human situation. Especially, effects of maternal protein intake during pregnancy have been evaluated in various subprimate animals, such as the pig (Atinmo, et al., 1974a and 1974b; Pond, et al., 1968, 1969, and 1973; Rippel, et al., 1965), miniature piglet (Tumbleson, et al., 1972), cat (Simonson, et al., 1972), guinea pig (Young and Widdowson,1975), and rat (Berg, 1967; Nelson and Evans,1953; Venkatachalam and Ramanathan, 1964; Zeman, 1967; Zeman and Stanbrough, 1969). Most of these studies show that reproductive efficiency is reduced as deficiency is increased in severity, but they also indicate that there are differences among species within subprimates in the effects of maternal protein restriction on reproductive performance. In large mammals such as pig, relatively little effects of maternal nutrients restriction on reproductive performance have been observed, compared with small mammals such as rat. For example, a protein-free diet given throughout pregnancy to pigs resulted in normal reproductive performance and viability of the progeny whose weight was two-thirds of the control value (Pond, 1968), but such a diet given to the pregnant rats did not maintain the pregnancy (Berg, 1967; Nelson and Evan, 1953; Zamenhof, et al., 1971). Therefore, one might speculate that the

imposition of insults in different mammals may produce variable degrees of adverse effects on reproductive performance of female animals depending on whether the mammal is large or small and whether the mammal is a primate or nonprimate.

Some general comparisons can be made, however, though the risk of extrapolating results across species boundaries is recognized. In studies of the effects of feeding protein-deficient diets to pregnant female animals, decreased litter size (Nelson and Evans, 1953; Young and Widdowson, 1975), increased resorptions (Nelson and Evans, 1953), decreased weight and/or length of individual pups (Allen and Zeman, 1971; Atinmo, et al., 1974b; Curtiss, 1953; Hastings-Roberts and Zeman, 1977; Nelson and Evans, 1953; Pond, et al., 1968 and 1969; Seegers, 1937; Young and Widdowson, 1975; Zamenhof, et al., 1971; Zeman, 1967 and 1970), decreased neonatal survival rate (McCoy, 1940; Stevenson and Ellis, 1957), and decreased postnatal growth of pups (McCoy, 1940; Pond, et al., 1969) have been demonstrated. A decreased individual organ weight has also been demonstrated in newborn pups whose mothers were fed a protein-deficient diet during pregnancy (Allen and Zeman, 1971; Atinmo, et al., 1974a; Tumbleson, et al., 1972; Zeman, 1967 and 1970; Zeman and Stanbrough, 1969). Tumbleson, et al. (1972) fed Sinclair (S-1) miniature sows either 0.4 or 16% protein diet througout gestation, and reported the order of vulnerability of the various organs of progeny as follows (from least to most affected): brain, eye, kidney, heart, pancreas, liver, lung, spleen, tibia, and tongue. Zeman (1967) also obtained similar results with rats by feeding protein-deficient diet during pregnancy. The protein-restricted diet during pregnancy had a greater effect on liver and kidney than that on the heart, brain, and thymus. Although the brain is generally considered the organ that is least affected by nutritional manipulation, Simons and Johnston (1976) demonstrated that protein deficiency

during prenatal and neonatal periods delayed brain maturation.

It is interesting to speculate the relationship of the difference in sizes of these tissues to possible difference in degree of maturation. The question also arises as to whether the sizes of tissues are related to the functional ability. By examining 1,000 dead human fetuses and infants, Potter and Thierstein (1943) found that the development of kidney glomeruli was primarily related to size of kidney, and postulated that organ size is related to the degree of maturation. Further, it has been reported that functional ability of several tissues of progeny from protein-deficient mother is significantly affected (Shrader and Zeman, 1969; Zeman, 1968). Although the effects on the function of the kidneys of the protein-deficient young cannot be determined by morphological and histological studies alone, Wachstein and Bradshaw (1965) suggested that the morphological development, in general, reflects the degree of maturity and funtional adequacy found in kidneys of newborn and growing animals. There were definite morphological and histochemical differences between kidneys from the animals in the control and protein-restricted groups (Zeman, 1968). Kidneys from the protein-restricted animals have fewer and less well-differentiated glomeruli, a greater portion of connective tissue, and relatively fewer collecting tubules. Reduced activities of acid and alkaline phosphatase enzymes were also noted in kidneys of protein-deficient young. These few and immature glomeruli of kidneys from protein restricted pups were closely related to altered kidney function, with marked reduction in glomerular filtration rate and depressed urine excretion (Hall and Zeman, 1968).

Another effect of maternal protein restriction during pregnancy is depressed skeleton development (Hastings-Roberts and Zeman, 1977; Shrader and Zeman, 1973; Zeman, 1967). This effect is evidenced by the decreased crown-rump

length (Zeman, 1967) and significantly fewer ossification center sites in the fetuses (Hastings-Roberts and Zeman, 1977) of protein-restricted dams as compared with the fetuses of control group. Moreover, increased postnatal food supply achieved by reduction of litter size did not give compensatory changes in the time of appearance of ossification sites (Shrader and Zeman, 1973). These results indicate that the time of appearance of ossification center is significantly delayed by prenatal protein restriction, and ossification center formation is influenced more by prenatal diet than by postnatal nutrition.

By manipulating the diet of pregnant rats, Hsueh, et al. (1967) attempted to define a critical component which, when deficient, results in the previously reported postnatal growth-stunting of the progeny (Chow, 1964; Chow and Lee, 1964) with 50% overall restriction of dietary intake. They restored certain dietary components to control levels, and observed the effect of this restoration. Restoration of vitamins and minerals failed to improve the growth-stunting effect of progeny. Restoration of the caloric intake by the addition of sucrose resulted in less marked but still signifinant postnatal growth-stunting. When both protein content in diet and caloric intake were restored to control level, however, there was a significant improvement in postnatal growth of progeny, suggesting that protein is the critical dietary component in maternal diet during gestation.

Previous results of animal studies clearly indicated that reproductive efficiency of female animals can be greatly altered by varying the protein content in maternal diet during pregnancy. Furthermore, it indicates that severe dietary protein restriction during pregnancy is detrimental to fetal growth and development regardless of species. If primates (monkeys) and subprimates (pigs, rats, and many others) are both intended for use as nutritional models for the human, in prenatal growth study, the discrepancies

between them and the human must be considered when extrapolating the results of animal studies. As Payne and Wheeler (1967) have emphasized, for example, the metabolic stress of pregnancy imposed on the primate mother is much less than that for other subprimate species due to its single fetus and its long gestation period.

3. Fetal cellular growth in intrauterine protein restriction

Where changes occur in sizes of organisms, it is important to consider the nature of the changes. Although the functional ability may reflect such changes, there must be a controlled technique to quantify certain events that occur during prenatal growth in animals.

The growth of an organism might consist in increase in the number of cells or increase in the size of cells or both (Winick and Noble, 1965). A new approach to the study of structural growth and development has been applied to define cellular changes. Since DNA has been shown to be relatively constant within a single nucleus in any species (6.2 pg for the rat) and the number of nuclei is equal to the number of diploid cells (Enesco and Leblond, 1962), it is possible to calculate, with reasonable accuracy, the number of diploid cells in a given organ or tissue by dividing the total DNA by the amount of DNA per nucleus. Furthermore, the weight, protein, or RNA contents per cell provide a mean figure for the material associated with the nucleus as well as the cell size. Therefore, this technique could provide cellular parameters that would give additional insights to weight or length data. In the study of normal growth of rat during prenatal and postnatal periods (Winick and Noble, 1965), timing and tissue differences in growth were demonstrated by determining total animal and individual organ DNA, RNA, protein, and weight at various times from 10 days after conception to maturity. Early prenatal growth in the rat

proceeded entirely by a proportional increase in DNA, RNA, protein, and weight. The rate of DNA synthesis decreased at different times for different organs.

Although there is some limitation (Enesco and Leblond, 1962) to the use of this technique in the study of the embryo and fetus which undergo rapid mitosis, the interrelationship between intrauterine malnutrition and structural growth and development of fetus has been explored with this technique by many investigators (Altinmo, et al., 1974a; Zamenhof, et al., 1968 and 1971; Zeman and Stanbrough, 1969). The study of the effects of prenatal protein restriction on cell number and cell size in the rat showed that cell size or cell number or both could be affected by prenatal protein restriction in various organs. Zeman and Stanbrough (1969) fed pregnant rats either 30 or 6% casein diet throughout gestation and found significant decreases in total DNA contents in carcass, liver, brain, kidneys, heart, and thymus of protein-deficient youngs at birth. However, no differences in weight/DNA and protein/DNA ratios were observed in all tissues studied except in liver of newborn protein-deficient animals. The results of this study indicate that the effect of maternal protein deficiency on body and organ size was primarily the result of the decrease in cell number. The reduced cell number in these organs was not reversed by increased postnatal feeding up to 21 days of age (Zeman, 1970), suggesting that the adverse change due to prenatal protein deficieny could persist at least until weaning.

Winick and Noble (1966a) suggested, in a study of cellular response in rat during malnutrition at various ages, that recovery of normal cellular growth of malnourished animals by increased feeding depends on the age of animal (growth phase) at the onset of malnutrition, and recovery is less likely when malnutrition occurs very early. They also suggested that early malnutrition impeded cell division and the animal did not recover by increased feeding. However, malnutrition at a later stage of growth resulted in a reduction of cell

size from which the animal could recover. Therefore, it is expected that newborn animals subjected to protein deprivation during gestation could be permanently retarded in growth.

B. Effects of maternal protein deficiency on placental growth and its association with fetal growth

Between conception and birth the nutritional needs of the fetus are met by three different mechanisms (Moghissi, 1978): during the preimplantation phase, the blastocyst presumably absorbs nutrients from the reproductive tract fluids through its outer layer of cells, the trophoblast; from the time of implantation, a sinusoidal space is formed between the fetal and maternal side and apparently the embryo receives nutrients directly from the maternal blood until the establishment of the placental circulation; when the placenta is developed, the fetus receives its nutrients via placental circulation. Thus the placenta has a central function in pregnancy, being involved in the transfer of nutrients to the fetus and in the inducing of certain metabolic changes in the maternal organism that are essential for fetal survival and well being.

In animal studies, it is possible to monitor the effects of various maternal stresses on the growth of both placenta and fetal organs. In the human, however, the fetus is not available for these types of studies, and the placenta which is accessible after birth is the only organ available for the study. Therefore, if the pattern of changes in cellular growth of placenta is correlated with cellular growth of fetal tissues in animals, similar changes in human placenta would give a clue to similar maternal stresses on human fetus.

It has been shown that normal placental growth in rat proceeds in the same way as in the fetal organs of the rat (Winick and Noble, 1966b). Initially, DNA and protein increase proportionally, resulting in a constant

protein/DNA ratio until the 16th day of gestation. On the 16th day of gestation the protein/DNA ratio rises, indicating that for a short time both cell division and cell enlargement are occuring simultaneously. On the 17th day of gestation DNA synthesis stops and protein synthesis continues to rise at the same rate, and therefore the protein/DNA ratio increases markedly. The same general pattern of cellular growth holds for human placenta (Winick, et al., 1967), although the timing is somewhat different. Winick, et al. (1967) demonstrated that the human placenta increases proportionally in total weight, protein, and RNA until term. However, DNA rises at a uniform rate and then abruptly levels off when the placenta reaches about 300g or the fetus 2,300g, suggesting that the phase of growth is by enlargement of already existing cells and cell division stops in human placenta about one month prior to term. These patterns will serve as a base for studies to explore abnormal prenatal growth in human.

Abnormal cellular growth in human placentas from 17 infants with low birth weight but with no malformations has been demonstrated (Winick, 1967). This study investigated the possibility of a biochemical abnormality in placentas from low birth weight infants by comparing the same parameters for placentas of normal infants. Placentas from infants with intrauterine growth failure were examined for weight, protein, DNA, and RNA contents, and a proportional reduction in weight of the placenta and in its DNA and protein contents, but markedly elevated RNA/DNA ratio were observed. These data indicated that intrauterine growth failure in infants is associated with reduced placental weight accompanied by reduction of cell numbers. A significant correlation between placental growth and fetal growth has also been demonstrated in the studies of both normal (Winick, et al., 1967) and low birth weight infants (Winick, 1967). These data suggest that the placenta may be used even in the human as a guide to fetal changes following maternal stresses such as

maternal malnutrition.

There are interesting evidences of damage to the placenta, due to inadequate maternal nutrition during pregnancy (Campbell, et al., 1953; Hastings-Roberts and Zeman, 1977; Koshy, et al., 1975; Winick, 1971; Zamenhof, et al., 1971). A reduction in rat placental weight with reduced cellular contents (DNA contents) at the 21st day of gestation was observed (Winick, 1971). This is due to inadequate protein intake (5% protein in maternal diet) during pregnancy. Similarly, Hastings-Roberts and Zeman (1977) observed that the restriction of protein intake (4% casein diet) during pregnancy resulted in the production of smaller placentas with a significant reduction both in cell number and cell size. Winick (1971) found that cell number in protein-restricted rat placenta was reduced by 13 days after conception, cell size remained normal, and RNA/DNA ratio was markedly elevated. Zamenhof, et al. (1971) also observed that protein-free diet fed to pregnant rats for five consecutive days during the last half of gestational period (from day 10 to 15) limited placental growth (25% weight reduction compared to control animals). On the contray, Campbell, et al. (1953) found that a protein-free diet given to pregnant rats for the last 6 days of pregnancy resulted in a very moderate decrease in placental weight with a considerable loss in maternal weight. This was apparently brought about by drawing on the maternal reserves laid down during the first 14 days of pregnancy.

It has been suggested that decreased DNA synthesis found in placenta from protein-restricted animal was closely associated with reduction in DNA polymerase activity (Velasco and Brasel, 1972). In the study of DNA polymerase activity of rat placentas, it was found that protein restriction during gestation reduced placental DNA contents only after 14 days of gestation, and the DNA contents were 70% of normal value by the 19th day of gestation.

Moreover, the reduced DNA synthesis in protein-deficient rat placenta was preceded by a reduction in DNA polymerase activity, suggesting that DNA polymerase activity is correlated with the rate of DNA synthesis.

These data support the speculation that maternal protein restriction during gestation might be an important contributing factor to abnormal placental growth as well as fetal growth failure. Furthermore, they suggest that growth pattern of placenta may indirectly reflect the fetal condition.

C. Effects of protein deficiency on maternal organism during pregnancy

In studies of protein deficiency during pregnancy, relatively less attention has been given to the mother compared to the offspring. During normal pregnancy, changes in sizes of various maternal organs occur (Herring, 1920; Souders and Morgan, 1957), and they are often accompanied by biochemical changes (Naismith, 1966; Poo, et al., 1939; Smith and Walsh, 1975). In a study of organ weights of pregnant rats, Harring (1920) reported that the liver was greatly enlarged by pregnancy, while the heart, kidneys, and spleen are not significantly affected. In addition, Souders and Morgan (1957) found an increase in weights of the uterus and ovaries compared with those of unmated littermate controls, but adrenal and thymus weights were decreased relative to body weight. The changes in sizes of various maternal organs indicate that important biochemical changes occur in these organs during pregnancy, and the increase in size of the liver is associated with considerable increases in fat (especially, triglyceride), protein, and RNA contents, and slight increase in DNA contents (Smith and Walsh, 1975; Naismith, 1966). A considerable increase in protein synthesis was also observed in various species of animals during normal pregnancy (Blaxter, 1964), due to the significant increase in nutritional demands for expansion of maternal tissues as well as growth of fetus. During

pregnancy the formation of the fetus draws materials from the mother for protein formation, while at the same time the enlargements of maternal tissues require a considerable preponderance of protein anabolism over catabolism. Thus the protein intake of women should be significantly increased during pregnancy (FAO/WHO, 1956; NAS/NRC, 1980).

Since it was believed that the fetus was parasitic to the mother, and had the ability to draw from the mother all of its dietary needs regardless of maternal nutritional status, pregnant women are likely to be susceptible to protein deficiency. However, Wallace (1948a) pointed out on the basis of animal data (sheep) that competition for available nutrients exists between the fetus and its mother when these are in short supply. Since it is generally considered that protein deficiency during pregnancy leads to a reduction in maternal reserves of pregnancy, it is interesting to observe the effects of a protein restriction on maternal nutritional status during pregnancy and to correlate them with fetal growth.

The impact of nutritional insult upon the individual organs of the maternal body might be different. It has been demonstrated, with nonpregnant (Addis, et al., 1936; Robinson, 1948; Wainio, et al., 1959) and pregnant (Wallace, 1948a) animals, that the liver is the organ in which the greatest degree of change was observed during nutritional manipulation. Addis, et al. (1936) found that during a 7-day fast the rat lost 40% of the original protein content in the liver, from 18 to 28% in heart, kidneys, and alimentary, 8% in muscle, skin, and skeleton, and 5% in brain. Similarly, Robinson (1948) showed, in a study of nonpregnant sheep, that the weight of the liver is particularly sensitive to a change in nutritional level in diet. The same general pattern of changes in maternal organs was found in pregnant animals (Wallace, 1948a).

In a study of pregnant ewes which were given various amounts of food

during pregnancy (Wallace, 1948a), increased liver weight that occurs as a result of pregnancy was not maintained by restricted food intake during pregnancy. Furthermore, the results of this study pointed out that liver weight and protein contents in the liver were more sensitive to nutritional manipulation than the large protein mass of the muscles. The liver illustrates tissues with labile cytoplasmic proteins, most of them are components of various enzymes which rise or fall in quantity as dietary protein increases or decreases (Muramatsu, et al., 1962; Wainio, et al., 1959). These labile proteins contribute amino acids to the metabolic pool for protein anabolism.

These data indicate that the impact of protein restriction might differ from one tissue to another, and it is particularly marked in the liver. Furthermore, they suggest that a quantitative examination of biochemical changes in maternal liver could provide information about maternal nutritional status during pregnancy.

D. Exposure to ultrasonic energy during pregnancy and its biological effects on organism

Ultrasonic energy has emerged as a possible stress to the human because of its rapid commercial development and increasing application in clinical practice of medicine. Over the last two decades, ultrasonic energy has been used for obstetric diagnosis, and it has gradually replaced radiologic techniques (Gottesfeld, 1978). The diagnostic ultrasonic techniques in obstetrics employ ultrasound as a means of obtaining information about the structure and functions of the organs of the maternal and fetal bodies, such as fetus, placenta, and uterus. Its use in obstetrics includes determination of multiple pregnancy (Picker, et al., 1977), fetal sex (Stocker and Evans, 1977) and position, age of the fetus, and localization of the placenta (Gottesfeld,

1978). It is also used to diagnose fetal anomalies by identifying all the major organs, including heart, liver, kidneys, and bladder (Taylor, 1977).

There are several methods of using ultrasound for diagnostic purposes. These methods are A-scan, B-scan, Rapid B-scan, Time-motion scan, Stop-action scan, and ultrasonic Doppler scan. In most techniques, echo information is obtained from the tissue structure and then displayed in an appropriate manner. The most common ultrasonic diagnostic method is a technique involving the reflection of continuous wave ultrasound and the phenomenon known as the Doppler effect (McDicken, 1976). The Doppler effect is a change in the observed frequency of a wave because of the motion of source, and thus the motion can be detected by a change in ultrasonic frequency due to the motion of the source.

While diagnostic ultrasonic techniques are widely used and actually offer much useful information, it is necessary to assess its possible biological effects on the organism. The degree of its effects on the organism might be related to several factors: ultrasonic intensity (or power); ultrasonic frequency; irradiation time; and mode of irradiation (pulsed waves or continuous waves). Adverse effects of ultrasound on biologicl systems could include degradation of biological molecules, destruction of cells and cell organelles, and formation of lesions in the tissues (O'Brien, et al., 1972). The thermal effect (heat results), the mechanical effect (shearing stresses), and the cavitation (oscillating gas bubble formation) have been implicated as a mechanism inducing biological damage associated with the ultrasonic effects (McDicken, 1976).

There are interesting experimental data which suggest that there are possible risks of ultrasonic energy to the organism. In a study of CF1 mice exposed to ultrasonic energy (at spatial peak average intensities ranged from 0.5 to 5.5 W/cm^2 for durations ranging from 10 to 300 seconds) during gestation, especially

during the period of neurogenesis and early organogenesis (O'Brien, 1976), it was reported that significant fetal weight reduction was evident at the 18th day of gestation. However, Januzik (1976) was not able to observe the negative effect of ultrasonic energy (at spatial peak intensity of 2.5 W/cm^2 for 20 seconds) on fetal weight (on 18th day of gestation) by using a different strain of mice (LAF1/J mice) from those used by O'Brien (1976). In the study by Januzik (1976), the effects of ultrasonic irradiation on postpartum weight changes after in utero exposure of ultrasonic energy were also examined, and significant body weight reductions were observed in irradiated pups at the 21st day of postconception. These previous data suggest that there must be a certain degree of risks to the fetus from ultrasonic energy exposure during the prenatal period. The risks of ultrasonic energy exposure during the reproductive life of an individual might be serious, and the safety for the fetus under stress is still in question. Therefore, a reliable assessment of risks and benefits associated with exposure to ultrasound is necessary.

In summary, it is still unclear whether the results obtained using animal models can be directly extrapolated to humans where nutrition and socioeconomic factors are interrelated. Nevertheless, animal studies have been almost exclusively used to assess the effects of maternal stresses on fetal growth and development because of the ease with which experimental conditions are controlled. Many animal studies dealing with the restriction of maternal protein intake during gestation and its effects on the reproductive performance of the mother have indicated that inadequate protein consumption during pregnancy could result in poor reproductive efficiency and fetal growth failure.

At the same time, ultrasonic energy has emerged as a possible stress to the human in utero with its rapidly increasing application for obstetric diagnosis, and there is evidence that suggests possible risks to the fetus from

ultrasonic energy exposure during prenatal period.

Although evidence supports separate adverse effects of either maternal protein deprivation or exposure to ultrasonic energy during pregnancy on fetal growth, there are no data that link combined effects of these two stresses on fetal growth and development.

Since human fetus is not available for investigation, it is important to consider whether placental growth pattern indicates fetal condition. There is evidence which suggests that growth pattern of placenta may indirectly reflect the fetal condition, but evidence supporting this theory is inconclusive. Therefore, a detailed study of placenta in conjunction with fetal growth as influenced by maternal exposure to protein restriction and ultrasonic energy would yield important information which may help to resolve the controversy.

In addition to fetal growth and development, maternal tissue changes and their relationship to fetal growth have to be considered to determine whether maternal tissues serve as a barrier for providing fetal protection against environmental stresses. Tremendous voids in our knowledge exists concerning the combined effects of ultrasonic energy exposure during pregnancy and maternal nutritional deprivation. Much more research is needed particularly that which focuses on the relationship between maternal tissue changes and fetal growth as affected by maternal protein restriction and ultrasonic energy exposure.

Chapter III

Materials and Methods

A. Experimental design

1. Experiment 1 : The influence of dietary protein and fat levels on fetal growth in mice

This experiment was performed to standardize the control diet which would be used in the following experiments.

In this experiment, pregnant mice were fed purified diets ad libitum starting on day zero of pregnancy. These purified diets contained various levels of casein (ranging from 6 to 20%) and fat (either 5 or 15%), and the composition of each diet is shown in Table 1. The caloric density of diets was either 4.01 or 4.51 Kcal/g diet depending on the level of fat in diets.

No animal was exposed to ultrasonic energy in Experiment 1.

2. Experiment 2 : The combined effects of ultrasonic energy exposure on day 4 of gestation and protein-deficient diet on fetal growth in mice

This experiment was conducted to determine the adverse effects of ultrasonic energy and/or protein-deficient diet on fetal growth. The design of this experiment was 2X2 factorial (Figure 1) where two levels of maternal nutritional condition (adequate and restricted protein) and two levels of ultrasonic energy exposure (actual and sham irradiation) were applied to pregnant mice.

The compositions of purified diets used in this experiment are shown in Table 2. A purified diet containing 12% casein was considered to be a control

Table 1

Composition of diets[1] used in experiment 1

Diet: Fat (%) / Casein (%)	5 / 6	5 / 12	5 / 20	15 / 6	15 / 12	15 / 20
	% diet[2]					
Casein	6.0	12.0	20.0	6.0	12.0	20.0
Methionine	0.3	0.3	0.3	0.3	0.3	0.3
Corn oil	5.0	5.0	5.0	15.0	15.0	15.0
Corn starch	51.3	51.3	51.3	51.3	51.3	51.3
Sucrose	31.4	25.4	17.4	21.4	15.4	7.4
Salt mixture[3]	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin mixture[4]	1.0	1.0	1.0	1.0	1.0	1.0
Caloric density (kcal/g diet)	4.01	4.01	4.01	4.51	4.51	4.51

Should'nt each column add up to 100%. They do - OK

[1] Prepared in a 2% agar gel (1:1, dry diet:water)

[2] On the basis of dry weight

[3] Rogers-Harper mineral mixture, catalog #170760, Teklad test diet, Madison, Wisconsin

[4] Vitamin fortification mixture, catalog #40060, Teklad test diet, Madison, Wisconsin

Nutrition (adequate or restricted)

Ultrasonic Energy (sham or actual)		
	Adequate Protein Sham Irradiation	Restricted Protein Sham Irradiation
	Adequate Protein Actual Irradiation	Restricted Protein Actual Irradiation

Figure 1. Experimental design

Table 2

Composition of Diet[1] used in experiment 2

Diet	Control	Protein-restricted
	% diet[2]	
Casein	12.0	6.0
Methionine	0.3	0.3
Corn oil	15.0	15.0
Corn starch	51.3	51.3
Sucrose	15.4	21.4
Salt mixture[3]	5.0	5.0
Vitamin mixture[4]	1.0	1.0

[1]Prepared in a 2% agar gel (1:1, dry diet:water)

[2]On the basis of dry weight

[3]Rogers-Harper mineral mixture, Catalog #170760, Teklad Test Diet, Madison, Wisconsin

[4]Vitamin fortification mixture, Catalog #40060, Teklad Test Diet, Madison, Wisconsin

diet in this experiment, since 12% casein diet was recommended for satisfactory reproduction of rat at the time (NAS/NRC, 1972), and the 12% casein diet with 15% fat was considered to be indicated for satisfying the nutritional demands of pregnant mice on the basis of maternal liver lipid and fetal composition data from Experiment 1. The protein-restricted diet contained 6% casein. All diets contained 15% fat, since an improvement of reproductive performances of mice was observed with increasing dietary fat level from 5 to 15% in the previous experiment.

Every pregnant animal was exposed to the irradiation procedure, either actual or sham irradiation, on the 4th day of gestation.

3. Experiment 3 : The combined effects of ultrasonic energy exposure on day 8 of gestation and protein-deficient diet on both maternal and fetal mice

The design of this experiment was the same as that in Experiment 2 (2X2 factorial) where two levels of maternal nutritional condition (adequate and restricted protein) and two levels of ultrasonic energy exposure (actual and sham irradiation) were applied to pregnant mice.

Since 18% casein diet was recommended recently by the National Academy of Science (1978) for satisfactory reproduction of mice, and an improvement of reproductive performances of mice was observed with increasing dietary fat from 5 to 15% in the previous study, the purified control diet (protein adequate diet) used in this experiment contained 18% casein and 15% fat, with 4.5 Kcal/g of caloric density (Table 3). The purified protein-restricted diet, which was isocaloric with the control diet, contained 6% casein and 15% fat.

Every pregnant animals used in this study was subjected to the irradiation procedure, either actual or sham irradiation, on the 8th day of

Table 3

Composition of diets[1] used in experiment 3

	control	protein restricted
	% diet[2]	
casein	18.0	6.0
methionine	0.5	0.5
corn oil	10.0	10.0
lard[3]	5.0	5.0
corn starch	51.3	51.3
sucrose	9.2	21.2
salt mixture[4]	5.0	5.0
vitamin mixture[5]	1.0	1.0

[1] prepared in a 2% agar gel (1:1, dry diet:water)

[2] on the basis of dry weight

[3] lard, catalog #902140, Nutrition Biochemical, Inc., Cleveland, Ohio

[4] Rogers-Harper mineral mixture, catalog #170760, Teklad test diet, Madison, Wisconsin

[5] Vitamin fortification mixture, catalog #40060, Teklad test diet, Madison, Wisconsin

gestation.

B. General procedures

1. Animals

a. Species

Conventionally reared LAF1/J mice (Jackson Lab, Bar Harbor, Maine) who were proven breeders and weighed between 24 and 31g were employed.

b. Mating procedure

A male LAF1/J mouse was placed with three females of the same species in a cage for approximately two weeks. The females were examined to determine pregnancy. When a female was pregnant, that female and the male in that cage were considered proven breeders and eligible for use in the study.

Proven breeders were mated by placing five females and two males together for a two-hour period (from 8:00 a.m. to 10:00 a.m.) and successful mating was ascertained by observing the presence of vaginal plugs. The day on which vaginal plugs were observed was considered day zero of pregnancy. On day zero, the pregnant females were divided into groups and assigned to one of the dietary regimens.

c. Housing

The animals were kept in a temperature-controlled room ($20^{o}C$) with alternate 12-hour light-dark periods. Pregnant females were housed individually in polypropylene "shoe-box" cages with Sanicel bedding (Paxton Processing Company, Inc., Paxton, Illinois).

2. Diets

All animals were fed a commercial lab feed (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois) until they were mated, and purified diets were used during the gestation period in all experiments. Food and water were available ad libitum until the time of sacrifice.

According to the manufacturer, commercially prepared Lab feed consisted of natural foods, and by proximal analysis contained 24% of crude protein, 4% of crude fat, and 4.5% of crude fiber. Vitamins and minerals were also present in amounts adequate for reproduction. Composition of purifided diets used in Experiments 1, 2, and 3 are shown in Tables 1, 2, and 3, respectively.

Dietary composition of major dietary constituents of purified diets was verified by proximal analysis in this laboratory. Protein content was calculated as 6.25 times the nitrogen content which was measured by Kjeldahl analysis (Oser, 1965). Fat was extracted for 16 hours by the Soxhlet method (Horwitz, 1975a), by using ethyl ether at 30-60^{o}C and fat content was determined gravimetrically. Ash was also determined gravimetrically following complete combustion in a muffle furnace at 550^{o}C for 8 hours (Horwitz, 1975b).

All purified diets were incorporated into a 2% agar gel to reduce wastage and to facilitate the determination of food intakes. The agar-based diet was color-coded by treatment to minimize possible errors in experimental feeding procedures.

The food consumtion of each animal was determined. Each animal was housed separately and given a weighed portion of gel diet. On the following day, any uneaten food was collected, dried at 70^{o}C, and weighed. The food intake of each animal was calculated on dry weight basis and food intake was recorded on a daily basis for each animal.

3. Animal irradiation

The following irradiation procedure was used in Experiments 2 and 3. The pregnant mice were anesthetized with "Metofane" (methoxyfluorane, Pitman-Moore, Inc., Washington Crossing, N.J.). At desired anesthetic condition, hairs on the total abdomen, both the sides and dorsal surface, were removed with electric clippers, and a commercial dipilatory ("Neet", Whitehall Lab, Inc., N.Y., N.Y.) was applied to remove stubbles. The animals were mounted on the holder in a "spread-eagle" fashion and positioned in irradiation tank. The irradiation was performed in the transmitting medium ($37^{o}C$, saline solution) at an ultrasonic frequency of 1MHz. The spatial peak intensity for each exposure was 2.5 W/cm^2 (actual irradiation) and 0.0 W/cm^2 (sham irradiation) for a duration of 20 seconds. The irradiation procedure included six exposures which permits exposure over the entire abdominal region. The irradiation was monitored by a computer, which permits a blind study by selecting the irradiated and sham animals in random fashion. Sham irradiated animals received identical treatments, but did not receive actual ultrasonic energy.

After irradiation the mice were wrapped with a tissue to prevent them from becoming chilled during their recovery, and placed in individual cages.

4. Animal analysis

a. Experiment 1: The influence of dietary protein and fat levels on fetal growth in mice

All animals were killed by cervical dislocation (The Universities Federation for Animal Welfare, 1976) on day 18 of gestation, because it is generally considered that the average gestational period of mice is 19 days (Rugh, 1968). At the time of dissection, maternal liver and all fetuses were

excised and immediately frozen at -25°C for subsequent compositional analysis.

Uteri of dams were carefully examined for the presence of implantation sites to determine whether the sites were normal or in the process of resorption.

Since no correlation between litter size and fetal weights was found in this experiment, all fetuses were eligible for compositional analyses. Fetus were selected at random from the midsection of both uterine horn for analyses, because fetal weight varies within individual uterine horn as well as between the two horns (Barr, et al., 1970).

Randomly chosen fetuses from each mother were assayed for DNA, RNA, protein, lipids, and ash contents. Maternal liver was also analyzed for lipids contents. The nucleic acids were isolated by a modification of the method of Schmidt-Thanhouser (Fleck and Munro, 1962), in which the homogenate was precipitated with trichloroacetic acid, washed with alcohol-ether mixtures, and dissolved in 0.3N NaOH solution. The resulting solution was used for the determination of DNA and RNA. DNA was measured by the modified-Ceriotti method of Hubbard et al. (1970) with calf thymus DNA (Sigma Chemical Company, St. Louis, Mo.) as the standard. In this method indole reacts with sugar moiety, deoxyribose, of DNA molecules and gives a yellow color. RNA was determined by the colorimetric method of Ceriotti (1955) with calf liver RNA (Sigma Chemical Company, St. Louis, Mo.) as the standard. Orcinol was used, in this method, to develop a blue-green color with furfural formed from ribose when ribose is heated with $FeCl_3$-HCl solution. Total lipids were determined by the method of Folch, et al. (1957). Protein was measured colorimetrically with Biuret reagent (Leshner and Litwin, 1972) by using bovine serum albumin (Sigma Chemical Company, St. Louis, Mo.) as the standard. The ash content of each fetus was measured gravimetrically after complete combustion in a muffler furnace at 550°C

for eight hours (Horwitz, 1975b). The ratios of protein to DNA, RNA to DNA, lipids to DNA, lipids to RNA, protein to RNA, and protein to lipids were calculated as a mean of assessing cellular changes.

b. Experiment 2: The combined effects of ultrasonic energy exposure on day 4 of gestation and protein-deficient diet on fetal growth in mice

The same experimental procedures for animal analysis were used in this experiment as those used in Experiment 1.

c. Experiment 3: The combined effects of ultrasonic energy exposure on day 8 of gestation and protein-deficient diet on both maternal and fetal mice

The same general experimental procedures were used in this experiment for animal analysis.

During gestation, all maternal animals were weighed at regular intervals (every other day from day zero to day 18 of gestation), and records of weight gain were kept for each animal. Besides maternal liver and fetuses, placentas were also collected for subsequent compositional analysis at the time of dissection. At the time of sacrifice of animals the following data were recorded: 1) maternal body weight; 2)maternal liver weight; 3)weight of uterus and its contents; 4)litter size; 5)number of resorption sites per mother; 6)individual fetal weight; and 7)individual placental weight. A placental to fetal ratio for each animal was calculated from these data. The weights of uterine tissue and fluids were also calculated by subtracting the weights of fetuses and placentas from weights of uterine mass. Randomly chosen fetuses and placentas from each mother were assayed for DNA, RNA, protein, lipids, and ash

contents. Maternal liver was also analyzed for DNA, RNA, protein, and lipids contents.

To assess fetal skeletal development, one to two randomly selected fetuses from each mother were fixed in 70% ethanol, cleared in 0.1% KOH solution, and stained with alizarin red S (Tipton and Burt, 1977). The cleared specimens were inspected for skeletal development and deformities, and missing ossification centers were counted against the reference control animal.

5. Statistical analysis

Analysis of variance (2X2 factorial) and regression and correlation statistics (Steel and Torrie, 1960) were used in data evaluation. Student's t-test was also used to determine whether the correlation coefficient obtained from the correlation analysis was statistically significant. A probability value of less than 0.05 was taken as the level of significance.

Chapter IV

The Influence of Dietary Protein and Fat Levels on and Fetal Growth in Mice

A. Results

1. Maternal food and caloric intakes

Data on food and caloric intakes of maternal animals fed purified diets containing various levels of protein and fat during gestation are presented in Table 4. At each level of dietary fat, pregnant mice consumed similar quantities of food regardless of dietary protein level. However, maternal food intake during gestation dropped significantly when the dietary fat level (and thus the caloric density of the diet) was increased from 5 to 15%. As a result, total caloric intakes were not affected by changes in the dietary fat level.

2. Maternal liver lipid changes

The maternal liver lipid accumulation (Table 4) was influenced by the levels of dietary protein and/or fat. At each level of dietary protein, increasing dietary fat level from 5 to 15% resulted in a significant decrease in liver lipid content. The level of dietary protein, likewise, had a significant influence on maternal lipid content. At 5% of dietary fat, there was a significant decrease in liver lipid content as the casein level was increased from 6 to 20%. When dietary fat level was increased from 5 to 15%, increasing dietary casein level from 6 to 12% resulted in a significant decrease in liver lipid content, but no further change in liver lipid content was observed with further increasing casein content to 20%.

Table 4

Maternal characteristics of mice fed diets containing varying level of protein and fat[1, 2]

Diet: Fat (%)	5			16		
Casein (%)	6	12	20	6	12	20
Number of dams in group	10	12	4	11	10	4
Food intake[3] (g/day)	4.5±0.1[a]	4.4±0.1[a]	-[4]	3.9±0.2[b]	4.0±0.2[b]	4.1±0.3[b]
Caloric intake (Kcal/day)	17.8±0.4	17.4±0.2	-[4]	17.1±0.9	17.8±0.9	18.0±1.6
Liver lipids[5] (g/100g)	23.23±1.79[a]	19.17±2.36[ab]	17.22±1.39[b]	12.85±0.85[c]	9.27±0.44[cd]	7.11±0.46[d]

[1]Results are means ± S.E.

[2]Values bearing different superscript letters differ significantly at $p<0.05$

[3]Dry weight basis

[4]Data lost during experiment

[5]Wet weight basis

Table 5

Gestational performance of mice fed diets containing varying level of protein and fat[1, 2]

Diet: Fat (%) / Casein (%)	5 / 6	5 / 12	5 / 20	15 / 6	15 / 12	15 / 20
Resorptions of implantations						
Number of resorptions per dam	1.4±0.4	1.0±0.3	1.0±0.4	2.1±0.4	2.0±0.4	0.3±0.2
% resorptions of implantations per dam	12.1±3.5[a]	9.7±3.4[a]	9.1±3.7[a]	20.5±3.3[a]	19.2±3.9[a]	3.9±2.9[b]
Litters of implantations						
Litter size	9.1±0.3	9.4±0.4	8.8±1.0	8.1±0.6	8.2±0.8	9.3±0.8
% litters of implantations per dam	87.9±3.5[a]	90.3±3.4[a]	90.9±3.7[a]	79.5±3.3[a]	80.8±3.9[a]	96.1±2.9[b]
Fetal weight (g)	1.00±0.03	1.04±0.03	0.99±0.05	0.93±0.04	1.01±0.02	1.03±0.05

[1]Results are means ± S.E.

[2]Values bearing different superscript letters differ significantly at $p<0.05$

3. Gestational performance

Results of the gestational performance of mice fed different dietary regimen (Table 5) show that the levels of dietary fat and protein had no influence on the number of resorption sites per dam, litter size, and fetal weight. However, when the data were expressed as percentage resorptions of implantations and percentage litters of implantations per dam, marked influences of the fat and protein contents in maternal diet were noted. This is due to the fact that by presenting data in this way it is possible to compensate for the variations in the number of implantations, which tend to occur independently of changes in dietary protein and fat intakes during gestation. The influence of dietary protein level on percentage resorptions and percentage litters of implantations was evident only at the high level of dietary fat (15%). When diets contained 15% fat, percentage resorptions and percentage litters of implantations were significantly decreased and increased, respectively, as the casein level in diet was increased from 6 to 20%.

4. Fetal composition

Results in Table 6 illustrate that both the protein and fat levels in diet during gestation cause marked changes in fetal protein contents. When diets contained 5% fat, no significant change in fetal protein content was found with increased dietary casein level from 6 to 12%. However, there was a significant increase in fetal protein content with increased dietary casein level from 6 to 20% in these 5% fat diets. In contrast, when diets contained 15% fat, a significant increase in fetal protein content was observed when casein level in diet was increased from 6 to 12%. When casein content was further increased to 20% in this 15% fat diet, however, no further change in

Table 6

Major constituents of fetus of mice fed diets containing varying level of protein and fat[1, 2, 3]

Diet: Fat (%)	5			15		
Casein (%)	6	12	20	6	12	20
	mg/g fetus					
Protein	95.6 ± 2.7[a]	97.6 ± 2.9[ab]	110.1 ± 6.6[bc]	99.1 ± 3.3[ab]	115.1 ± 5.2[c]	120.0 ± 2.5[c]
Lipids	20.5 ± 0.4	21.5 ± 0.5	21.4 ± 0.2	21.6 ± 0.3	21.2 ± 0.3	20.4 ± 0.2
Ash	19.2 ± 0.2	19.8 ± 0.3	19.1 ± 0.3	19.9 ± 1.0	20.2 ± 1.2	20.3 ± 0.1

[1]Results are means ± S.E.

[2]Wet weight basis

[3]Values bearing different superscript letters differ significantly at $p < 0.05$

Table 7

Parameters of fetal cellular growth of mice fed diets containing varying level of protein and fat[1, 2, 3]

Diet: Fat (%) / Casein (%)	5			15		
	6	12	20	6	12	20
DNA (mg/fetus)	2.31± 0.03[a]	2.07± 0.38[a]	2.52±0.20[ab]	2.22±0.12[a]	2.81±0.17[bc]	3.23±0.14[c]
RNA (mg/fetus)	6.85±0.14[a]	7.80±0.42[a]	9.73±0.10[b]	7.80±0.40[a]	9.17±0.25[b]	9.90±0.50[b]
Protein/DNA (mg/mg)	41.43±1.43	43.05±4.67	41.25±3.56	40.25±1.86	40.28±1.71	38.33±1.27
RNA/DNA (mg/mg)	2.96±0.06	3.23±0.28	3.59±0.15	3.00±0.16	3.04±0.25	3.08±0.18

[1]Results are means ± S.E.

[2]Wet weight basis

[3]Values bearing different superscipt letters differ significantly at $p < 0.05$

fetal protein content was observed. Neither dietary fat nor dietary protein level influenced fetal fat and ash contents (Table 6).

The influence of dietary protein level on fetal DNA content (Table 7) was shown only at the high level of dietary fat (15%). When diets contained 15% fat, fetal DNA content was significantly increased as the casein level was increased from 6 to 12%. As casein content was further increased to 20% in these 15% fat diets, the fetal DNA content did not continue to increase. The change in fetal RNA content was the same as that observed in fetal protein and DNA contents. A significant increase in fetal RNA content was observed with an increase in the casein level from 6 to 20% in 5% fat diets, while fetal RNA content was increased when the casein level was increased from 6 to 12% in 15% fat diets. The ratios of protein to DNA and RNA to DNA (Table 7) were not significantly different among dietary groups.

B. Discussion

Nutrient-deficient diets often have the effect of producing a caloric deficit due to a decrease in total food intake. In this study, however, no significant change was observed in food or caloric intake of pregnant animals with a change in the protein content of diets at each level of dietary fat. These findings are similar to those reported by other investigators (Zeman, 1967; Zamenhof, et al., 1971) studying protien deficiency during pregnancy. In contrast, significantly reduced food intakes of protein-deficient animals during pregnancy have also been reported (Hastings-Roberts and Zemam, 1977). In this study, a change in the level of dietary fat, likewise, did not result in changes in caloric intakes among groups. Therefore, it can be concluded that any changes in maternal and fetal organisms with altered dietary protein and/or fat levels are due to differences in respective nutrient intakes rather than altered

caloric intakes.

Results from the present study show that the amount of fat accumulation in the maternal liver of mice is reduced with an increase in both dietary protein and fat content. Liver lipid accumulation at low dietary protein level has been attributed to interference with the intracellular metabolism of lipids, since impairment of lipid transport due to decreased synthesis of the protein portion of the lipoprotein molecule has been suggested as a possible cause for fatty liver in the case of protein deficiency (Flores, et al., 1970; Seakins and Waterlow, 1972; Truswell, et al., 1969). It has also been speculated that fatty changes in the liver on low-protein diets might be due to a net increase in the amount of lipid per gram of tissue caused by a reduction in liver cell size (Edozien and Switzer, 1978), rather than changes in intracellular metabolism of the lipids. On the other hand, the observed reduction in the liver lipid accumulation caused by additional fat in diet may be due to a decrease in lipogenesis when animals are fed high dietary fat levels (Platka-Bird and Bennink, 1978).

When diet contained minimal amount of protein (6% casein), increasing the dietary fat content from 5 to 15% had no effect on any of the parameters measured for gestational performance (litter size, percent litters of implantations, number of resorption sites, and percent resorptions of implantations) and fetal growth (fetal weight and fetal body composition). This is probably because increasing the fat level lowers food intake, and consequently increasing fat content could exaggerate a deficient state of protein, as well as lead to a deficient state of other nutrients such as vitamins and minerals. When diet contained sufficient amount of protein (20% casein), however, increasing the dietary fat content improved both gestational performance and fetal growth.

Percentages of resorptions and litters of implantations of mice were not altered by an increase in the level of dietary protein when diets contained low level of dietary fat (5%). However, at the high level of dietary fat (15%), an increase in dietary casein from 6 to 20% improved the outcome of gestation, as indicated by a significant decrease in percentage resorptions and an increase in percentage litters of implantations. These differences in the outcome of gestation with different levels of dietary fat indicate that some fat, besides supplying the essential fatty acid, is needed for optimal gestational performance. Other investigators (Knapka, et al., 1978) similarly noted that an increase in the fat content of the diet from 4 to 12% improved reproductive performance at the high level of dietary protein, as measured by increased litter sizes and weaning weights of pups in their study using other inbred strains of mice (BALB/cAnN, C3H/HeN, C57BL/6N, and DBA/2N).

As shown in Table 6, the protein content was low in fetus from mice fed low-protein diets (6% casein), regardless of dietary fat level. However, the level of fat in diets had a significant effect on fetal protein content as dietary protein level was increased. When diets contained low levels of fat (5%), an increase in fetal protein content was observed only by a large increase in dietary protein level (from 6 to 20% casein). At the high level of dietary fat (15%), however, increased fetal protein content was noted with a small increase in dietary protein (from 6 to 12% casein). These results indicate that dietary protein utilization was affected by the dietary fat content as well as by the level of dietary protein.

A significant increase in fetal DNA content (Table 7), without a change in protein/DNA ratios, was found when the casein level was increased from 6 to 12% in high fat diets. This indicated that the result of protein restriction on fetal cellular growth was primarily a change in cell number, rather than cell

size. Similar findings were reported for rats (Zeman and Stanbrough, 1969). The increase in the fetal RNA content (Table 7) was also observed as dietary protein and/or fat level increased, and the increase in fetal RNA content was closely associated with increase in fetal protein content.

The results of this study indicate that protein metabolism is influenced by the level of dietary fat, and that high fat diets are utilized more efficiently than low fat diets. Even though there is yet insufficient evidence to show whether the protein-sparing action occurs primarily through protein synthesis or other reactions in protein metabolism, instances in which certain phases of protein metabolism can be influenced by dietary fat have been reported. For example, lower excretion of urinary nitrogen (Samuels, et al., 1948) was found in rats receiving high fat diets after fasting. This suggests that the extent of protein catabolism is reduced by dietary fat. It was also observed that rats made more efficient use of low protein diets when the basal diets contained 30% of fat than when diets contained only 3% of fat (French, et al., 1948).

The results of this study clearly demonstrated that both the dietary protein and fat levels significantly influence fetal growth of LAF1/J mice. Dietary fat influenced protein utilization and gestational performance of these mice. Optimal fetal growth was obtained with the diet containing 20% casein with 15% fat as indicated by increased fetal protein, DNA and RNA contents. Normal maternal liver lipid content in this 20% protein and 15% fat diet also indicates that this diet is optimal for reproductive performance of LAF1/J mice.

Chapter V

The Combined Effects of Ultrasonic Energy Exposure on Day 4 of Gestation and Protein-Deficient Diet on Fetal Growth in Mice

A. Results

1. Characteristics of maternal animals

Food and caloric intakes of maternal animals were essentially the same in all groups (Table 8 and Appendix 1). However, the differences in maternal body weight gain, liver weight, and liver lipid contents among groups were noticeable due to protein restriction during gestation (Table 8 and Appendix 1). The body weight gain of maternal animal fed protein-deficient diet was significantly lower than that of animal fed protein-adequate (control) diet. In addition, the weight of maternal liver as well as liver lipid contents were significantly low in the protein-restricted group.

In contrast, all maternal parameters tested in this experiment were not influenced by either the ultrasonic energy exposure or the interaction of protein restriction and ultrasonic energy exposure.

2. Gestational performance

The results of gestational performance of mice are summarized in Table 9 and the analysis of variance of these data is shown in Appendix 2. The introduction of protein-restricted diet and ultrasonic energy to pregnant animals had no effect on litter size and resorptions of implantations. On the other hand, the weight of fetus from protein-restricted mother was significantly lower than that of the controls, regardless of irradiation conditions. No

Table 8

Characteristics of maternal mice[1]

Nutrition	Control		Protein-restricted	
Ultrasonic Energy	Sham	Actual	Sham	Actual
Number of dams in group	4	9	11	7
Food intake[2] (g/day)	9.3 ± 0.3	10.2 ± 0.3	10.0 ± 0.2	9.7 ± 0.2
Caloric intake (kcal/day)	41.9 ± 1.4	46.0 ± 1.4	45.1 ± 0.9	43.7 ± 0.9
Body weight gain (g)[3], *	19.0 ± 1.5	19.7 ± 0.8	14.8 ± 1.5	16.4 ± 1.8
Liver weight (g)**	2.31 ± 0.11	2.21 ± 0.06	1.97 ± 0.06	1.78 ± 0.26
Liver lipids[4], ** (g/100 g)	10.30 ± 2.32	9.61 ± 0.71	15.10 ± 0.99	14.8 ± 1.58

[1]Results are means ± S.E.

[2]Dry weight basis

[3]Maternal body weight gain throughout the gestational period

[4]Wet weight basis

*A significant nutritional effect was shown at the level of $p < 0.05$

**A significant nutritional effect was shown at the level of $p < 0.01$

Table 9

Gestational performance of mice on day 18 of gestation[1]

Nutrition	Control		Protein-restricted	
Ultrasonic Energy	Sham	Actual	Sham	Actual
Resorptions of implantations				
Number of resorptions per dam	2.8 ± 0.9	2.2 ± 0.5	1.8 ± 0.5	1.8 ± 0.7
% resorptions of implantations per dam	21.2 ± 6.4	17.1 ± 4.3	15.7 ± 4.0	15.0 ± 7.1
Litters of implantations				
Litter size	9.6 ± 0.4	9.8 ± 0.6	9.8 ± 0.5	10.0 ± 0.8
% litters of implantations per dam	78.8 ± 6.4	82.9 ± 4.3	84.4 ± 4.0	85.0 ± 7.1
Fetal weight (g)**	0.95 ± 0.02	0.99 ± 0.02	0.87 ± 0.02	0.82 ± 0.03

[1]Results are means ± S.E.

**A significant nutritional effect was shown at the level of $p < 0.01$

combined effect of protein restriction and ultrasonic energy exposure on gestational performance was observed in this experiment.

3. Fetal growth

Data summarizing the results of major constituent analyses of fetus are shown in Table 10. The analysis of variance of these data (Appendix 3) revealed that maternal protein deprivation during pregnancy had significant adverse effect on fetal protein content, but not on lipids nor ash contents of fetus. However, the effects of ultrasonic energy exposure and its interaction with protein deprivation were not significant for fetal protein, lipids, and ash contents.

Data concerning fetal cellular growth measurements are presented in Table 11, and the analysis of variance of these data is shown in Appendix 4. The fetal RNA content was markedly reduced by the separate effect of either protein restriction or ultrasonic energy exposure, although no significant difference in fetal DNA content was observed among groups. No effect of interaction of these two stresses, however, were noticed on fetal RNA contents. Further, there were significant reductions in protein/DNA ratios as well as RNA/DNA ratios of fetuses when maternal animals were fed protein-restricted diet during gestation. However, the effects of ultrasonic energy exposure and its interaction with protein restriction were negligible for both the protein/DNA and RNA/DNA ratios.

B. Discussion

It has often been conjectured that protein-deficient diet has the effect of decreasing the total food intake, but evidence supporting this theory has not

Table 10

Major constituents of fetal mice on day 18 of gestation[1]

Nutrition	Control		Protein-restricted	
Ultrasonic Energy	Sham	Actual	Sham	Actual
	mg/g fetus			
Protein*	113.2 ± 6.4	100.7 ± 2.8	95.9 ± 2.4	92.0 ± 3.6
Lipids	17.0 ± 0.8	15.9 ± 0.4	16.8 ± 0.6	16.8 ± 1.0
Ash	17.2 ± 0.8	16.5 ± 0.5	16.3 ± 0.5	18.0 ± 1.4

[1]Results are means ± S.E.

*A significant nutritional effect was shown at the level of $p < 0.05$

Table 11

Parameters of fetal cellular growth of mice on day 18 of gestation[1, 2]

Nutrition	Control		Protein-restricted	
Ultrasonic Energy	Sham	Actual	Sham	Actual
DNA (mg/fetus)	2.36 ± 0.18	2.36 ± 0.12	2.43 ± 0.09	2.03 ± 0.11
RNA*, ** (mg/fetus)	10.60 ± 0.39	9.79 ± 0.24	8.21 ± 0.19	7.34 ± 0.40
Protein/DNA** (mg/mg)	51.2 ± 5.2	43.8 ± 2.8	37.8 ± 1.9	39.8 ± 1.5
RNA/DNA** (mg/mg)	4.57 ± 0.37	4.22 ± 0.21	3.43 ± 0.16	3.63 ± 0.18

[1]Results are means ± S.E.

[2]Wet weight basis

*A significant effect of ultrasonic energy was shown at the level of $p < 0.05$

**A significant nutritional effect was shown at the level of $p < 0.01$

been convincing. While protein-deficient diet has been reported to result in decreased food intakes in various species of pregnant animals (Berg, 1967; Kohrs, et al., 1976; Pond, et al., 1968), no significant difference in food intake between control and protein-deficient groups was observed during gestation by other investigators (Zamenhof, et al., 1971; Zeman, 1967). Since we observed that maternal food intakes of low-protein diet groups did not differ from those of control groups, any changes observed in maternal and fetal organisms with protein-deficient diet could be directly attributable to low-protein intakes in this experiment.

The maternal body weight gain during gestation was significantly affected by protein restriction, whereas maternal weight gain did not appear to be affected by either exposure to ultrasonic energy or its interaction with protein restriction. Protein-restricted animals gained less body weight during gestation than the control animals did. This inferior maternal body weight gain in protein-restricted animals may be due to significant reductions in maternal liver and fetal weights in this experiment. These results would be compatible with previous findings which show a net loss of maternal body weight as well as decreased fetal weight as a result of maternal protein deprivation during gestation (Hastings-Roberts and Zeman, 1977).

As previously reported (experiment 1), maternal protein restriction resulted in a significant increase in accumulation of maternal liver lipids in this experiment. Liver lipid accumulation in protein-restricted animals may be a result of the impairment of lipid transport due to decreased synthesis of protein part of the lipoprotein molecules, because low plasma triglyceride levels have been frequently observed in protein-deficient subjects (Flores, et al., 1970; Seakins and Waterlow, 1972).

There were no significant differences in both litter size and

resorptions among the groups. The results indicate that a 6% casein diet can maintain normal size litters throughout gestation, and ultrasonic energy exposure on day 4 of gestation has no effect on the maintenance of pregnancy. However, protein restriction during gestation produced fetus which was smaller than control, and the fetus from protein-restricted mother had significantly lower protein contents than the control animals did. Experiments with rats (Zeman, 1967), in which pregnant animals were fed a diet containing either 24 or 6% casein as the sole source of protein, yielded similar results to those observed in this experiment. Pregnant rats fed protein-restricted diet produced pups with decreased birth weight and decreased liver and kidney weights with respect to total body weight.

Although no significant differences in fetal weight were observed in this experiment that are due to ultrasonic energy exposure, the results suggest that fetal weight reduction is at least partly due to ultrasonic irradiation when protein is restricted. Similar trend was also noticed in fetal protein contents. These results suggest that there is a certain degree of risks to the fetus from ultrasonic energy exposure during prenatal period, and the adverse effect of ultrasonic energy might manifest itself during the postnatal period. This speculation is supported by previous findings by Januzik (1976). In the study of LAF1/J mice exposed to ultrasonic energy during gestation, Januzik (1976) was not able to observe the negative effect of ultrasonic energy on fetal weight on the 18th day of gestation, but a significant body weight reduction was observed in irradiated pups on the 21st day of postconception.

The growth of an organism consists of an increase in the number of cells or growth in the size of cells or both (Winick and Noble, 1965), and thus many studies have been concerned with cellular changes in fetal organism (Zamenhof, et al., 1968 and 1971; Zeman and Stanbrough, 1969). The size of cells in the

fetus of protein-restricted animals, as indicated by the protein/DNA ratios, was reduced in this experiment. The RNA contents per cell, as indicated by RNA/DNA ratios, was also significantly decreased by protein restriction. The decreased amount of fetal RNA content by either protein restriction or ultrsonic energy exposure may be related to the decreased protein synthesis, since the trend of change in RNA/DNA ratios and protein/DNA ratios was in the same general direction in this experiment. Cell number, as indicated by total DNA contents, was somewhat reduced by ultrasonic energy exposure when protein was restricted, but the difference was not marked enough to show statistical difference.

The results of this experiment suggest that both the maternal nutrition and fetal growth can be impaired by protein restriction during pregnancy. Moreover, ultrasonic energy exposure on day 4 of gestation could have adverse effects on the growth of fetal mice, especially on the fetal cellular growth.

Chapter VI

The Combined Effects of Ultrasonic Energy Exposure on Day 8 of Gestation and Protein-Deficient Diet on both Maternal and Fetal Mice

A. Results

1. Characteristics of maternal animals

a. Maternal food and caloric intakes during gestation

Data concerning daily food and caloric intakes of maternal animals during gestation are presented in Figure 2, and the analysis of variance of these data is presented in Appendices 5 (for food intake) and 6 (for caloric intake). The data clearly show that protein content in the diet had a significant influence on maternal food intake during gestation, while the effect of ultrasonic energy exposure on maternal food intake was negligible. During the first half of pregnancy, maternal food intakes were not changed markedly as pregnancy progressed, and differences in food intakes among groups were not significant. When pregnancy progressed, however, there was an increase in food intakes in all groups. The increase in food intakes was more marked in control diet groups (groups CS and CA) than that in protein-restricted diet groups (groups RS and RA), and this trend was maintained for the remainder of pregnancy. Consequently, the total food intakes of the control diet groups significantly exceeded those of the protein-restricted diet groups over the period of the pregnancy.

Since protein-restricted diet was isocaloric with the control diet, the decrease in food intake due to restriction of protein content in diet resulted in a significant reduction in caloric consumptions.

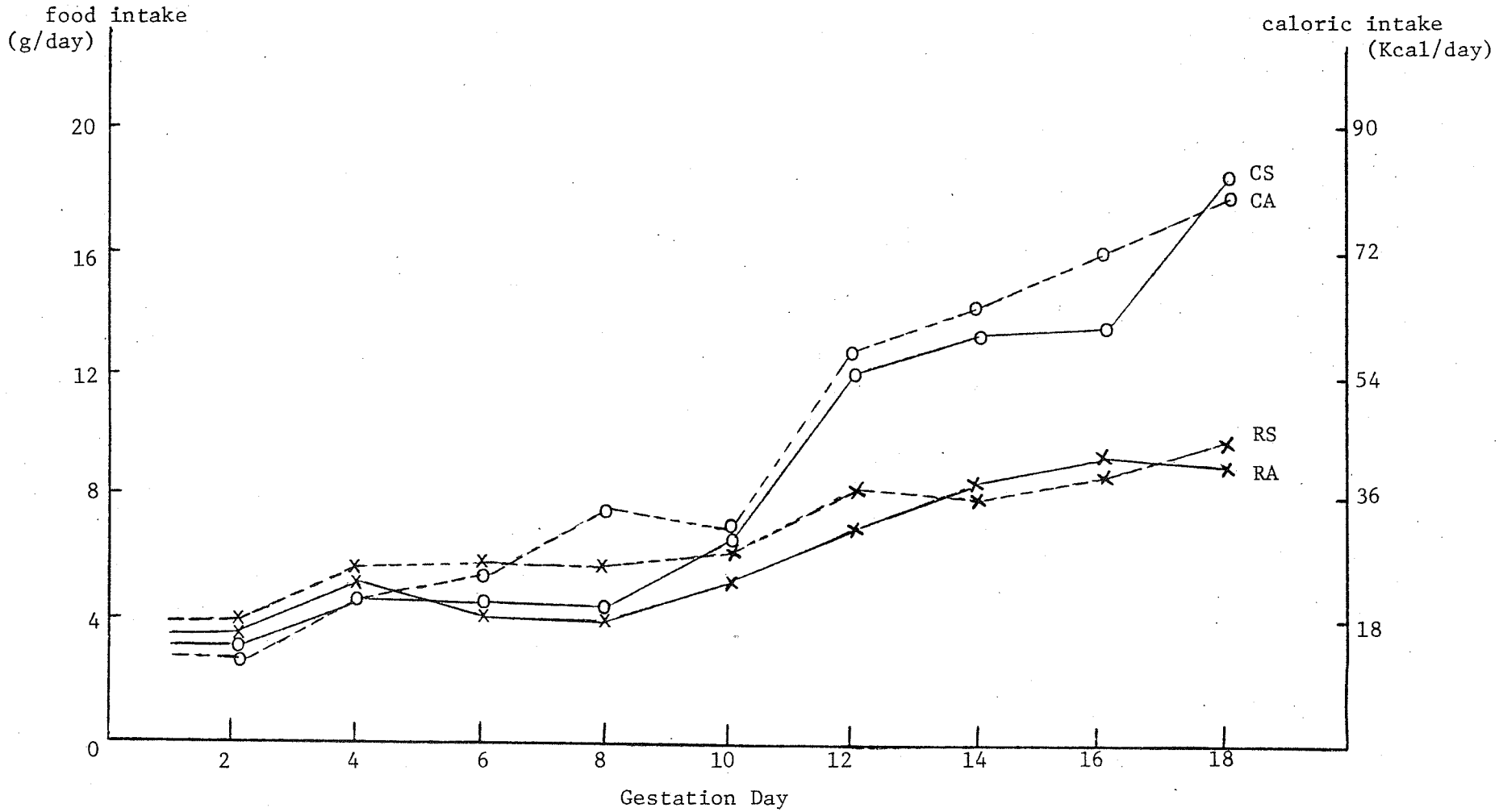

Figure 2. Daily food and caloric intakes of maternal mice during gestation[1, 2, 3, 4]

[1]Results are means of 6 to 9 dams.
[2]Food intakes are on the basis of dry weight.
[3]CS, control diet-sham irradiation; CA, control diet-actual irradiation; RS, protein restricted diet-sham irradiation; RA, protein restricted diet-actual irradiation
[4]A significant nutritional effect on maternal daily food and caloric intakes was shown at the level of $p < 0.01$ after gestation day 10

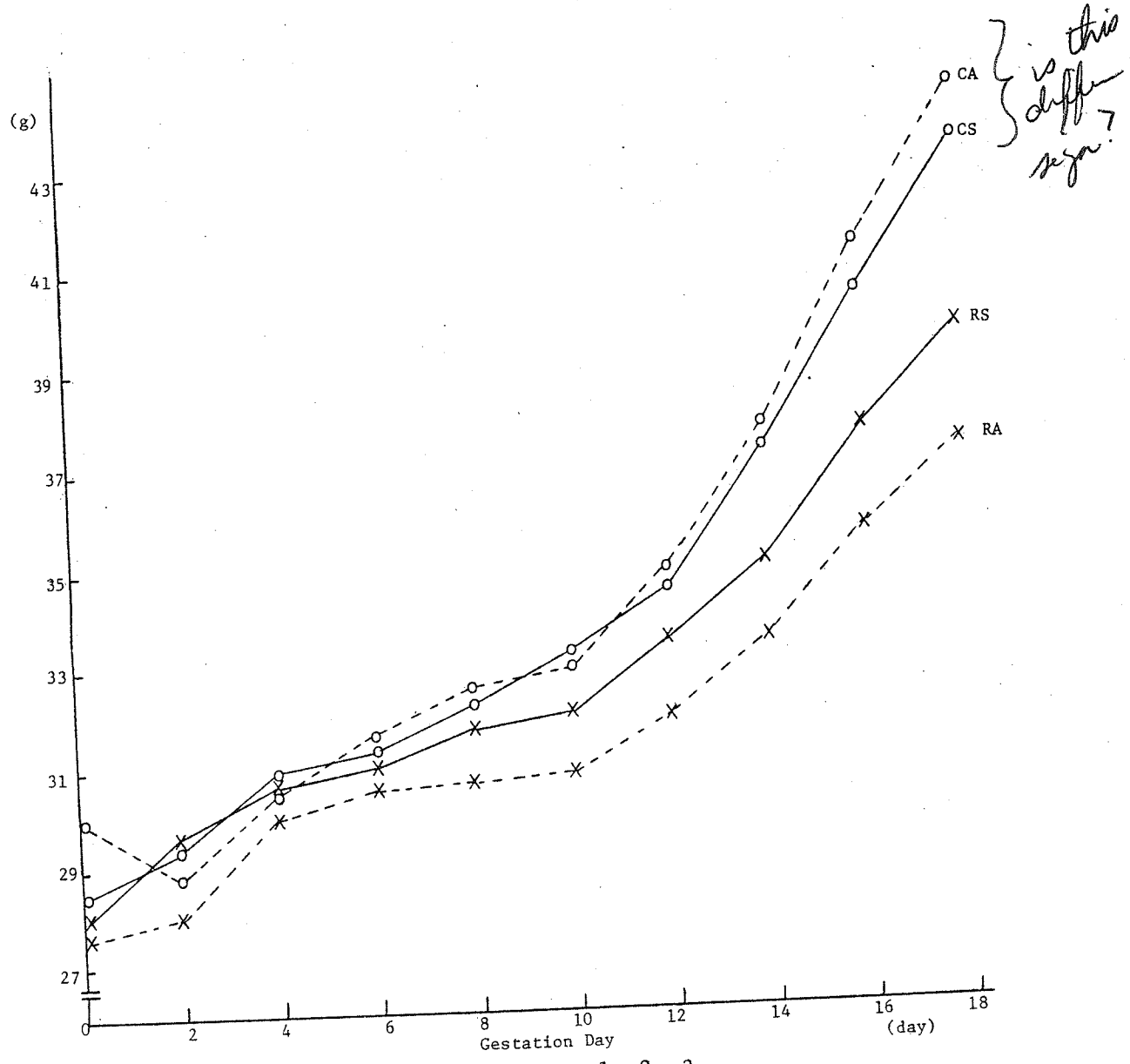

Figure 3. Maternal body weight during gestation[1, 2, 3]

[1]Results are means of 6 to 9 dams.
[2]CS, control diet-sham irradiation; CA, control diet-actual irradiation; RS, protein restricted diet-sham irradiation; RA, protein restricted diet-actual irradiation
[3]A significant nutritional effect on maternal body weights was shown at the level of either $p < 0.05$ or $p < 0.01$ after gestation day 8

The analysis of variance presented in Appendices 5 and 6 shows that neither ultrasonic energy nor its interaction with protein-deficient diet had any effect on maternal food and caloric intakes during gestation.

b. Maternal body weight gain during gestation

There was an increase in maternal body weight in all groups as pregnancy progressed (Figure 3). The general pattern of increase in maternal body weight consisted of minimal increase during the first half of pregnancy and a progressive linear rate of increase through the latter half of pregnancy. During the latter half of pregnancy, considerable increase in maternal body weight was observed in all groups, but this increase was significantly lower in low-protein diet groups than that in controls (Appendix 7). Therefore, total body weight gain in protein-restricted diet groups was significantly lower than that in control groups over the period of pregnancy (Table 12 and Appendix 8).

Maternal net body weight and maternal net body weight changes (both gram and percentage) were also significantly affected by the levels of protein in diets, and those values were low in animals fed protein-restricted diet during gestation (Table 12 and Appendix 8).

In contrast, no interaction was observed between protein restriction and ultrasonic energy exposure on maternal body weight gain in this experiment.

c. Weights of the uterus and its contents

Data on weights of total uterine mass and its component parts are summarized in Table 13, and the analysis of variance of these data is presented in Appendix 9. Although no significant effect of ultrasonic energy exposure and its interaction with protein-restricted diet was observed on the weights of the

Table 12

Maternal body weight gain during gestation[1, 2]

Nutrition	Control		Protein-restricted	
Ultrasonic Energy	Sham	Actual	Sham	Actual
Number of dams	6	8	9	9
Total body weight gain (g)**	14.9 ± 1.0	16.0 ± 1.3	11.5 ± 1.2	9.7 ± 2.3
Net body weight (g)[3,**]	34.2 ± 0.6	34.1 ± 0.8	32.0 ± 1.4	29.7 ± 1.2
Maternal net body weight change (g)[4,**]	5.56 ± 0.63	5.45 ± 0.59	4.02 ± 0.74	3.28 ± 0.42
Maternal net body weight change (%)[5,**]	19.7 ± 2.5	19.2 ± 2.2	14.4 ± 2.5	11.8 ± 1.4

[1]Results are means ± S.E.

[2]Animals were exposed to ultrasonic energy on day 8 of gestation

[3]Maternal body weight on gestation day 18 minus weight of uterine and its contents

[4]Maternal net body weight on gestation day 18 minus initial body weight

[5]Net maternal body weight change (g) divided by initial body weight (g)

**A significant nutritional effect was shown at the level of $p < 0.01$

Table 13

Weights of the uterus and its contents[1]

Nutrition	Control		Protein-restricted	
Ultrasonic Energy	Sham	Actual	Sham	Actual
Number of dams	6	8	9	9
Uterine mass[2]				
g	9.14 ± 1.22	10.53 ± 1.55	7.51 ± 1.22	7.12 ± 1.06
%[4]	20.97 ± 1.10	22.98 ± 2.87	18.53 ± 2.65	19.09 ± 2.82
Fetuses				
g*	5.90 ± 0.59	7.02 ± 1.22	4.96 ± 0.93	4.54 ± 0.83
%[4]	13.48 ± 1.01	15.19 ± 2.28	12.20 ± 2.05	12.17 ± 2.20
Placentas				
g*	0.61 ± 0.02	0.65 ± 0.10	0.47 ± 0.08	0.48 ± 0.07
%[4]	1.40 ± 0.05	1.42 ± 0.19	1.16 ± 0.16	1.29 ± 0.20
Uterine Tissue and Fluids[3]				
g*	2.64 ± 0.13	2.86 ± 0.33	2.07 ± 0.27	2.12 ± 0.19
%[4]	6.08 ± 0.20	6.36 _ 0.68	5.18 ± 0.62	5.66 ± 0.49

[1]Results are means ± S.E.
[2]Total weights of uterine tissue and its contents (fetuses, placentas, and fluids)
[3]Weights of uterine tissue and fluids after removal of fetuses and placentas
[4]Results expressed as a percentage of total maternal body weight
*A significant nutritional effect was shown at the level of $p < 0.05$

uterus and its contents, some effect of protein-restricted diet on these parameters was noted. The animals fed low-protein diet showed somewhat lower weight of the total uterine mass than those fed control diet did, but the differences among groups were not sufficiently marked to show statistical significance. When the results were expressed in terms of the component parts of the uterine mass, however, the significant difference in the weight of each component part was observed among groups. Total weights of fetuses and placentas from mother fed protein-restricted diet were significantly reduced compared to those from controls. The weights of the amniotic fluid and uterus itself were also significantly reduced by lowering the protein content in diet.

When the weights of uterus and its components were expressed as a percentage of total maternal body weight on day 18 of gestation, however, there was no significant difference among groups (Table 13 and Appendix 9).

The total weights of the uterine mass and its component parts were also calculated on a per pup basis (Table 14). The weight of uterine mass were significantly reduced by lowering the protein content in diet. It was due to a significant decrease in individual fetal weight in low-protein diet group. The weights of placenta and uterine tissue per pup were not significantly influenced by protein content in maternal diet (Appendix 10). In addition, there was a noticeable change in the weights of fetuses, placentas, and uterine mass when expressed on per pup basis due to ultrasonic energy exposure during gestation, although the effect of ultrasonic energy exposure on these weights were not marked enough to show statistical significance at the level of $P<0.05$. A certain degree of reduction in the weights of fetus, placenta, and uterine mass was observed due to ultrasonic energy exposure on day 8 of gestation, especially when the protein content in maternal diet was limited. When these data were expressed as a percentage contribution to the total maternal weight on day 18 of

Table 14

Fetal, placental, and uterine weights per pup[1]

Nutrition	Control		Protein-restricted	
Ultrasonic Energy	Sham	Actual	Sham	Actual
Number of dams	6	8	9	9
Weight of uterine mass per pup[2]				
g**	1.52 ± 0.04	1.55 ± 0.03	1.47 ± 0.05	1.34 ± 0.04
% body weight[4]	3.51 ± 0.04	3.59 ± 0.26	3.79 ± 0.25	3.62 ± 0.12
Mean fetal weight				
g**	0.97 ± 0.05	1.02 ± 0.06	0.91 ± 0.03	0.82 ± 0.06
% body weight[4]	2.28 ± 0.07	2.30 ± 0.14	2.34 ± 0.11	2.19 ± 0.15
Mean placental weight				
mg	102.5 ± 6.1	96.4 ± 2.7	94.1 ± 6.4	88.8 ± 4.7
% body weight[4]	0.24 ± 0.02	0.22 ± 0.01	0.24 ± 0.02	0.24 ± 0.01
Weight of uterine tissue and fluid per pup[3]				
g	0.44 ± 0.01	0.46 ± 0.05	0.46 ± 0.06	0.46 ± 0.06
% body weight[4]	1.03 ± 0.05	1.08 ± 0.15	1.22 ± 0.20	1.25 ± 0.16
Fetus/placenta (mg/mg)	9.75 ± 0.95	10.57 ± 0.48	10.04 ± 0.71	9.19 ± 0.51

[1]Results are means ± S.E.

[2]Total weights of uterine tissue and its contents divided by litter size

[3]Weights of uterine tissue and fluids divided by litter size

[4]Percent contribution of individual fetal and placental weights and of the remaining uterine tissue and fluids per pup as a percentage of maternal body weight

**A significant nutritional effect was shown at the level of $p < 0.01$

gestation, however, no difference was observed among groups (Table 14 and Appendix 10).

The ratios of fetal to placental weights were the same for all groups (Table 14 and Appendix 10).

d. Characteristics of maternal liver

Data relating to liver weight and total contents of lipid and protein in maternal liver are reported in Table 15, and the analysis of variance of these data are presented in Appendix 11. The liver weight of maternal animal maintained on protein-deficient diet during gestation were significantly reduced as compared with that of controls, but there was no effect of dietary protein level on the relative weight of liver (liver weight/body weight). The concentrations of lipids in the liver of maternal mice fed low-protein diet were significantly higher than those of controls. Also the protein content of livers from protein-restricted dams was significantly reduced. Accordingly, a significant reduction in the protein to lipid ratio was observed in the protein-restricted diet groups. The livers from animals fed protein-deficient diet were pale or yellow in color, and they were often fragile.

Neither ultrasonic energy exposure alone nor in combination with a protein-deficient diet had any effect on liver weight, liver weight to body weight ratio, and lipid and protein contents in maternal liver.

The cellular parameters of maternal liver are shown in Table 16, and the analysis of variance of these parameters is presented in Appendix 12. Animal fed protein-restricted diet had higher concentration of liver DNA (mg DNA/g liver) than those fed control diet, while there was no difference in total DNA content in the maternal liver (mg DNA/liver) among groups. In contrast, the concentration of liver RNA (mg RNA/g liver) as well as the total RNA content (mg

Table 15

Characteristics of maternal liver of mice[1, 2]

Nutrition	Control		Protein-restricted	
Ultrasonic Energy	Sham	Actual	Sham	Actual
Number of dams	6	8	9	9
Liver weight (g)*	2.03 ± 0.08	1.93 ± 0.10	1.80 ± 0.11	1.77 ± 0.06
Liver weight/maternal body weight (mg/g)	46.9 ± 0.5	43.4 ± 1.8	45.7 ± 1.8	47.7 ± 1.6
Lipids (mg/g liver)**	59.7 ± 6.0	67.8 ± 6.6	106.0 ± 11.1	106.8 ± 9.7
Protein (mg/g liver)**	232.3 ± 13.7	226.7 ± 9.9	204.0 ± 5.1	203.2 ± 7.6
Protein/Lipids (mg/mg)**	3.98 ± 0.22	3.49 ± 0.22	2.05 ± 0.16	2.00 ± 0.15

[1]Results are means ± S.E.

[2]Wet weight basis

*A significant nutritional effect was shown at the level of $p < 0.05$

**A significant nutritional effect was shown at the level of $p < 0.01$

Table 16

Cellular parameters of maternal liver of mice[1, 2]

Nutrition	Control		Protein-restricted	
Ultrasonic Energy	Sham	Actual	Sham	Actual
Number of dams	6	8	9	9
DNA (mg/liver)	6.83 ± 0.34	6.45 ± 0.34	6.65 ± 0.46	6.61 ± 0.48
DNA (mg/g liver)*	3.36 ± 0.11	3.24 ± 0.12	3.71 ± 0.17	3.67 ± 0.21
RNA (mg/liver)	16.08 ± 1.11	15.11 ± 1.02	14.37 ± 0.95	13.60 ± 0.56
RNA (mg/g liver)	7.88 ± 0.31	7.81 ± 0.24	7.97 ± 0.15	7.68 ± 0.27
RNA/DNA (mg/mg)	2.35 ± 0.06	2.43 ± 0.10	2.18 ± 0.12	2.13 ± 0.13
Protein/DNA (mg/mg)**	69.38 ± 4.55	70.37 ± 2.98	55.55 ± 1.90	56.60 ± 4.10
Protein/RNA (mg/mg)*	29.52 ± 1.38	28.99 ± 0.73	25.69 ± 0.86	26.82 ± 1.59
Lipid/DNA (mg/mg)**	17.94 ± 2.11	21.18 ± 2.41	28.41 ± 2.37	29.55 ± 2.94

[1]Results are means ± S.E.

[2]Wet weight basis

*A significant nutritional effect was shown at the level of $p < 0.05$

**A significant nutritional effect was shown at the level of $p < 0.01$

RNA/liver) in the liver did not differ among groups. Although no difference in the amount of RNA per cell (RNA/DNA ratio) was observed among groups, the relative cellular protein (protein/DNA ratio) and lipid (lipid/DNA ratio) contents in liver were significantly affected by the level of protein in diet. The protein-deficient diet caused a decrease in cellular protein contents in maternal liver but an increase in its cellular lipid content. The reduced ratios of protein to RNA contents in liver were also observed in protein-restricted diet groups compared with those in control groups.

No significant effect of ultrsonic energy exposure and its interaction with protein-deficient diet was observed on any parameters tested for cellular changes in maternal liver.

2. Gestational performance

Table 17 and Appendix 13 show the adverse effect of protein-restricted diet on gestational performance as measured by the parameters reported in this experiment, although there was little effect of ultrasonic energy exposure or its interaction with protein-restricted diet on these parameters. A decrease in dietary protein content resulted in an increase in the number of resorption sites per dam, but it did not affect the litter size. When data were expressed in terms of percentage of implantation, however, both percentage resorptions and percentage litters of implantations were markedly affected by protein level in diet. The percentage resorptions and percentage implantations were significantly increased and decreased, respectively, due to protein-restricted diet.

Table 17

Gestational performance of mice[1]

Nutrition	Control		Protein-restricted	
Ultrasonic Energy	Sham	Actual	Sham	Actual
Number of dams	6	8	9	9
Resorptions of implantations				
Number of resorptions per dam*	2.2 ± 0.5	1.0 ± 0.4	2.7 ± 0.9	3.3 ± 0.6
% resorptions*	24.3 ± 5.5	13.4 ± 6.4	31.9 ± 8.6	39.8 ± 5.8
Litters of implantations				
Litter size	6.0 ± 0.4	6.9 ± 1.1	5.3 ± 1.0	5.3 ± 0.9
% litters*	75.7 ± 5.5	86.6 ± 6.4	68.1 ± 8.6	60.2 ± 5.8

[1]Results are means ± S.E.

*A significant nutritional effect was shown at the level of $p < 0.05$

3. Fetal body composition and cellular growth

On the 18th day of gestation, the fetal body composition did not differ significantly among groups (Table 18 and Appendix 14). However, protein-deficient diet during gestation had a significant adverse effect on fetal cellular growth (Table 19 and Appendix 15). Total DNA, RNA, and protein contents of fetus from the mother fed protein-deficient diet were significantly less than those of controls. Moreover, the fetal contents of DNA, RNA, and protein were somewhat reduced due to ultrasonic energy exposure when dietary protein was restricted, although the reduction was not marked enough to show statistical significance at the level of $P<0.05$. No difference among groups was observed in protein/DNA, weight/DNA, RNA/DNA, and protein/DNA ratios in fetus.

In the examination of skeletons of fetuses on day 18 of gestation, some adverse effects of protein-restricted diet and/or ultrasonic energy exposure were observed on the number of missing ossification sites, but the difference among groups was not marked enough to show statistical significance at the level of $P<0.05$ (Table 19 and Appendix 15).

4. Placental cellular growth

While either ultrasonic energy or its interaction with protein-deficient diet had little influence on parameters tested for placental cellular growth, the protein-deficient diet had a significant adverse effect on some of the parameters measured in this experiment (Table 20 and Appendix 16). Total protein and RNA contents of placenta from animals fed protein-restricted diets during gestation were significantly reduced compared with those from animals fed control diets. In addition, placental RNA content was somewhat reduced due to ultrasonic energy exposure during gestation, but the reduction was not marked

Table 18

Body composition of fetal mice on gestation day 18[1, 2, 3]

Nutrition	Control		Protein-restricted	
Ultrasonic Energy	Sham	Actual	Sham	Actual
Number of dams	6	8	9	9
Water (%)	85.0 ± 0.3	84.9 ± 0.8	84.9 ± 0.3	85.1 ± 0.2
Protein (%)	12.0 ± 0.1	11.8 ± 0.2	11.0 ± 0.2	10.6 ± 0.1
Lipids (%)	2.1 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.1 ± 0.2
Ash (%)	1.81 ± 0.04	1.78 ± 0.03	1.79 ± 0.06	1.76 ± 0.07

[1]Results are means ± S.E.

[2]Wet weight basis

[3]Body compositional data do not differ significantly among groups at the level of $p < 0.05$

Table 19

Parameters of fetal cellular growth and bone development[1, 2]

Nutrition	Control		Protein-restricted	
Ultrasonic Energy	Sham	Actual	Sham	Actual
Number of dams	6	8	9	8
DNA (mg/fetus)**	3.39 ± 0.11	3.40 ± 0.10	2.69 ± 0.17	2.28 ± 0.18
RNA (mg/fetus)**	6.15 ± 0.18	6.09 ± 0.17	4.63 ± 0.22	4.43 ± 0.19
Protein (mg/fetus)**	130.4 ± 5.8	131.1 ± 6.6	95.8 ± 3.6	91.6 ± 6.0
Weight/DNA (mg/mg)	329.7 ± 14.6	328.6 ± 18.8	328.7 ± 21.0	355.3 ± 43.7
Protein/DNA (mg/mg)	38.8 ± 2.4	38.7 ± 2.0	36.9 ± 2.3	41.5 ± 3.4
RNA/DNA (mg/mg)	1.82 ± 0.07	1.80 ± 0.06	1.74 ± 0.06	1.99 ± 0.13
Protein/RNA (mg/mg)	21.2 ± 0.7	21.4 ± 0.6	20.0 ± 1.2	20.6 ± 0.9
# of missing ossification centers per pup	2.67 ± 1.89	4.63 ± 0.91	4.50 ± 1.26	8.05 ± 2.92

[1]Results are means ± S.E.

[2]Wet weight basis

**A significant nutritional effect was shown at the level of $p < 0.01$

Table 20

Parameters of placental cellular growth of mice[1]

Nutrition Ultrasonic Energy	Control		Protein-restricted	
	Sham	Actual	Sham	Actual
Number of dams	6	8	8	8
Protein (mg/placenta)**	18.16 ± 1.28	16.14 ± 0.52	14.45 ± 0.25	15.37 ± 0.90
DNA (µg/placenta)	379.7 ± 22.6	349.6 ± 19.3	341.0 ± 11.1	320.1 ± 17.0
Weight/DNA (mg/mg)	271.3 ± 10.8	281.4 ± 17.2	261.3 ± 13.0	293.1 ± 16.9
Protein/DNA (mg/mg)	49.1 ± 2.4	46.3 ± 3.7	42.7 ± 1.6	47.5 ± 2.4
RNA (µg/placenta)*	379.7 ± 22.6	349.6 ± 19.3	341.0 ± 11.1	320.1 ± 17.0
Protein/RNA (mg/mg)	24.4 ± 0.8	23.0 ± 0.7	24.0 ± 0.8	23.2 ± 1.0
RNA/DNA (mg/mg)	2.01 ± 0.08	2.00 ± 0.13	1.80 ± 0.10	2.06 ± 0.11

[1]Results are means ± S.E.

*A significant nutritional effect was shown at the level of $p < 0.05$

**A significant nutritional effect was shown at the level of $p < 0.01$

enough to show statistical significance. The placental DNA content was also decreased by protein-restricted diet and/or ultrasonic energy exposure, although the effects of these two stresses on placental DNA content were not statistically significant.

The ratios of weight to DNA, protein to DNA, protein to RNA, and RNA to DNA were not significantly different among groups.

5. Relationship between maternal nutritional status and fetal growth

Correlation between parameters measured for maternal nutritional status and fetal growth are presented in Table 21. While maternal net body weight gain as well as maternal liver weight were not correlated with fetal weight, a positive correlation was obtained between maternal total weight gain and fetal weight. The maternal liver protein content was also positively correlated with fetal protein content on day 18 of gestation.

6. Relationship between maternal nutritional status and placental growth

There was no correlation between placental growth and any parameters measured as indicative of maternal nutritional status in this experiment (Table 22).

7. Relationship between fetal growth and placental growth

On day 18 of gestation, there was a positive correlation between placental protein/DNA ratio and fetal protein/DNA ratio (Table 23). However, no relationship was observed between placental and fetal contents of protein, DNA, and RNA. No statistically significant relationship was revealed between placental weight and fetal weight.

Table 21

Correlations between maternal nutritional status and fetal growth[1]

	Regression Equation	r^2	t value[3]
Maternal weight gain and fetal weight	Y = 0.02X + 0.67	0.49	3.05**
Maternal net weight gain and fetal weight	Y = 0.003X + 0.91	0.04	0.05
Maternal liver weight and fetal weight	Y = 0.08X + 0.78	0.12	0.69
Maternal liver protein content and fetal protein content	Y = 0.13X + 59.3	0.46	7.93**

[1]Results calculated from 30 to 32 sets of data

[2]Correlation coefficient

[3]t values presented with level of significance, **$p < 0.01$

Table 22

Correlations between maternal nutritional status and placental growth[1]

	Regression Equation	r^2	t value[3]
Maternal weight gain and placental weight	Y = -0.33X + 98.9	-0.09	-0.47
Maternal net weight gain and placental weight	Y = 1.28X + 89.0	0.17	0.92
Maternal liver weight and placental weight	Y = 8.87X + 78.2	0.16	0.87
Maternal liver protein content and placental protein content	Y = 0.005X + 13.9	0.16	0.87

[1]Results calculated from 30 to 32 sets of data

[2]Correlation coefficient

[3]No t value showed significance at $p < 0.05$

Table 23

Correlations between fetal growth and placental growth[1]

	Regression Equation	r^2	t value[3]
Placental weight and fetal weight	Y = 0.003X + 0.65	0.27	1.51
Placental protein content and fetal protein content	Y = 3.33X + 58.64	0.34	1.92
Placental DNA content and fetal DNA content	Y = 0.004X + 1.49	0.32	1.79
Placental RNA content and fetal RNA content	Y = 0.002X + 4.10	0.17	0.90
Placental protein/DNA ratio and fetal protein/DNA ratio	Y = 0.42X + 19.93	0.44	2.61*

[1]Results calculated from 30 to 32 sets of data

[2]Correlation coefficient

[3]t values presented with level of significance, *$p < 0.05$

B. Discussion

In the present experiment, a significant reduction in daily food intake of pregnant animal was observed with a lowered protein content of the diet. This finding was noted by other investigator in studies with the rhesus monkey, swine, and the rat (Berg, 1967; Kohrs, et al., 1976; Pond, et al., 1968). However, protein-deficient diets do not always have the effect of decreasing total food intake, and other investigators (Zamenhof, et al., 1971; Zeman, 1967) report no significant difference in food intake between control and protein-deficient diet groups during gestation. Since we observed a significant reduction in maternal food intake in protein-deficient diet groups during gestation, it can be concluded that the protein-deficient diet had the effect of producing deficits of calories as well as all other dietary nutrients.

The reduced body weight gain of maternal mice was observed in low-protein diet group over the period of gestation. The poor weight gain is likely due to the reduced total food intake (including that of total daily protein and caloric consumptions), which in turn was caused by the low-protein diet. The reduced maternal body weight gain of the protein-restricted diet group was accompanied by the reductions in maternal net weight gain and the weights of uterus and its component parts, such as fetuses, placentas, and amniotic fluid and uterine tissue itself. These findings are comparable to those in previous study (Hasting-Roberts and Zeman, 1977), where the effect of protein-deficient diet during pregnancy on maternal body weight and weights of the products of conception were investigated in the rat. They fed pregnant rats either control (24% casein diet) or protein-restricted diet (4% casein diet), and found a significant low food intake, net loss of maternal body weight, reduced uterine tissue weight, and smaller placentas and fetuses due to protein deprivation in maternal diet during gestation.

In view of the significant reduction in maternal liver weight in animals fed protein-deficient diet during gestation, it appears that maternal tissue was used to compensate for deficits of calories and perhaps other dietary nutrients. Moreover, the absence of a significant differences in the ratio of liver weight to body weight among groups indicates the proportional decrease in liver weight to body weight. A significant impact of protein-deficient diet on the chemical composition of the maternal liver was also found in this experiment. The lipid content was higher in the liver of animals fed protein-deficient diet than in control animals, while liver protein content was reduced in animals fed protein-deficient diet.

The significant reduction of liver protein content in the animal fed protein-deficient diet could be possibly explained by labile cytoplasmic proteins in the liver which could fall in quantity when dietary protein is restricted (Muramatsu, et al., 1962; Wainio, et al., 1959). These labile proteins would contribute amino acid to the metabolic pool for protein anabolism during pregnancy. In addition, the results of this experiment also indicate that protein anabolism in liver was also influenced by the protein content in diet. When pregnant mice were fed low-protein diet during gestation, there was a reduction in protein/RNA ratio, indicating a decrease in the quality of ribosomes (degrading polysomes into monosomes) as well as amino acid supply.

On the other hand, liver lipid accumulation found in low-protein diet group might be partly attributed to interference with the intracellular metabolism of lipids, since impairment of lipid transport due to decreased synthesis of the protein portion of the lipoprotein molecule has been suggested as a possible cause for fatty liver in the case of protein deficiency (Flores, et al., 1979; Seakins and Waterlow, 1972; Truswell, et al., 1969).

When the data were expressed in terms of relative amounts of lipid and

protein per cell (lipid/DNA and protein/DNA), it became evident that animals fed protein-restricted diet had an increased cellular content of lipid but a decreased protein content per cell.

The results also indicate that the level of protein in diet during gestation can cause cellular changes in maternal liver. Pregnant animal fed low-protein diet during gestation had an increased concentration of liver DNA (DNA/g liver), but the total DNA content in liver (DNA/liver) was not changed in this animal. These results suggest that mice fed protein-deficient diet had smaller size of liver cells than the controls, but cell number was not affected by lowering protein content in diet during pregnancy.

The data on gestational performance of the dam indicate a pronounced effect of protein-deficient diet on the maintenance of pregnancy as measured by significant increases in number of resorptions per dam and percent resorptions of implantations and significant decrease in percent litters of implantations. However, such results differ from those reported by Zemam (1967), who fed pregnant rats either 24 or 6% casein diet, and found normal maintenance of pregnancy in the rats fed protein-restricted diet. This apparent discrepancy found in gestational performance between two studies can be explained by either species difference (mice vs. rats) or significant reduction in food intake in protein-restricted animals in our study. Zeman (1967) used rats as an experimental animal model and was not able to find significant difference in food intake between control and protein-restricted groups, so that the additional effects of restricted calorie and other nutrients on gestational performance were eliminated in this study. Therefore, it can be concluded that the impact of protein restriction on gestational performance of animals might differ from one species to another, and reduction in food intake during pregnancy is an important factor in maintaining pregnancy.

The result of average fetal weight measurements is in agreement with those of other investigators (Nelson and Evans, 1953; Zeman, 1967). The restriction of protein content in diet during pregnancy in mice resulted in the production of young which had a lower body weight than control on day 18 of gestation. Further, the proportional reduction in fetal body size was indicated in this experiment, since no significant differences were observed in percent fetal body composition between protein-restricted and control groups.

The results of this experiment also indicated the critical need for dietary protein during gestation for fetal and placental cellular growth. A comparison of DNA contents in the fetuses of control and protein-deficient animals indicates that there are fewer cells in the fetus from protein deficient animal than those from control. However, there was no change in cell size of fetus, as indicated by either weight/DNA or protein/DNA, between protein-restricted and control animals. Moreover, the amount of protein found in fetus was also decreased with decreased level of protein in maternal diet during gestation. Since a significant reduction in fetal content but no change in protein/RNA were observed in this experiment, it can be assumed that a decrease in the fetal RNA content is responsible for the reduction in fetal protein content found in protein-restricted group. While there was no significant difference in averge placental weight and DNA content of placenta among groups, the decrease in both protein and RNA contents in placenta was the same as that observed in fetus when pregnant mice were fed protein-restricted diet. These results emphasize the importance of protein content in maternal diet during pregnancy for normal cellular growth of both the fetus and placenta.

Although it is generally assumed that nutritional needs for fetal growth can be provided at the expense of the maternal organism, our present study showed positive correlation between maternal liver protein content and fetal

protein content. This result suggests that competition for available nutrients might exist between the fetus and its mother under our experimental condition, and thus significant adverse effects of nutritional deficiency during pregnancy could occur on both maternal and fetal organisms when the nurients are in short supply. It has also been indicated that the trends of fetal growth and plcental growth are in the same general direction. Results from this study support for this idea. A significant positive correlation between placental protein/DNA ratio and fetal protein/DNA ratio was noted. On the other handno difference in the ratio of fetal weight to placental weight among groups was found.

Our experimental data also suggest that there are possible risks of ultrasonic energy exposure to the fetal and placental organisms. The tendency toward a decreased fetal and placental weights on day 18 of gestation was noticeable with ultrasonic energy exposure, especially when protein content was limited in maternal diet during gestation. The trends for the differences in the DNA and RNA contents of both fetus and placenta were similar to the trends observed for weights of these products of conception. However, the difference was not marked enough to show statistical significance. A larger number of animals might be necessary to reveal statistically significant differences, but the important point is that abnormal growth of both the fetus and placenta could result from ultrasonic energy exposure during prenatal period. Adverse effects of ultrasonic energy exposure on fetal and placental organisms may arise from degradation of biological molecules and destruction of cell and cell organelles (O'Brien, et al., 1972).

Chapter VII

Summary

The influence of dietary protein and fat levels on fetal growth in mice was investigated in Experiment 1 to standardize the control diet which would be used in the following experiments. The results of Experiment 1 indicated that both the dietary protein and fat levels significantly influence fetal growth of LAF1/J mice. In addition, dietary fat influenced protein utilization of these mice. Optimal fetal growth was obtained with the diet containing 20% casein with 15% fat as indicated by increased fetal protein, DNA, and RNA contents. Normal maternal liver lipid content in the 20% protein and 15% fat diet also indicates that this diet is optimal for reproductive performance of LAF1/J mice.

Experiments 2 and 3 were performed to determine the adverse effects of ultrasonic energy and/or protein-deficient diet on maternal and fetal mice. The results of these experiments suggest that the maternal nutrition and fetal and placental growths can be impaired by protein restriction in diet during pregnancy. Although it is generally assumed that nutritional needs for fetal growth can be provided at the expense of the maternal organism, the results of these experiments suggest that competition for available nutrients might exist between the fetus and its mother under our experimental condition, and thus significant adverse effects of nutritional deficiency during pregnancy could occur on both maternal and fetal organisms when the nutrients are in short supply. It has also been indicated that the trends of fetal and placental growth are in same general direction.

Moreover, our experimental data also suggest that there are possible risks of ultrasonic energy exposure to the fetal and placental organisms, as

indicated by the tendency toward a decreased fetal weight, placental weight, and DNA and RNA contents of both fetus and placenta, especially when protein content was limited in maternal diet during gestation.

References

Addis, T., L.J. Poo, W. Lew. 1936. The quantities of protein lost by the various organs and tissues of the body during a fast. J. Biol. Chem. 115:101-110.

Allen, L.H. and F.J. Zeman. 1971. Influence of increased postnatal food intake on body composition of pregnancy of protein-deficient rats. J. Nutr. 101:1311-1318.

Antonov, A.N. 1947. Children born during the seige of Leningrad in 1942. J. Pediat. 30:250-259.

Atinmo, T., W.G. Pond, and R.H. Barnes. 1974a. Effect of maternal energy vs. protein restriction on growth and development of progeny in swine. J. Ani. Sci. 39:703-711.

Atinmo, T., W.T. Pond, and R.H. Barnes. 1974b. Effect of dietary energy vs. protein restriction on blood constituents and reproductive performance in swine. J. Nutr. 104:1033-1040.

Barr, M., Jr., R.P. Jensh, and R.L. Brent. 1970. Prenatal growth in the albino rat: effects of number, intrauterine position. Am. J. Anat. 128:413-428.

Berg, B.N. 1965. Dietary restriction and reproduction in the rat. J. Nutr. 87:344-348.

Berg, B.N. 1967. Maintenance of pregnancy in protein-deprived rats by transitory protein supplements during early gestation. J. Nutr. 92:66-70.

Blaxter, K.L. Protein metabolism and requirements in pregnancy and lactation: In Mammalian protein metabolism, edited by H.N. Munro and J.B. Allison. Academic Press, N.Y., N.Y. 1964. vol II, pp.173-223.

Burke, B.S., V.V. Harding, and H.C. Stuart. 1943a. Nutrition studies during pregnancy. IV.Relation of protein content of mother's diet during pregnancy to birth length, birth weight and condition of infant at birth. J. Pediat. 23:506-515.

Burke, B.S., V.A. Beal, S.B. Kirkwood, and H.C. Stuart. 1943b. The influence of nutrition during pregnancy upon the condition of the infant at birth. J. Nutr. 26:569-583.

Campbell, R.M., I.R. Innes, and H.W. Kosterlitz. 1953. Some dietary and hormone effects on matenal, feotal and placental weights in the rat. J. Endocrin. 9:68-75.

Ceriotti, G. 1955. Determination of nucleic acids in animal tissues. J. Biol. Chem. 214:59-70.

Cheek, D.B., A.B. Holt, W.T. London, J.H. Ellenberg, D.E. Hill, and J.L. Sever. 1976. Nutritional studies in the pregnant rhesus monkey: the effect of

protein-calorie or protein deprivation on growth of the fetal brain. Am. J. Clin. Nutr. 29:1149-1157.

Chow, B.F. 1964. Growth of rats from normal dams restricted in diet in previous pregnancies. J. Nutr. 83:289-292.

Chow, B.F. and C.J. Lee. 1964. Effect of dietary restriction of pregnancy rats on body weight gain of the offspring. J. Nutr. 82:10-18.

Curtiss, C. 1953. Effects of a low protein intake on the pregnant rat. Metabolism 2:344-353.

Ebbs, J.H., F.F. Tisdall, and W.A. Scott. 1941. The influence of prenatal diet on the mother and child. J. Nutr. 22:515-526.

Edozien, J.C. and B.R. Switzer. 1978. Fatty liver in experimental protein-energy malnutrition in the rat. Exp. Molecul. Path. 29: 1-11.

Enesco, M. and C.P. Leblond. 1962. Increase in cell number as a factor in the growth of the organ and tissues of the young male rat. J. Embryol. Exp. Morphol. 10:530-562.

FAO/WHO expert group. Protein requirements. World Health Organization technical report series, No. 301. W.H.O., Washington, D.C., 1965.

Fleck, A. and H.N. Munro. 1962. The precision of ultraviolet absortion measurements in the Schmidt-Thanhauser procedure for nucleic acid estimation. Biochimica Et. Biophysica Acta 55:571-583.

Flores, H., N. Pak, A. Maccioni, and F. Monckeberg. 1970. Lipid transport in Kwashiorkor. Br. J. Nutr. 24:1005-1011.

Folch, J., M. Lees, and G.H.S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

French, C.E., A. Black, and R.W. Swift. 1948. Further experiments on the relation of fat to economy of food utilization: III. Low protein intake. J. Nutr. 35:83-88.

Gottesfeld, K.R. 1978. Ultrasound in obstetrics. Clin. Obst. Gynecol. 21:311-327.

Hall, S.M. and F.J. Zeman. 1968. Kidney function of the progeny of rats fed a low protein diet. J. Nutr. 95:49-54.

Hastings-Roberts, M.M. and F.J. Zeman. 1977. Effects of protein deficiecy, pair-feeding, or diet supplementation on maternal, fetal and placental growth in rats. J. Nutr. 107:973-982.

Herring, P.J. 1920. The effect of pregnancy upon the size and weight of the some of the organs of the body. Brit. Med. J. 2:886.

Horwitz, W. Crude fat or ether extract. In Official methods of analysis of the

association of official analytical chmists. AOAC, Washington, D.C. 1975a. pp 134-135.

Horwitz, W. Ash-official final action. In Official methods of analysis of the association of official analytical chemists. AOAC, Washington, D.C. 1975b. p 566.

Hsueh, A.M., C.E. Agustin, and B.F. Chow. 1976. Growth of young rats after differential manipulation of maternal diet. J. Nutr. 91:195-200.

Hubbard, R.W., W.T. Mathew, and D.A. Dubowik. 1970. Factors influencing the determination of DNA with indole. Anal. Biochem. 38:190-201.

Januzik, S.J. 1976. Two studies of biological effects due to ultrasonic irradiation. Thesis. University of Illinois, Urbana, Illinois.

Knapka, J.J., K.P. Smith, and F.J. Judge. 1977. Effects of crude fat and crude protein on reproduction and weaning growth in four strains of inbred mice. J. Nutr. 107:61-69.

Kohrs, M.B., A.E. Harpen, and G.R. Kerr. 1976. Effects of a low-protein diet during pregnancy of the rhesus monkey: I. Peproductive efficiency. Am. J. Clin. Nutr. 29:136-145.

Kohrs, M.B., G. Scheffler, and G.R. Kerr. 1979. Effects of a low-protein diet during pregnancy of the rhesus monkey: II. Physiological adaptation of the infant. Am. J. Clin. Nutr. 32:1206-1213.

Koshy, T.S., V.R. Sara, T.L. King, and L. Lazarus. 1975. The influence of protein restriction imposed at various stages of pregnancy of fetal and placental development. Growth 39:497-506.

Lechtig, A., J.P. Habicht, H. Delgado, R.E. Klein, C. Yarborough, and R. Martorell. 1975. Effect of food supplementation during pregnancy on birth weight. Pediat. 56:508-520.

Leshner, A.I. and V.A. Litwin. 1972. Brief communication: A simple method for carcass analysis. Physiol. and Behavior 9:281-282.

McCoy, R.H. 1940. Effects of different levels of protein in the diet of the rat. J. Biol. Chem. 133:lxiv-lxv (Abstr.).

McDicken, W.N. Diagnostic ultrasonics: principles and use of instruments. John Wiley & Sons, N.Y., N.Y., 1976.

Moghissi, K.S. 1978. Maternal nutrition in pregnancy. Clin. Obst. Gynecol. 21:297-310.

Muramatsu, K. and K. Ashida. 1962. Effects of dietary protein level on growth and liver enzyme activities of rats. J. Nutr. 76:143-150.

Naismith, D. 1966. The requirement for protein, and the utilization of protein and calcium during pregnancy. Metabolism 15:582-595.

NAS/NRC. Nutrient Requirements of Laboratory Animals, 2nd edition. National Academy of Sciences, Washington, D.C. 1972.

NAS/NRC. Nutrient Requirements of Laboratory Animals, 3rd edition. National Academy of Sciences, Washington, D.C. 1978.

NAS/NRC. Recommended Dietary Allowances, 9th edition. National Academy of Sciences, Washington, D.C. 1980.

Nelson, M.M. and H.M. Evans. 1953. Relation of dietary protein levels to reproduction in the rat. J. Nutr. 51:71-84.

O'Brien, W.D.,Jr. Uitrasonically induced fetal weight reduction in mice. In Ultrasound in medicine, ed. by D. White and R. Barnes. 1976. Vol. 2, pp.531-532.

O'Brien, W.D.,Jr., M.L. Shore, R.K. Fred, and W.M. Leach. On the assessment of risk to ultrasound. In Ultrasonics symposium proceedings. IEEE, Inc., N.Y., N.Y. 1972. pp.486-490.

Oser, B.L. Total nitrogen: Kjeldahl method. In Hawk's physiological chemistry, 14th edition. McGraw-Hill Book Company, N.Y., N.Y. 1965. pp 1214-1220.

Payne, P.R. and E.R. Wheeler. 1967. Comparative nutrition in pregnancy. Nature 215:1134-1136.

Picker, R.H., D.H. Smith, and D.M. Saunders. 1977. A new method of aminocentesis using ultrasound in multiple pregnancy to assess the second twin. Obst. Gynecol. 50:589-591.

Platka-Bird, L. and M.R. Bennink. 1978. Relationship of level and type of dietary fat to fetal and maternal rat lipogenesis and lipid deposition. J. Nutr. 108:1422-1430.

Pond, W.G. 1973. Influence of maternal protein and energy nutrition during gestation on progeny performance in swine. J. Ani. Sci. 36:175-182.

Pond, W.G., W.C. Wagner, J.A. Dunn, and E.F. Walker, Jr. 1968. Reproduction and early postnatal growth of progeny in swine fed a protein-free diet during gestation. J. Nutr. 94:309-316.

Pond, W.G., D.N. Strachan, Y.N. Sinha, E.F. Walker, Jr., J.A. Dunn, and R.H. Barnes. 1969. Effect of protein deprivation of swine during all or part of gestation on birth weight, postnatal growth rate and nucleic acid content of brain and muscle of progeny. J. Nutr. 99:61-67.

Poo, L.J., W. Lew, and T. Addis. 1939. Protein anabolism of organs and tissues during pregnancy and lactation. J. Biol. Chem. 128:69-77.

Potter, E.L. and S.T. Thierstein. 1943. Glomerular development in the kidney as an index of fetal maturity. J. Pediat. 22:695-706.

Riopelle, J., C.W. Hill, and S. Li. 1975. Protein deprivation in primates:

V.Fetal mortality and neonatal status of infant monkeys born of deprived mothers. Am. J. Clin. Nutr. 28:989-993.

Rippel, R.H., O.G. Rasmussen, A.H. Jensen, H.W. Norton, and D.E. Becker. 1965. Effect of level and source of protein on reproductive performance of swine. J. Ani. Sci. 24:203-208.

Robinson, P. 1948. The effect of supermaintenance and submaintenance diets of mature Border Leiceter-Cheviot ewes. J. Agr. Sci. 38:345.

Rugh, R. The mouse: its reproduction and development. Burgess Publishing Company, Minneapolis, Minnesita. 1968.

Samuels, L.T., R.C. Gilmore, and R.M. Renecke. 1948. The effect of previous diet on the ability animals to do work during subsequent fasting. J. Nutr. 360:639-651.

Seakins, A. and J.C. Waterlow. 1972. Effects of low-protein diet on the incorporation of amino acids into rat serum lipoproteins. Biochem. J. 129:793-795.

Seegers, W.H. 1937. The effect of protein deficiency on the course of pregnancy. Am. J. Physiol. 119:474-479.

Shrader, R.E. and F.J. Zeman. 1969. Effect of maternal protein deprivation on morphological and enzymatical development of neonatal rat tissue. J. Nutr. 99:401-421.

Shrader, R.E. and F.J. Zeman. 1973. Skeletal development in rats as affected by maternal protein deprivation and postnatal food supply. J. Nutr. 103:792-801.

Simons, S.D. and P.V. Johnston. 1976. Prenatal and postnatal protein restriction in the rat: effect on some parameters related brain development, and prospects for rehabilitation. J. Neurochem. 27:63-69.

Simonson, M., H.M. Hanson, and D.A. Bordie. 1972. Replication of effects of maternal malnourishment in another species. Fed. Proc. 31:688 (Abst.).

Smart, J.L., B.P.F. Adlard, and J. Dobbing. 1972. Effect of maternal undernutrition and other factors on birth weight in the rat. Biol. Neonate 20:236-244.

Smith, C.A. 1947. Effects of maternal undernutrition upon the newborn infant in Holland (1944-1945). J. Pediat. 30:229-243.

Smith, R.W. and A. Walsh. 1975. Composition of liver lipids of the rat during pregnancy and lactation. Lipids 10:643-645.

Souders, H.J. and A.F. Morgan. 1957. Weight and composition of organs during the reproductive cycle in the rat. Am. J. Physiol. 191:1-7.

Steel, R.G.D. and J.H. Torrie. Principles and procedures of statistics with special reference to the biological sciences. McGraw-Hill Book Company,

Inc., N.Y., N.Y. 1960.

Stevenson, J.W. and N.R. Ellis. 1957. Effect of gestation diets and creep feeding on livability and weight gains of suckling pigs. J. Ani. Sci. 16:877-884.

Stoker, J. and L. Evans. 1977. Fetal sex determination by ultrasound. Obst. Gynecol. 50:462-466.

Taylor, K.J.W. 1977. The ultrasound boom. The Yale J. Biol. Med. 50:325-326.

The University Federation for Animal Welfare. The UFAW handbook on the care and management of laboratory animals. Churchill Livingstone, N.Y., N.Y. 1976. p162.

Thomson, A.M. and F.E. Hytten. 1961. Calorie requirements in human pregnancy. Proc. Nutr. Soc. 20:76-83.

Tipton, P.W. and M.E. Burt. 1977. A method for mechanized staining of rat and mouse foetuses for teratological examination. Lab. Ani. 11:265-267.

Truswell, A.S., D.L. Hansen, C.E. Watson, and P. Wannenburg. 1969. Relation of serum lipids and lipoproteins to fatty liver in Kwashiokor. Am. J. Clin. Nutr. 22:568-576.

Tumbleson, M.E., O.W. Tinsley, K.W. Hicklin, and J.B. Mulder. 1972. Fetal and neonatal development of Sinclair (S-1) miniature piglets affected by maternal dietary protein deprivation. Growth 36:373-387.

Velasco, E.G. and J.A. Brasel. 1972. DNA polymerase activity in malnourished rat placenta. Fed. Proc. 31:687 (Abst.).

Venkatachalam, P.S. and K.S. Ramanathan. 1964. Effect of protein deficiency during gestation and lactation on body weight and composition of offspring. J. Nutr. 84:38-42.

Wachstein, M. and M. Bradshaw. 1965. Histochemical localization of enzyme activity in the kidneys of three mammalian species during their postnatal development. J. Histochem. Cytochem. 13:44-56.

Wainio, W.W., J.B. Allison, and L.T. Kremzner. 1959. Enzymes in protein depletion. J. Nutr. 67:197-204.

Wallace, L.R. 1948a. Growth of lambs before and after birth in relation to the level of nutrition. J. Agr. Sci. 38:93.

Wallace, L.R. 1948b. The growth of lambs before and after birth in relation to the level of nutrition. J. Agr. Sci. 38:243-302.

Williams, P.F. 1945. Importance of adequate protein nutrition in pregnancy. J.A.M.A. 127:1052-1055.

Winick, M. 1967. Cellular growth of human placenta: III. Intrauterine growth

failure. J. Pediat. 71:390-395.

Winick, M. 1971. Cellular changes during placental and fetal growth. Am. J. Obst. Gynecol. 109:166-176.

Winick, M. and A. Noble. 1965. Quantitative changes in DNA, RNA, and protein during prenatal and postnatal growth in the rat. Develop. Biol. 12:451-466.

Winick, M. and A. Noble. 1966a. Cellular response in rats during malnutrition at various ages. J. Nutr. 89:300-306.

Winick, M. and A. Noble. 1966b. Quantitative changes in ribonucleic acids and protein during normal growth of rat placenta. Nature 212:34-35.

Winick, M., A. Coscia, and A. Noble. 1967. Cellular growth in human placenta: I. Normal placental growth. Pediat. 39:248-251.

Young, M. and E.M. Widdowson. 1975. The influence of diets deficient in energy, or in protein, on conceptus weight, and the placental transfer of a non-metabolizable amino acids in the guinea pig. Biol. Neonate 27:184-191.

Zamenhof, S., E.V. Marthens, and F.L. Margolis. 1968. DNA (cell number) and protein in neonatal brain: Alteration by maternal dietary protein restriction. Science 160:322-323.

Zamenhof, S., E.V. Marthens, and L. Grauel. 1971. DNA (cell number) and protein in neonatal rat brain: Alteration by timing of maternal dietary protein restriction. J. Nutr. 101:1265-1269.

Zeman, F.J. 1967. Effect on the young rat of maternal protein restriction. J. Nurt. 93:167-173.

Zeman, F.J. 1968. Effect of maternal protein restriction on the kidney of the newborn young of rats. J. Nutr. 94:111-116.

Zeman, F.J. 1970. Effect of protein deficiency during gestation on postnatal development in the young rat. J. Nutr. 100:530-538.

Zeman, F.J. and E.C. Stanbrough. 1969. Effect of maternal protein deficiency on cellular development in the fetal rat. J. Nutr. 99:274-282.

Appendix 1

Analysis of variance for characterisics of maternal mice[1]

Source	df	Mean Squares				
		Food intake	Caloric intake	Body Wt. gain	Liver Wt.	Liver lipids
Nutrition (N)	1	0.09	1.8	80.4*	0.59**	175.7**
Ultrasonic energy (U)	1	0.56	11.4	13.9	0.01	1.7
N x U	1	2.31	47.0	3.0	0.18	0.3
Error	27	0.57	11.6	18.0	0.05	8.3

[1]Mean squares presented with level of significance

*$p < 0.05$

**$p < 0.01$

Appendix 2

Analysis of variance for gestational performance of mice[1]

Source	df	Mean squares: Resorptions of implantations: Number of resorptions	Mean squares: Resorptions of implantations: % resorptions	Mean squares: Litters of implantations: Litter size	Mean squares: Litters of implantations: % Litters	Fetal Wt.
Nutrition (N)	1	3.3	157.3	0.02	160.6	0.10**
Ultrasonic energy (U)	1	0.7	390.1	0.02	404.7	0.01
N x U	1	0.4	80.8	0.45	82.2	0.02
Error	27	2.5	213.9	3.71	213.9	0.01

[1]Mean squares presented with level of significance

**$p < 0.01$

Appendix 3

Analysis of variance for major constituents of fetal mice[1]

Source	df	Mean squares		
		Protein	Lipids	Ash
Nutrition (N)	1	1044.7*	0.64	0.70
Ultrasonic energy (U)	1	421.1	1.37	1.64
N x U	1	114.1	1.68	9.00
Error	27	148.9	2.65	3.74

[1]Mean squares presented with level of significance *$p < 0.05$

Appendix 4

Analysis of variance for fetal cellular growth parameters[1]

Source	df	Mean squares			
		DNA	RNA	Protein/DNA	RNA/DNA
Nutrition (N)	1	0.11	35.71**	473.1**	4.94**
Ultrasonic energy (U)	1	0.25	5.02*	43.7	0.03
N x U	1	0.25	0.01	134.4	0.47
Error	27	0.11	0.71	59.8	0.36

[1]Mean squares presented with level of significance

*P < 0.05, **p < 0.01

Appendix 5

Analysis of variance for daily food intake of maternal mice during gestation[1, 2]

Source	df	Mean squares Gestation day 8-10	10-12	12-14	14-16	16-18
Nutrition (N)	1	8.45	168.36**	234.23**	243.16*	575.66**
Ultrasonic energy (U)	1	1.09	9.42	0.06	8.41	0.11
N x U	1	0.47	1.37	1.83	23.57	1.88
Error	28	10.97	21.42	16.90	19.98	22.33

[1]Mean squares presented with level of significance

**$p < 0.01$

[2]Maternal daily food intakes do not differ significantly among groups until gestation day 10

Appendix 6

Analysis of variance for daily caloric intake of maternal mice during gestation[1, 2]

Source	df	Mean squares Gestation day 8-10	10-12	12-14	14-16	16-18
Nutrition (N)	1	135.8	2707.3**	3766.4**	3910.0**	9256.7**
Ultrasonic energy (U)	1	17.5	151.5	0.9	135.3	1.8
N x U	1	7.6	22.0	29.4	379.0	30.2
Error	28	176.4	344.4	271.8	321.3	359.1

[1]Mean squares presented with level of significance

**$p < 0.01$

[2]Maternal daily caloric intakes do not differ significantly among groups until gestation day 10

Appendix 7

Analysis of variance for maternal body weight during gestation[1, 2]

Source	df	Mean squares				
		Gestation day				
		10	12	14	16	18
Nutrition (N)	1	18.2*	28.3*	80.5**	134.1**	244.8**
Ultrasonic energy (U)	1	4.5	2.7	3.0	2.1	2.1
N x U	1	1.7	5.2	7.7	18.8	25.7
Error	28	4.2	6.1	7.1	9.4	15.1

[1]Mean squares presented with level of significance

*$p < 0.05$, **$p < 0.01$

[2]Maternal body weights do not differ significantly among groups until gestation day 8

Appendix 8

Analysis of variance for maternal body weight gain during gestation[1, 2]

Source	df	Mean squares			
		Total wt. gain	Net body wt.	Maternal net body wt. change	
				g	%
Nutrition (N)	1	176.7**	81.7**	22.6**	316.3**
Ultrasonic energy (U)	1	1.4	11.8	1.4	18.1
N x U	1	16.2	9.1	0.8	9.2
Error	28	9.3	8.9	3.0	37.5

[1]Mean squares presented with level of significance, **$p < 0.01$

[2]Initial maternal body weights do not differ significantly among groups at the level of $p < 0.05$

Appendix 9

Analysis of variance for weights of the uterus and its contents[1]

Source	df	Mean squares							
		Uterine mass		Fetuses		Placentas		Uterine tissue & fluids	
		g	% Body Wt.	g	% Body Wt.	g	% Body Wt.	g	% Body Wt.
Nutrition (N)	1	49.4	77.7	37.84*	35.9	0.175*	0.22	3.34*	5.11
Ultrasonic energy (U)	1	1.9	12.8	0.98	5.4	0.007	0.04	0.15	1.12
N x U	1	6.2	4.2	4.67	5.9	0.003	0.02	0.07	0.08
Error	28	12.2	56.4	7.33	34.7	0.040	0.23	0.51	2.55

[1]Mean squares presented with level of significance

*$p < 0.05$

Appendix 10

Analysis of variance for fetal, placental and uterine weights per pup[1]

Source	df	Mean squares: Uterine mass per pup g	Uterine mass per pup % Body Wt.	Mean fetal wt. g	Mean fetal wt. % Body Wt.	Mean placental wt. g	Mean placental wt. % Body Wt.	Uterine tissue and fluid per pup g	Uterine tissue and fluid per pup % Body Wt.	Fetus/ Placenta
Nutrition (N)	1	0.142**	0.199	0.142**	0.005	498.1	0.001	0.001	0.2663	3.600
Ultrasonic energy (U)	1	0.010	0.012	0.005	0.024	255.5	0.001	0.001	0.0158	0.002
N x U	1	0.003	0.132	0.044	0.056	12.1	0.000	0.001	0.0002	5.430
Error	28	0.020	0.330	0.020	0.130	216.6	0.002	0.02	0.21	3.360

[1]Mean squares presented with level of significance

**p < 0.01

Appendix 11

Analysis of variance for characteristics of maternal liver[1]

Source	df	Mean squares				
		Liver weight	Liver Wt./ Maternal body wt.	Lipids	Protein	Protein/ Lipids
Nutrition (N)	1	0.296*	19.1	14182.4**	5222.3**	22.76**
Ultrasonic energy (U)	1	0.033	3.6	151.4	81.4	0.56
N x U	1	0.010	58.1	106.0	43.2	0.38
Error	28	0.060	21.3	683.2	613.4	0.27

[1]Mean squares presented with level of significance

* $p < 0.05$, ** $p < 0.01$

Appendix 12

Analysis of variance for cellular parameters of maternal liver[1]

Source	df	Mean squares							
		DNA		RNA					
		mg/ liver	mg/ g liver	mg/ liver	mg/ g liver	RNA/DNA	Protein/ DNA	Protein/ RNA	Lipid DNA
Nutrition (N)	1	0.001	1.25*	20.05	0.01	0.410	1483.10**	70.04*	689.82**
Ultrasonic energy (U)	1	0.222	0.05	5.82	0.23	0.003	8.18	0.70	37.15
N x U	1	0.162	0.02	0.70	0.09	0.038	0.01	5.36	8.66
Error	28	1.470	0.22	6.51	0.45	0.100	92.46	11.53	52.98

[1]Mean squares presented with level of significance

*P < 0.05, **p < 0.01

Appendix 13

Analysis of variance for gestational performance[1]

Source	df	Mean squares			
		Resorptions of implantations		Litters of implantations	
		# resorption/dam	% resorptions	Litter size	% litters
Nutrition (N)	1	15.59*	2247.7*	9.50	2271.6*
Ultrasonic energy (U)	1	0.47	18.9	1.48	160.3
N x U	1	6.51	678.9	1.48	673.8
Error	28	3.52	391.4	6.74	391.7

[1]Mean squares presented with level of significance

*$p < 0.05$

Appendix 14

Analysis of variance for fetal body composition[1]

Source	df	Mean squares			
		Water	Protein	Lipids	Ash
Nutrition (N)	1	0.014	9.85	0.0003	0.017
Ultrasonic energy (U)	1	0.002	0.93	0.1593	0.025
N x U	1	0.146	0.13	0.1381	0.011
Error	27	1.34	2.53	0.14	0.02

[1]Body compositional data do not differ significantly among groups.

Appendix 15

Analysis of variance for fetal cellular growth and bone development[1]

Source	df	Mean squares							
		DNA	RNA	Protein	Wt./DNA	Protein/ DNA	RNA/DNA	Protein/ RNA	# of Missing ossification centers
Nutrition (N)	1	6.21**	19.16**	10391.9**	7570.1	1.4	0.02	7.35	125.2
Ultrasonic energy (U)	1	0.32	0.13	23.2	7522.3	39.9	0.11	1.31	13.1
N x U	1	0.32	0.04	44.8	8022.2	40.3	0.15	0.23	37.9
Error	27	0.17	0.30	236.3	4221.3	52.9	0.05	7.04	31.9

[1]Mean squares presented with level of significance

**$p < 0.01$

Appendix 16

Analysis of variance for placental cellular growth[1]

Source	df	Mean squares						
		Protein	DNA	Wt./DNA	Protein/ DNA	RNA	Protein/ RNA	RNA/DNA
Nutrition (N)	1	37.2**	8657.2	5.3	49.9	48599.7*	0.07	0.05
Ultrasonic energy (U)	1	2.2	4855.5	3239.1	7.4	409.6	8.93	0.11
N x U	1	16.1	166.5	868.8	106.6	22855.3	0.66	0.15
Error	26	4.3	2289.8	1748.7	55.3	7199.5	5.27	0.09

[1]Mean squares presented with level of significance

*P < 0.05, **p < 0.01