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THE EFFECT OF DIET DURING PREGNANCY ON LACTATION PERFORMANCE

by

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ABSTRACT

The purpose of this research was to determine the effect of dietary restriction during pregnancy on the ability of the rat to lactate. This was based on the thesis that a failure to increase "maternal fat stores" over gestation would reduce the energy available for milk production.

The study was conducted in two parts. In the first part, the effect of a protein or calorie deficiency during pregnancy on maternal body fat was determined. In the second part, rats were fed either a low calorie-adequate protein or a low protein-adequate calorie diet throughout pregnancy. After delivery they were maintained on a normal diet, pair-fed with a non-restricted nursing littermate, and allowed to suckle eight foster pups for twenty-one days.

The protein and lipid content of the dam's milk, and the rate of growth, carcass composition, and the weight of the litter's cerebellums were measured to determine whether adequate milk for growth and development of the pups was provided. The lipid content of the mother's carcass was analyzed to see if there was any change due to lactation.

From the findings of this study, it appears that the diet during pregnancy affects the weight gain and body fat content of the mother, as well as the birth weight of her pups. Body fat, in all dietary treatments, was catabolized during the suckling period. The weight loss during lactation was inversely

proportional to the fat content of the rat's body at the end of pregnancy.

Adequate lactation appears to involve an interaction of the diets during pregnancy and lactation, with a deficiency in one being overcome by an adequate diet in the other. The possibility exists of a transitory lag in milk production during initial lactation for those animals on a poor diet during gestation. It is likely that the fat deposited over pregnancy, in well nourished animals, is used to supplement early lactation.

During lactation the mother's diet affects her weight loss, and the gain of her pups, by influencing the volume of milk she produces. The protein and fat composition of the milks are not affected by diet during pregnancy, but rather are determined by the animal's genetic potential within a normal range.

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I   INTRODUCTION

Nutritionally, the case for breast-feeding is overwhelming. Gross differences in the basic composition of human milk and cow's milk have been known for many years, and this understanding has enabled cow's milk to be modified domestically and commercially for easier digestability, and to supply in an approximate manner the infant's requirements of known nutrients. However, recent research has re-emphasized the complex nature of human milk, made up of over 100 constituents which differ in proportion and chemical composition from the equally complex milks of other mammals. The biochemical properties of, and the differences between, human and cow's milk are imperfectly understood and remain a developing field. On teleological ground, however, nutritionists must advise mothers that the breast is best.

The secretion of milk occurs after delivery in most women. Complete failure to secrete milk is a rare event; most failures are due to inadequate continuation of secretion. In the human a number of factors, such as social, psychological, and culture, come into play in the establishment of successful lactation; these are not usually observed in animals. The general health and diet of the pregnant animal has been shown to affect its ability to lactate. If the same is true of humans, the diet and weight gain of the mother during her pregnancy may be considered as an important factor in the establishment of lactation. The pregnant woman has been shown to increase her body fat during

pregnancy, perhaps in anticipation of the energy demands of lactation, since lactation imposes a greater stress on the maternal organism than does pregnancy. In women who will not lactate, these fat stores are of no benefit and may be an etiological factor in the development of obesity. In the woman who is motivated to nurse her child, a lack of fat storage may be a causative factor in her failure to lactate successfully.

Animals have been used to demonstrate that the diet in the pre and post-natal period is particularly important for brain development, as well as for the development of other organ systems. If either the quantity or quality of milk is affected by insufficient fat stores in the mother's body, this could have severe effects on the offspring.

This study was designed to determine the effect of a diet restricted during pregnancy on the ability of the rat to lactate successfully, as measured by the growth, body composition and the weight of the pups' cerebellums. The composition of the dam's milk was also determined to see if there were any alterations due to the mother's diet during pregnancy.

2. REVIEW OF LITERATURE

2.1 Weight Gain and Alterations in Body Composition During Pregnancy

The weight gain in pregnancy, its nature and significance, must be considered in both well-fed and poorly-fed populations. In reports from the U.S.A. and Europe, average weight gains in well-nourished pregnant women commonly ranged between 10 and 11 kg. (Kerr, 1943; Tompkins and Wiehl, 1951; Humphreys, 1954; Ihrman, 1960; and Huter et al., 1965). None of these studies gave dietary information; however, the weight gains reported were similar to the mean gain of 10.3 kg. found in pregnant Australian women who were restricting their caloric intake to 2000 kcal. (English and Hitchcock, 1968). Thomson and Billewicz (1957) have shown that the best reproductive performance in terms of the incidence of perinatal mortality, pre-eclampsia, and low birth weight babies is associated with a weight gain of about 12.5 kg. This weight gain was observed in healthy Scottish primigravidae eating to appetite.

A limited amount of data reported from India and West Africa indicate that poorly fed pregnant women from rural communities gained between 5 and 6 kg. (Venkatachalm et al., 1960; Thomson et al., 1966). When pregnant mothers perform strenuous physical activity on very little food, they may fail to gain weight or may even loose weight during the gestation period (Thomson et al., 1966).

It has long been recognized that pregnant animals avidly retain nitrogen for growth of the fetus and reproductive tissues. In the rat, the actual retention of nitrogen has been measured. Naismith (1966) observed a considerable accumulation of protein in the maternal carcass during the first 14 days of gestation when competition from the fetuses is minimal. The magnitude of this reserve is determined by the protein content of the diet. During the last 7 days, when there is rapid fetal growth, the stores are depleted. This catabolism of protein in late pregnancy occurs irrespective of the concentration of protein in the diet. On a diet of 13% protein the rat ends her pregnancy with no more protein in her muscles than in those of her non-pregnant controls. On a low protein diet (8%) the rat loses not only her smaller stores of protein, but also her own body carcass protein. A more recent study by Naismith and Morgan (1976) found that supplementing the protein intake from days 6 to 10 in pregnant rats increased substantially the weight and cellularity of their fetuses at term. This observation confirms the hypothesis that protein is stored in early pregnancy for use during the period of rapid fetal growth in the rat.

Several investigators, using the nitrogen balance technique, have suggested that in the human, in addition to the protein deposited in the reproductive tissues (fetus, uterus, breast) and blood, a very appreciable accumulation of nitrogen occurs in "maternal stores". Johnstone et al (1972) studied pregnant women from various socio-economic levels and found a net nitrogen retention of 0.9 g./day. The existence of significant protein storage during pregnancy is questioned in the human since nitrogen balance techniques tend

to overestimate the intake and underestimate the output of nitrogen. The retention of nitrogen in the maternal stores is not in agreement with other more direct estimates of the composition of body gains.

Thomson and Hytten (1971) estimated the relative contribution of the various tissues to the weight gained in pregnancy. On the basis of these estimates and their own data on body water, obtained using dilution techniques (Hytten et al, 1966), they were able to show that if protein is stored it is stored dry; a physiological impossibility, as muscle protein is always found associated with water. Four kilograms of the maternal weight gain is, however, almost certainly depot fat. This fat accumulation occurs very rapidly before mid-pregnancy and it slows down during the second half of pregnancy when most of the weight gain is accounted for by increases in the fetus and reproductive organs (Hytten and Leitch, 1971). Direct demonstration of the storage and loss of fat has not been attempted in humans. However, Naismith (1971) found that rats maintained throughout pregnancy on a high protein diet showed no net gain in carcass protein, but there was a 40% increase in their body fat. In comparison, a gain of 4 kg. of fat in the average woman represents an increase of about 30% in body fat.

Apart from pregnancy, fat storage on this scale is unknown in healthy adults. It is probably due to the effect of progesterone on the hypothalamic centres which changes the energy balance either by increasing food intake or decreasing the energy output (Beaton, 1966). Carcass analysis of rats given daily injections of progesterone showed an increase in body weight above controls,

which was due to an increase in body fat (Galletti and Klopper, 1964).
Hytten (1974) has suggested that most pregnant women experience a surge of
appetite towards the end of the first trimester which tends to decline as preg-
nancy proceeds, but food intake probably remains fairly constant throughout at
least the last two-thirds of pregnancy at some 200 kcal. above the non-pregnant
daily average. Emerson et al (1972), using indirect calorimetry, found the caloric
requirement to increase above non-pregnant requirements from 10 kcal. in the
first trimester to 85 kcal. in the second, and 220 kcal. in the third. Blackburn
and Calloway (1975) have found no decrease in physical activity of pregnant
American women. It thus appears that the fat stores are a result of an increased
appetite due to progesterone.

Taggart et al (1967) measured the skinfold thickness at 7 sites and total
body water from 10 weeks gestation to 6 to 8 weeks post-partum in Scottish
women. They found skinfold thickness increased around the trunks and legs, up
to about 30 weeks, stabilized, and decreased abruptly about the time of delivery.
At 6 to 8 weeks post-partum the measurements were almost at the level of week
10 of pregnancy, although body dry weight remained above pre-pregnancy levels.
These findings would seem to suggest fat deposition is a normal phenomenon in
pregnancy, however according to Taggart fat is deposited during the first three-
quarters of pregnancy rather than the first half as suggested by Hytten and
Leitch (1971).

It has been suggested that fat accumulated during pregnancy acts as a
buffer against possible deprivation in late pregnancy. As an anticipatory

mechanism it is analogous to fat storage in mammals before hibernation, and in birds before migration. Coltart and Williams (1976) found an increased lipolysis within adipose tissue in late pregnancy as well as an impaired glucose tolerance; this makes more free fatty acids available for maternal energy needs, so sparing maternal glucose for fetal use. Scow, Chernick and Smith (1958) were among the first to describe an increase in ketone-body levels in late pregnancy in the rat, suggesting an increase in fat catabolism in late pregnancy. Data on the composition of eviscerated rat carcass showed carcass fat to decrease at the time that protein retention in the fetus was increasing (Beaton et al, 1954).

Thomson and Billewicz (1957) suggested there was a compensating mechanism whereby most women lose this extra fat after parturition. English and Hitchcock (1968) found that Australian women return to their pre-pregnancy weight by six months post-partum on caloric intakes of 2460 and 1880 kcal. for lactating and non-lactating women respectively. Dennis and Bytheway (1965) found a slightly greater rate of weight loss among lactating women as compared with non-lactating women. Thomson et al (1970) studied the energy intakes and caloric expenditure of lactating women and concluded that when these women ate to appetite, the milk supply was usually subsidized to some extent (300 kcal./day) from body fat which has been laid down during pregnancy. Naismith and Ritchie (1975) found breast feeding in British women induced the catabolism of their body fat, even when their energy intakes exceeded the recommended intake for lactation. This would seem to suggest that fat stores may be necessary to maintain normal lactation. It is well known that neutral fat can

provide the energy for milk synthesis (Kon and Cowie, 1961). If weight gain has been limited during pregnancy, what will be the effect on lactation?

In the human, it appears that there is no storage of protein, but rather a 4 kg. storage of fat when the diet during pregnancy is adequate. Some of the fat stored in the carcass is used for energy in late pregnancy when the demands of the fetus are the greatest, but the remaining fat stores could quite possibly be necessary to provide energy for milk synthesis during lactation. If this is true a body fat gain of 4 kg. would be desirable to ensure adequate initial lactation.

2.2 The Effect of Diet During Pregnancy on Lactation

If lactation is supported to some extent from stores laid down in pregnancy, it might be expected that increasing storage during pregnancy would be associated with increasing adequacy of lactation. There is considerable evidence from animal experiments to support this hypothesis. The food supply during pregnancy affects subsequent lactation performance in the cow, pig and sheep, and a loss of maternal tissue weight has been shown to result in a decrease in lactation in these species.

DeGeeter et al (1973) showed that pigs from gilts fed low protein diets during gestation displayed a trend towards slower postweaning weight gains. The quantity of milk produced was significantly reduced. In an earlier study the same group of researchers observed a carryover effect of gestation treatment to lactation. Reduced gains in pigs were observed when they were nursed by sows

fed a low protein diet during gestation but a high protein diet during lactation (DeGeeter et al, 1972). Pond et al (1968) reported that the milk production of sows fed protein-free diets during pregnancy was adversely affected. They observed a significantly lower body weight at six weeks in experimental pigs and in pigs transferred to the experimental sows at two days of age from control sows, compared with that of sows nursing control pigs of their own or from a reciprocall transfer. Vermidahl et al (1969) observed that the average daily gains per pig during the suckling period was significantly greater for pigs from dams fed adequately during gestation. These well-fed sows produced more milk and ate less when fed at libitum during lactation than gilts given a deficient diet during pregnancy and allowed to eat ad libitum during lactation. Mahan and Mangan (1975), however, found that pigs fed low protein diets during gestation can lactate efficiently if adequate protein is available during lactation.

Buitrago et al (1974) found that the net weight gain of pigs from mating to parturition was significantly correlated with the level of energy intake. Performance of the gilts during lactation in terms of body weight changes was opposite from the trend observed in gestation: gilts that gained more weight during gestation lost most weight during lactation. Average weight gain per pig during lactation, when all gilts were fed a normal diet, was about equal for all groups and the final weight at weaning was not different among treatments. This research would seem to indicate that a poor diet during pregnancy can be overcome if adequate food is provided during lactation.

The practice of increasing the food intake of dairy cows in late pregnancy, with the object of increasing milk yield in the ensuing lactation period, is frequently followed although critical information is limited and often, seemingly, contradictory. Broster, Foot and Line (1970) reported on a series of five experiments on the effect of level of feeding in pregnancy on milk production. Pre-calving and post-calving feeding were found to contribute jointly towards the attainment of the animal's inherited yield potential. Small inadequacies in the pre-partum feeding level can be offset by more generous feeding after calving; generous pre-calving feeding allows post-calving feeding to be minimized. There are limits to this interchange: too severe a restriction of intake before calving may raise the animal's requirements after calving beyond its food intake capacity and yield falls.

Thomson and Thomson (1953) found the milk yield of ewes was decreased from 20 gallons to 11 gallons when ewes were fed half the quantity of nutrients supplied to controls during gestation. Substantial increases in nutrients offered to the deficient animals immediately after lambing did not increase the milk supply quickly enough to be of full benefit to the lambs, indicating that the diet during pregnancy can affect early lactation in ewes.

Several researchers working with rats have found that feeding low protein or low calorie-low protein diets throughout gestation and lactation resulted in lactation failure (Zeman, 1967; Widdowson and Cowen, 1972; Chow and Rider, 1973). Naismith (1971) showed that, during lactation, rats fed on a 25 % casein diet lost no body protein, but lost almost 70% of their body fat. This

would seem to indicate that on a high protein diet, optimum use is made of dietary protein for the synthesis of milk protein, the additional energy for milk formation being supplied from oxidation of the fat accumulated during pregnancy. In contrast, rats fed a low protein diet (10% casein) contributed 10% of their body protein, in addition to calories from body fat, toward the synthesis of a much reduced volume of milk.

Venkatachalam and Ramanathan (1966) suggested that feeding a low protein diet for one week during pregnancy to rats would decrease the output of milk. Stewart et al (1975) found in each generation of malnourished female rats there were some dams who failed to produce milk: their mammary glands showed little or no sign of activity on macroscopic examinations. When their litters died their stomachs and intestines showed no evidence of milk ingestion. Even in those undernourished animals that reared their young, the mammary glands were underdeveloped and the milk supply was low. Malasan (unpublished data, in Beaton, 1966) found when dams who had free access to food during pregnancy (and presumably had appreciable fat stores) were restricted to one-half of the ad libitum intake during lactation, they were able to maintain relatively normal lactation for 12 days. It is suggested that at this point the maternal stores had been exhausted; and after this time milk secretion was inadequate to maintain growth. This same researcher found food restriction during pregnancy did not, in itself, impair the ability to lactate; dams restricted to 50% ad libitum during pregnancy and fed ad libitum during lactation showed no impairment of lactation (Malasan, unpublished data, in Beaton, 1966).

Studies by Ebbs and Kelly (1942) demonstrated a relationship between diet during pregnancy and lactation success in three groups of low income Canadian mothers. They found that those with a supplemented diet and those who were taught a good diet had a higher rate of breast-feeding success than those with a poor diet. A "good" rating for breast feeding was used when the baby was completely breast fed and the baby progressed normally; "fair" was used when supplemental feeding was necessary, and the progress of the baby was not as rapid as normal. "Poor" indicated either very slow progress, or marked loss of weight on breast milk, or a poor supply of breast milk, or artificial feeding. The differences in lactation performance were apparent at one, three, and five months after delivery.

Woodhill et al (1955) investigated the influence of maternal diet on the incidence and duration of lactation in a group of Australian women. They found that 74% of women with good or excellent diets in the later half of pregnancy breast fed their babies through the seventh month of life compared with only 6% of women with very poor diets. The same trend was evident in relation to diets studied during the first half of pregnancy. Hankin and Symonds (1965) found antenatal diets of Australian women had an effect on both the establishment and the length of lactation. Naismith and Ritchie (1975) reported on two English mothers, who gained only 4.5 and 5.0 kg. respectively during pregnancy, and failed to provide enough milk to satisfy their infants when their caloric intake during lactation was low. Hytten (1954) was unable to find any evidence that the nature of the diet taken by Scottish women during

pregnancy had any influence on the yield or composition of breast milk on the seventh day after delivery, or on the incidence of failure to breast feed due to an inadequate supply of breast milk. English and Hitchcock (1968) found a caloric restriction during pregnancy had no effect on the ability to lactate if the calorie intake was increased during lactation.

There appears to be a relationship between the diet during pregnancy and the ability to lactate successfully. This may be related to the fat store normally deposited during pregnancy being available to supply the energy needed for milk production. There is, however, a variation in the response of the mothers... some may decrease the volume of milk produced, while others appear to produce milk at the expense of their own bodies. It does appear that the deficiency of a maternal fat reserve can be overcome by providing adequate calories and protein during the lactation period.

2.3 The Relationship Between Diet During Lactation and the Composition of Milk

In the human, the composition of milk can differ between individuals and even within the same women during the course of a feed, between each breast and with the stage of lactation (Hytten and Leitch, 1971). The volume rises rapidly during the first week, and more slowly thereafter, while the lactose content rises during the first week to a level which is almost constant for the duration of lactation (Hytten, 1954). The concentration of total lipid varies extensively between individuals, but a trend toward higher lipid concent-rations after six months has been observed (Underwood et al, 1970). The protein

level falls at six weeks postpartum and changes very little thereafter (Underwood et al, 1970).

It has been suggested that there may be a racial difference in the composition of breast milk; however, when the breast milk of Bantu and white women in the same population was analyzed there was no significant difference in the protein, fat and lactose content of the milk (Prinsloo et al, 1970). The volume of milk secreted varies considerably. In the human the volume can vary from less than 500 ml./day to about 3500 ml./day (Munro and Allison, 1964).

There is quite a range of literature dealing with the effect of diet during lactation on the composition of milk. The effect of dietary protein on milk quality and quantity has received a great deal of attention, as has the effect of dietary fats on lipid composition, but the extent to which caloric intakes influence the composition of milk remains a relatively unexplored field in human nutrition. However, in the cow, energy is one of the most limiting factors to high milk production, and the available energy during early lactation plays an important role in establishing the level of milk production that will continue throughout the lactation period (Schmidt, 1971). In cows, increasing protein and calorie intake increases the casein content and milk yield, and slightly decreases the percentage of fat (Schmidt, 1971). Rook and Witter (1968) found a fall in milk production with under-nutrition of sows during lactation. This was associated with a decreased lactose content and an increased content of fat and protein. Similar results were found by Lodge (1959). Increasing

calories significantly increased the milk yield; however, animals on a lower protein and calorie diet produced a milk with a higher protein and fat content with the result that there was no significant difference in gross energy output. Trigg et al (1974) found widely varying responses in lactating beef cows when fed low calorie diets during lactation. Some cows rapidly declined in milk yield, some maintained output but lost considerable weight, while others were intermediate with respect to these characteristics.

There have been attempts to assess the effects of famine or near famine on lactation, and several of these studies have indicated that in times of deprivation the quantity of milk decreases and the duration of the lactation period becomes shortened. Descaine (1871) reported that during the siege of Paris, of 43 women attempting to feed their infants, 16 had insufficient milk and 12 babies died of hunger. Twelve others were thought to have enough milk but it was produced at the expense of the mothers' bodies. Records kept at the Landesfrauen-Klinik, Wuppertal, showed that the average daily secretion of milk on the seventh day postpartum was about two ounces less in 1945-6 than it had been in 1938 (Gunther, 1968). Smith (1947) mentions a slight decrease in the quantity of breast milk, but not in the duration of lactation in Holland during the hungry period in World War II. Antonov (1947) came to the following conclusions during the siege of Leningrad: (1) in spite of hunger the mammary gland secretes milk if there is sufficient physiological stimulation, that is the capacity for breast feeding remains, and (2) the quantity of milk, however, decreases, and the duration of the lactation period becomes shorter.

How then do women in underdeveloped nations subsisting on inadequate diets appear to lactate successfully? Goplan (1956) found the milk produced by Indian women on low protein intakes to be adequate to meet the protein requirement of their children when expressed as the average protein intake per kg. of body weight. The birth weight and actual body weight of these infants were lower than those reported at the 50th percentile for American infants of corresponding ages. The two growth curves were, however, nearly parallel signifying that although the actual weights were different, the growth rates were not widely divergent. Similar findings were published by Sundararaj and Perura (1975) on Indian women who, because of local taboos, eat only bread and coffee for one to three months following delivery, and yet were able to lactate successfully as measured by the normal growth of their infants.

Rajalakshmi (1971) reviewed the literature dealing with the reproductive performance of poor Indian women. He suggested that successful lactation could be achieved on a diet consisting of 1500 kcal. and 40 g. protein. Indian women produce about 470 ml. of milk at one month and this increases to 700 ml. by three to four months, much below the Western average of 850 ml./day. In spite of the low volume of milk their infants doubled their birth weight by four months, which is within the Iowa norms. It thus appears that there is a wide variety of lactational ability among human mothers, with some women being able to feed their babies despite nutritional deprivation, but severe undernutrition reduces the proportion who can do so.

Studies of the variations in the composition of milk from different popu-
lations have shown conflicting results. Hanafy et al (1972) found Egyptian
malnourished mothers produced milk with a lower protein, fat and lactose content
than did apparently healthy mothers from within the same population. The
greatest variation was in the volume produced by the two groups, with the mal-
nourished mothers producing 22% less milk. The children of the two groups of
mothers differed significantly in weight. Baily (1965) found the milks of dif-
ferent groups of New Guinea women differed in protein and fat composition with
lactose showing very little variation. When compared with variations in dietary
intake of protein and calories the quantity of milk was affected more than the
quality.

The total protein content and amino acid composition of milk from mal-
nourished mothers have been found by some authorities not to be significantly
different from the milk of well-nourished mothers. Jelliff (1952) found the protein
content of breast milk from Nigerian mothers compared favourably with that of
women from other parts of the world. He reported no evidence of a decrease
in the protein content with prolonged lactation. On the other hand, Ogbeide
(1975) reported normal ranges of protein content in the breast milk of Nigerian
mothers until six months (1.2 g./100 ml.), but from seven to twenty-two months
over 50% of a sample of 105 apparently healthy mothers produced milk with an
average protein content of 0.772%. It is thought that in the absence of suf-
ficient dietary protein to sustain satisfactory lactation, the protein required
for milk synthesis must be derived from the mother's own tissue (Kon and Cowie,
1961). Nageshwara Rao and Nageshwara Rao (1974) found that lactating

women on a daily intake of 42 g. vegetable protein went into marked negative nitrogen balance, indicating the protein content of their milk was subsidized by catabolism of their own tissues. Perhaps in mothers on inadequate intakes the physiological ability of the organism to catabolize body protein is exceeded in prolonged lactation.

The major portion of the protein in milk is synthesized within the mammary gland from amino acids that are absorbed from the blood stream, and thus reflects the amino acid composition of the diet; however, gamma casein, blood serum albumin and the immune globulins are absorbed as preformed proteins from the blood (Jordan and Morgan, 1970). Srinevasan and Ramanathan (1954) found lower levels of cysteine in breast milk of Indian women than in American women. Lindblad and Rahimtoola (1974) found low levels of methionine and lysine in the milk of women whose diet lacked these two amino acids.

Several researchers have looked at the effect of dietary supplementation on the composition of breast milk. Deb and Coma (1962) found that supplementation of Indian womens' diets with casein, in the form of dry biscuits, did not increase the protein content of the milk, but it did increase the fat content and the whey to curd ratio in the milk. The increase in the whey portion of the milk is thought to be due to an increase in a serum albumin fraction. Kamarkar et al (1959) correlated the protein intakes of Indian mothers with the protein composition of their milk, and they found an initial increase in total nitrogen and protein nitrogen of the milk with increased protein intake, but intakes of protein above 40 grams had no effect on the milk protein. Supplementation with eggs and milk

to increase the protein intake of nursing mothers from 40 to 55 grams did not alter the milk constituents (Devadas et al, 1971). Goplan (1958) found that increasing the dietary protein in poor Indian womens' diet from 61 to 99 grams resulted in an appreciable increase in the 24-hour output of breast milk; however, this was attended by a dimunition in the percentage of protein in the milk. Increasing protein intake to 114 g. had no further effect. It thus appears that increasing protein intake above a minimum requirement affects primarily the volume of milk with only minor effects on its composition.

In rats, the volume of milk produced varied directly with the casein level of the diet up to 30%; however, the protein concentration of the milk showed no significant differences within a range of 5 to 50% dietary casein (Mueller and Cox, 1946).

The main component of milk fat is triglyceride, and this fraction of milk has been shown to vary in relation to diet. Insull et al (1959) found that neither the daily milk volume nor daily fat production appear to be influenced by changes in the mother's diet; however, with deficient caloric intake, milk fat closely resembles human depot fat, and during energy equilibrium milk fat closely resembles dietary fatty acid composition. Raising the mother's intake of polyunsaturated fatty acids has been shown to raise the polyunsaturated composition of the milk (Insull et al, 1959; Wellby et al, 1973). This increase in polyunsaturated fatty acids is accompanied by an increase in vitamin E levels, however no appreciable change in the cholesterol and phospholipid fraction occurs (Kramer et al, 1965; Potter et al, 1976).

It has been suggested that the most important dietary factor influencing the fatty acid composition of milk is the level of carbohydrate in the diet. High levels of carbohydrate favour the synthesis of lauric and myristic acid in the mammary gland and reduce the uptake of triglycerides from the blood. However, when there is a relatively low intake of carbohydrate the fatty acid composition of the diet is reflected in the milk (Read et al, 1965). On a low fat diet the $C_{20}-C_{22}$ polyenoic acids which have special relevance to brain growth were replaced by substantial percentage increases in acids of a 10 to 14 carbon chain length in the breast milk of East African mothers (Crawford and Stevens, 1974). An earlier study by Hansen et al (1970) found no differences in the composition of essential fatty acids in the breast milks of Danish and Ugandan mothers, although Ugandan milk did have a higher proportion of short chain fatty acids indicating synthesis within the mammary gland.

It thus appears that the volume of milk produced varies with the adequacy of the diet, therefore reducing the calories available to the suckling young when the mother is fed an inadequate diet during lactation. The lactose and protein content seem to be fairly constant within a normal range during early lactation regardless of diet. The protein content can, however, fall drastically to inadequate levels during prolonged nursing on a deficient diet. This is probably due to no labile protein remaining in the mother's body. Total lipid content varies extensively between individuals although the cholesterol, phospholipid and essential fatty acid composition are fairly constant. Both the fatty acid and amino acid composition of the milk reflect dietary intake.

2.4 Postnatal Growth

Growth can be defined as protein accretion, which is measured as an increase in cell number or cell size. Normal growth of the rat and its individual organs proceeds in three phases: (a) early prenatal and postnatal growth due to hyperplasia, (b) growth associated with hyperplasia and hypertrophy, and thereafter (c) growth due to hypertrophy. The hyperplastic growth begins at ten days after conception in the rat, and continues for a variable period in all organs (Winick and Noble, 1965). Eisen (1976) has shown the percentage of protein, ash, and fat to increase from birth to weaning in the rat, while the percentage water decreases. Bergen (1974) has pointed out that protein and fat are laid down at the same rate during early development, whereas later fat accumulates more rapidly. In the human infant as much as a third of the energy intake is accounted for by increments of protein and fat during the first two months of life, whereas after two months the value is less than 10% (Widdowson, 1974).

Changes in cell number or cell size, as well as changes in body composition with changes in age and weight of unselected animals fed ad libitum, provide a standard with which to compare the effects of nutrition during the suckling period. Most of the studies on lactation have relied on the method of restricting feed intake of pups by either limiting the maternal diet, increasing litter size, or intragastric feeding of limited amounts of milk.

Heggeness et al (1961) showed that preweaning weight gains of rats varied with the availability of the milk supply. These researchers rotated two

groups of pups between three dams with group one always going to a mother who had been without young for eight hours, and group two going to the lactating female who had been suckling group one animals. With increasing intakes of milk, pup carcass protein, ash, and fat content were found to be higher at weaning, with body fat showing the greatest difference. Allen and Zeman (1971) decreased litter size to four to determine the effect of increasing the milk available to nursing pups. There was a significant increase in total body fat, protein, and ash at weaning. Smart et al (1974) found rats restricted in intake from age 5 to 25 days weighed less and contained less body fat throughout life than normally fed animals. Wurtman and Miller (1976) were unable to show any significant difference in the weight of pups raised in litters of twelve or less, but when raised in litters of sixteen there was a decrease from controls in the total carcass DNA, protein, and lipid.

Chow et al (1968) showed that restricting the female rat to 50% ad libitum during pregnancy and lactation reduced birth weight by about 1 gram and drastically depressed subsequent growth of her progeny. However, feeding rats 50% of their ad libitum intake during pregnancy and ad libitum during lactation produced a less pronounced reduction in growth rate. Restriction to 50% of ad libitum intake only during lactation resulted in reduced preweaning growth with continued slow growth in adult life almost as severe as that caused by a restriction during both pregnancy and lactation (Chow and Rider, 1973). Barnes et al (1973) found no long-lasting effects of malnourishment during the period of gestation, however long-lasting effects were caused by postnatal

nutritional deprivation of rat pups. Ottinger and Tanabe (1968) were also unable to find any changes in adult body weight or behaviour of rats deprived during the prenatal period. Offspring whose mother was food deprived during the lactation period showed body weight deficits and increased errors in problem solving as compared with control rats.

Enwonwa and Glover (1973) found marked retardation in the growth of young suckled by malnourished mothers. The severity of the retarded growth varied with the size of the litter. The RNA, DNA, and protein contents of organs were reduced as a result of restricted intake during the suckling period. Winick and Noble (1966) reported a proportional decrease in the protein, DNA, and RNA content of several organs of pups raised in litters of 18, indicating a reduction in cell number without a change in cell size. These animals did not resume normal growth when adequately fed after weaning. Conversely, when Zeman (1970) reduced litter size to four, there was an increase in the size of the cells of the liver, kidney, and heart without an increase in cell number. Zeman reported no change in the brain; however, the litters were not reduced until day 7 at which time part of the growth of the brain would be complete. Smart et al (1974) found a reduced weight of the cerebellum in rats undernourished from 5 to 25 days as opposed to rats undernourished from conception to 5 days. An earlier study from the same laboratory (Smart et al, 1973) found the difference between forebrain and cerebellum weights in pups on an adequate or restricted diet during the suckling period to be highly significant. The final cerebellum weight was more affected by a restricted diet during the suckling period than was the forebrain.

Miller (1970) found that when neonatal rats were hand-fed different levels of protein, weight gain from birth to 5 days changed almost directly with protein concentration. When Miller looked at the relative importance of calories and protein on neonatal rat development he found the addition of calories from 50% to normal levels on an isonitrogenous diet had little effect on weight gain, but the addition of protein from 10% to normal levels on an isocaloric diet greatly increased weight gain. Czajka-Narins et al (1973) also found weight gains of rats to change almost linearly with protein intake from 0.6% to 9.6%. With a decreased protein intake the percentage of protein in the carcass was observed to decrease. Similar results were reported by Naismith and Morgan (1973). They found growth by hyperplasia to be influenced by the concentration of protein in the diet even when the intake of energy approximates maintenance. When 19-day-old weanlings were fed 25% of the normal caloric intake on either a low or high-protein diet for 16 days, rats on the high protein diet gained four times as much as their littermates on the low protein diet, and there was a significant increase in the protein and DNA content of the carcass, liver, kidney, and muscle with an increased protein intake. A comparison of the protein concentration of the milks of several species shows the percentage of calories from protein to be correlated to the growth rate per unit metabolic size (Payne and Wheeler, 1968). This would seem to indicate that total protein intake and not caloric intake is the determining factor for growth in all species.

Development appears to be altered by diet during the suckling period. Allen and Zeman (1971) reported on a decrease in the age of eye opening in

pups raised in small litters, and a percentage of fat at day 13 similar to that observed in controls at 21 days. Similarly, Smart et al (1974) showed a slower sexual development in pups of rats fed deficient diets during the nursing period. It has been suggested by Widdowson and McCance (1960) that sexual maturity is weight dependent rather than age dependent.

In summary, it appears that limiting the supply of nutrients available throughout the critical period of most rapid cellular proliferation, corresponding to the lactational period, can exert profound and long-lasting effects on the organism. Rats raised in litters which are larger than normal exceed the dam's lactational capacity, with the result that they suffer from protein-calorie undernutrition. During the nursing period growth is mainly due to cell division and any reduction in cell number due to a restricted intake of amino acids necessary for this growth will persist throughout life. The brain is one organ which will show the greatest reduction in cell number due to a deficient diet during lactation because its growth spurt occurs almost wholly within this period. In contrast, the liver's growth by cell division occurs from birth to 44 days and a reduced cell number due to a poor diet in the suckling period can be partially corrected by adequate feeding after weaning (Winick and Noble, 1965). If in the human infant, final body size, sexual maturity, and cellular development of organs are affected by the nursing diet, what will be the effect on the developing infant of any alterations in the mother's diet during pregnancy which affects her lactational performance?

3. EXPERIMENTAL DESIGN AND METHODS

The purpose of this study was to determine the effect of a diet restricted during pregnancy, on the ability of the rat to lactate successfully. The study was conducted in two parts. In the first part, the effect of a protein or calorie deficiency during pregnancy on maternal body fat was determined. In the second part, rats were fed either a low calorie-adequate protein or a low protein-adequate calorie diet throughout pregnancy. After delivery they were maintained on a normal diet, pair-fed with controls, and allowed to suckle eight foster pups until weaning. The protein and lipid content of the dam's milk, and the rate of growth, carcass fat content, and the litter's cerebella weight were assayed to determine if adequate milk for growth and development of the pups had been provided. The lipid content of the mother's carcass was analyzed to determine if there was any change due to lactation.

3.1 Procedure for Part One

Eight litters, each consisting of six female Sprague-Dawley breed 22-day-old, germ-free rats were purchased from the Bio-breeding Laboratories in Ottawa, Canada. The animals were maintained on Purina Laboratory Chow and tap water ad libitum from their arrival until mated. They were housed in an air-conditioned room maintained at 24°C., subjected to a light/dark cycle (light: 6:00-18:00). Littermates were divided into four groups when they reached 200 g., and housed in screen-bottom individual cages. The first group of rats

was non-pregnant controls (C), and these animals were fed a 15% casein diet to appetite. The three remaining groups of rats were mated with well-nourished 150-350 g. males, which were left in the cages with the females until the appearance of vaginal plugs which were taken to indicate the first day of pregnancy. The males were removed and the females kept on their diets throughout pregnancy.

The second group of rats was pair-fed with the C rats on a 25% casein diet to produce a calorie restricted-adequate protein group (LC). The third group of animals was the non-restricted pregnant group (NR), and they were fed a 15% casein diet to appetite. The fourth group was pair-fed with the NR group on an 8% casein diet to produce a protein restricted-adequate calorie group (LP). (see Figure 1).

The composition of the diets is given in Table I. The animals were fed weighed amounts of food at 9:00 and 17:00, with the diets being mixed into a paste with tap water to prevent spillage. Water was provided ad libitum. The animals were handled frequently, and were weighed on day one of pregnancy and at four-day intervals thereafter.

On the morning of the 22nd day of pregnancy the animals were weighed and then sacrificed by placing them in a bell jar saturated with anaesthetic grade ether. The fetuses were removed by Caesarian section and the number and litter weight recorded before they were disposed of. The gastrointestinal tract was removed from the dam, and the eviscerated weight recorded. The carcass was frozen immediately and then dried to a constant weight for 48 to 56 hours at 100°C. in a Gallenkamp oven. The lipid content of the carcass was determined by extraction with petroleum ether in a Soxhlet Apparatus.

Figure 1.   Schematic Model of Part One

Control (C)   Low Calorie (LC)

```
┌──────────────┐          pair          ┌──────────────┐
│     15%      │ ◄──────  fed  ───────► │     25%      │
│    Casein    │                        │    Casein    │
└──────────────┘                        └──────────────┘
```

adequate calories   restricted calories
and protein   adequate protein

Non-restricted (NR)   Low Protein (LP)

```
┌──────────────┐          pair          ┌──────────────┐
│     15%      │ ◄──────  fed  ───────► │      8%      │
│    Casein    │                        │    Casein    │
└──────────────┘                        └──────────────┘
```

adequate calories   adequate calories
and protein   restricted protein

TABLE I   Composition of diets g/100g

| | Casein content of the diet/100g* | | | |
	25%	15%	10%	8%
casein	25	15	10	8
peanut oil	15	15	15	15
corn starch	51.8	61.8	66.8	68.8
cellulose	2	2	2	2
vitamins	2.2	2.2	2.2	2.2
minerals	4	4	4	4

* The protein content of the mixed diets was verified by micro Kjeldahl analysis.

3.2 Procedure for Part Two

Eight litters of female Sprague-Dawley rats were purchased, raised, and mated in the same manner as described in Part One. The littermates were divided into three groups. The first group of rats was fed a 25% casein diet at the same level as fed to the Control group in Part One. These animals were on a low calorie-adequate protein diet (LC). The second group of rats were pregnant controls, and they were fed to appetite on a 15% casein diet, and were called the non-restricted group (NR). In Part One it was difficult to pair-feed the NR and LP groups because the LP animals would not eat the amount taken in by their NR littermates. Because of this the third group of rats in Part Two was fed a 10% casein diet to appetite. These animals were thus on a low protein-adequate calorie diet (LP). The feeding, weighing, and recording procedures described in Part One were followed in Part Two.

On the morning of the 21st day of gestation all animals were moved into individual plastic cages with wire grid tops to which rat bedding and shavings were added. Within 24 hours of birth the pups were removed from their mothers, and the litter number and weight recorded before the pups were disposed of. The mothers were each given eight foster pups, weighing a total of 50 to 60 grams, that had been born on the same day as their own pups. The foster pups' mothers had been maintained on Purina Laboratory Chow and tap water ad libitum throughout their gestation. The foster litters were composed of four females and four males.

All three experimental groups were weighed prior to fostering. The mothers and each individual pup were weighed at day 4, day 7, and then at weekly intervals throughout the 21-day lactation period. The foster pups were marked for identification at age four days. All three groups were fed a 15% casein diet during the lactation period. The NR mothers were fed to appetite and the LP and LC mothers were pair-fed with their NR littermate to prevent the restricted animals from eating more than their NR littermate.

The dams were removed from their pups for four hours prior to milking the dam at 14:00 hours on the seventh and fourteenth days of lactation. The pups were kept in an incubator at $37^{\circ}C$ for the four hours they were away from their mothers. The dams were anesthetized with anaesthetic grade ether, and given a parenteral injection of 1 unit of oxytocin in 0.5 ml. of 0.9% saline to facilitate milking. A Pasteur pipette was used to collect the milk which was immediately frozen and analyzed at a later date for protein and lipid. To analyze for protein, the milk protein was first solubilized by mixing .02 ml. of milk with .18 ml. of distilled water to give a 10% solution. This solution was drawn through the pipette, used to measure the milk, several times to remove any of the milk adhering to the glass. The 10% solution was combined with 0.8 ml. of 0.4 $\underline{M}$ KOH and then dissolved at $37^{\circ}C$. for 24 hours. A 0.2 ml. sample of the solubilized protein and 0.8 ml. of water were combined to give a 1 ml. protein solution, and the protein was estimated by the method described by Lowry et al (1956). The lipid content of the milk was estimated by using a modification of the method described by Wurtman and Miller (1976). A 0.5 ml. sample of milk was transferred by pipette to a tube that could be fitted with

a condensor.   Three 0.5 ml. volumes of distilled water were drawn through the same pipette to remove the milk residue that adhered to the side of the pipette. Then 4 ml. of methanol were added to the tube.   The tube was shaken and fitted with the condensor.   Then it was heated to 75°C. for 90 minutes.   The tubes were then cooled and transferred to a 15 ml. conical centrifuge tube and 8 ml. of chloroform and 1 ml. of water were added, after which the tubes were shaken, stoppered, and centrifuged for 20 minutes at 200 g.   The chloroform   phase was withdrawn by pipette and filtered through fast filter paper into a preweighed bottle.   After evaporation the weight of the lipid was determined.

On the morning of the 22nd nursing day the dams were weighed and then sacrificed by placing them in a bell jar saturated with anaesthetic grade ether.   The gastrointestinal tract was removed and the eviscerated weight recorded.   The carcass was prepared and analyzed for fat by the method described in Part One.   The individual pups were weighed and sacrificed in the same manner as their foster mothers.   The cerebella were removed and weighed. The pups were eviscerated and skinned, with the skin being carefully scraped with a scalpel to prevent fat loss.   The eviscerated and skinned carcasses (minus tail, ears, snout, and cerebellum) were frozen and then dried to a constant weight for 36-42 hours at 100°C. in a Gallenkamp oven.   The lipid content of the carcasses was determined by extraction with petroleum ether in a Soxhlet Apparatus.

The data were analyzed, for statistical significance, by the Student's T test distribution and the Pearson product moment correlation coefficient.

4. EXPERIMENTAL RESULTS

4.1 Results of Part One

The results for Part One of this experiment are shown in Tables II, III, IV and V. It is interesting to note that by attempting to pair-feed the NR and LP littermates, the NR animals were not allowed to eat to appetite due to the decreased food consumption of the LP animals. This resulted in the NR animals having an adequate energy intake for the first two weeks of gestation, and a calorie restricted diet during the third week. The overall effect of this design error was that the LC and NR animals ended pregnancy with essentially the same weight gain, and the same sized pups.

Although the LC rats consumed 11.5% fewer calories ($p < 0.05$) than the NR rats, their protein intake was actually 47.6% greater ($p < 0.001$). The NR animals had 20.0% more fat in their carcasses at the end of pregnancy than did the LC rats ($P < 0.05$). This difference was probably due to the higher caloric intake of the NR animals in the first two weeks of gestation when the demands of the fetuses were minimal, and if the NR rats had been allowed to eat to appetite throughout their pregnancy the difference in fat stores would have been even greater.

As would be expected, both the NR and LC rats gained significantly more weight ($p < 0.001$) than the C animals over the 21 days of their pregnancies; however, only the NR rats had significantly more fat in their carcasses (31.6%) than their virgin controls ($p < 0.02$).

TABLE II   Average daily food intake of dams during pregnancy*

Group	Daily food intake (g.)	Daily calorie intake (Kcal)	Daily protein intake (g.)
Control (C)	15.00 ± 0.46^a	66.46 ± 2.02^a	2.25 ± 0.07^a
Low calorie (LC)	15.00 ± 0.46^a	66.46 ± 2.02^a	3.75 ± 0.11^b
Non-restricted (NR)	16.94 ± 0.75	75.06 ± 3.32	2.54 ± 0.11
Low protein (LP)	16.66 ± 0.72	73.82 ± 3.20	1.33 ± 0.06^b

Mean values differ significantly from those for the

NR group: (a) $p < 0.05$, (b) $p < 0.001$

*Each value represents the mean and standard error for seven rats.

TABLE III   Dam's weight gain during pregnancy*

Group	Weight Gain
Control (C)	52.00 ± 7.50
Low calorie (LC)	119.00 ± 6.12^a
Non-restricted (NR)	118.00 ± 8.98^a
Low protein (LP)	$67.86 \pm 7.43^{b, c}$

Mean values differ significantly from control:
(a) $p < 0.001$.

Mean values differ significantly from non-restricted:
(b) $p < 0.002$.

Mean values differ significantly from low calorie:
(c) $p < 0.001$.

*Each value represents the mean and standard error for seven rats.

TABLE IV   Maternal carcass weight and fat content after pregnancy.*

Group	Carcass weight (g.)	Carcass fat content (g.)
Control (C)	253.36 ± 5.91	43.53 ± 2.77
Low calorie (LC)	256.50 ± 6.79	45.82 ± 4.66^{e}
Non-restricted (NR)	256.07 ± 8.18	57.27 ± 4.40^{b}
Low protein (LP)	$205.43 \pm 8.53^{a,d,g}$	$31.55 \pm 3.99^{c,f,h}$

Mean values differ significantly from controls:
(a) $p < 0.002$, (b) $p < 0.02$, (c) $p < 0.04$.

Mean values differ significantly from non-restricted:
(d) $p < 0.005$, (e) $p < 0.05$, (f) $p < 0.002$.

Mean values differ significantly from low calorie:
(g) $p < 0.002$, (h) $p < 0.005$.

*Each value represents the mean and standard error for seven rats.

The LP animals gained significantly less weight over gestation than did either their LC ($p < 0.001$) or their NR ($p < 0.005$) littermates. The weight gain was about the same as that of their virgin littermates; however, their mean carcass weight at the end of pregnancy was significantly lighter than any of the other three groups: C ($p < 0.005$), LC ($p < 0.005$), and NR ($p < 0.005$). The fat content of the LP rats was also significantly less than the other three groups: LC ($p < 0.05$), NR ($p < 0.01$), and C ($p < 0.05$). Thus, one would expect the energy needs for lactation in the LP rats would have to be met by the diet, as there were such small fat stores in their carcasses.

As shown in Table V, there were no significant differences in the number of pups per litter among the three dietary treatments, however the LP animals produced fetuses of significantly lighter weight than either the LC ($p < 0.005$) or the NR ($p < 0.05$) groups.

Table VI shows the effect of the maternal diet during gestation on fertility. Only 75.0% of the dams fed a low protein diet during pregnancy delivered pups as opposed to 87.5% of the low calorie and 98.8% of the non-restricted dams.

4.2   Results of Part Two

The average daily food intake of the dams during gestation, and the weight changes during pregnancy and lactation, are presented in Tables VII and VIII.   Table IX shows the number and average weight of pups delivered by the dams.   In order to overcome some of the difficulties encountered in Part One,

TABLE V   Number of fetuses per litter and average fetal weight*

Group	Number/litter	Average weight/fetus (g.)
Low calorie (LC)	10.71 ± 0.29 (10-12)	4.98 ± 0.09 (4.75-5.27)
Non-restricted (NR)	10.57 ± 1.17 (5-14)	5.02 ± 0.32 (4.29-6.00)
Low protein (LP)	11.00 ± 0.31 (10-12)	4.14 ± 0.20 (3.63-4.91)[a]

Mean value differs significantly from non-restricted and low calorie: (a) $p < 0.05$.

*Each value represents the mean, standard error, and range for six litters. The litters from one group of littermates was removed due to an extremely low value for the NR animal.

TABLE VI   Effect of maternal nutrition on fertility

Group	Number mated	Number delivered	Percent
Low calorie (LC)	24	21	87.5
Non-restricted (NR)	24	23	95.8
Low protein (LP)	24	18	75.0

when the attempt was made to pair-feed the NR and LP littermates, both groups were fed to appetite in Part Two, and the protein content of the low protein diet was increased from 8% to 10%. There were no significant differences between the calorie intake of the LP and NR dams in Part Two, and the calorie intake of the LP animals in Part One and Part Two were similar; however, due to the increased protein content of the low protein diet in Part Two, the LP animals increased their protein intake by 23.5% while the NR animals increased their protein and calorie intakes by only 5.0% above those reported in Part One.

These alterations in the protein and calorie intake resulted in increased weight gains over gestation, from those reported in Part One, of 10.0% in the NR animals and 43.3% in the LP animals. By contrast, the LC rats gained 5.0% less weight than the LC animals in Part One. Part of the increased weight gains of the NR and LP groups can be explained by an increase in fetal weights; however, it is likely that a large percentage of the LP animals' gain would be due to an increase in the fat stores. When calorie and protein intakes were correlated with the weight gain over gestation both the protein (r=0.5934; $p < 0.002$) and calorie (r=0.4500; $p < 0.02$) intakes were found to correlate significantly, but protein appears to be more significant than calories.

Once again the LC rats ate fewer calories (16.6%) and more protein (39.3%) than the NR animals. These differences were both highly significant ($p < 0.001$). However, unlike the findings in Part One, the LC animals in Part Two gained significantly less weight over gestation than did their NR littermates ($p < 0.05$).

TABLE VII   Average daily food intake of dams during pregnancy*

Group	Daily food intake (g.)	Daily calorie intake (g.)	Daily protein intake (g.)
Low calorie (LC)	14.83 ± 0.72[a]	65.60 ± 0.54[a]	3.70 ± 0.03[a]
Non-restricted (NR)	17.76 ± 0.35	78.67 ± 1.56	2.66 ± 0.05
Low protein (LP)	16.63 ± 0.59	73.66 ± 14.83	1.65 ± 0.06[a]

Mean values differ significantly from those for the
non-restricted group: (a) $p < 0.001$

*Each value represents the mean and standard error for eight rats.

TABLE VIII   Dam's weight change during pregnancy and lactation*

Groups	Weight when mated (g.)	Weight gain pregnancy (g.)	Weight loss lactation (g.)
Low calorie (LC)	207.0 $\pm$ 3.85	113.63 $\pm$ 4.01[a]	24.13 $\pm$ 6.80
Non-restricted (NR)	202.6 $\pm$ 3.76	129.75 $\pm$ 5.00	31.75 $\pm$ 9.81
Low protein (LP)	204.4 $\pm$ 4.49	97.25 $\pm$ 5.39[b,c]	7.94 $\pm$ 7.87[a]

Mean values differ significantly from non-restricted:
(a) $p < 0.05$, (b) $p < 0.005$.

Mean values differ significantly from low calorie:
(c) $p < 0.05$.

*Each value represents the mean and standard error for eight rats.

The LP rats ate 37.9% less protein than the NR dams ($p < 0.001$) and gained significantly less weight over their pregnancies than did either their NR ($p < 0.005$) or LC ($p < 0.05$) littermates. There were no significant differences in the number of pups per litter among the three dietary treatments; however, as was found in Part One, the LP animals produced pups of significantly lighter weight than either the LC ($p < 0.05$) or the NR ($p < 0.02$) groups. The average pup weight was correlated with the protein intake of its mother over gestation ($r=8776$; $p < 0.001$), but not to the caloric intake (Figure 2).

The performance of the rats during lactation in terms of body weight changes was opposite from the trend observed in gestation: the NR dams that gained the most weight during pregnancy lost the most weight during lactation whereas the LP animals that gained the least over gestation lost the least over lactation. There was a negative correlation ($r=-0.4403$; $p < 0.02$) between the weight gain over pregnancy and the weight change over lactation in all groups. As can be seen from Table VIII there was no significant difference between the weight losses of the LC and NR dams, but there was a significant difference between the weight losses of the LP and NR mothers ($p < 0.05$).

The three groups of mothers ended the 21 days of lactation with no significant differences in their carcass weights or in the fat content of their carcasses, as shown in Table X. However, all groups had a significantly lower mean percentage of body fat ($p < 0.005$) than did the non-pregnant control group in Part One.

TABLE IX   Number of pups/litter and average pup weight*

Group	Number/litter	Average weight/pup (g.)
Low calorie (LC)	11.50 ± 0.73 (8-14)	5.77 ± 0.27 (4.40-7.05)
Non-restricted (NR)	9.88 ± 0.55 (8-12)	5.85 ± 0.19 (5.14-6.66)
Low protein (LP)	10.63 ± 0.63 (8-13)	4.90 ± 0.33 (3.86-5.20)[a,b]

Mean values differ significantly from non-restricted:
(a) $p < 0.02$.

Mean values differ significantly from low calorie:
(b) $p < 0.05$.

*Each value represents the mean, standard error and range for eight litters.

Figure 2. The Relationship between the Mother's Protein
Intake during Pregnancy and the Birth Weight
of her Pups.

(r = 0.8776, p $<$ 0.001 : x = 3.23 + 0.8533.Y)

□ Low Calorie (LC),   △ Non-restricted (NR), ○ Low Protein (LP)

Average Birth Weight/Pup (g)

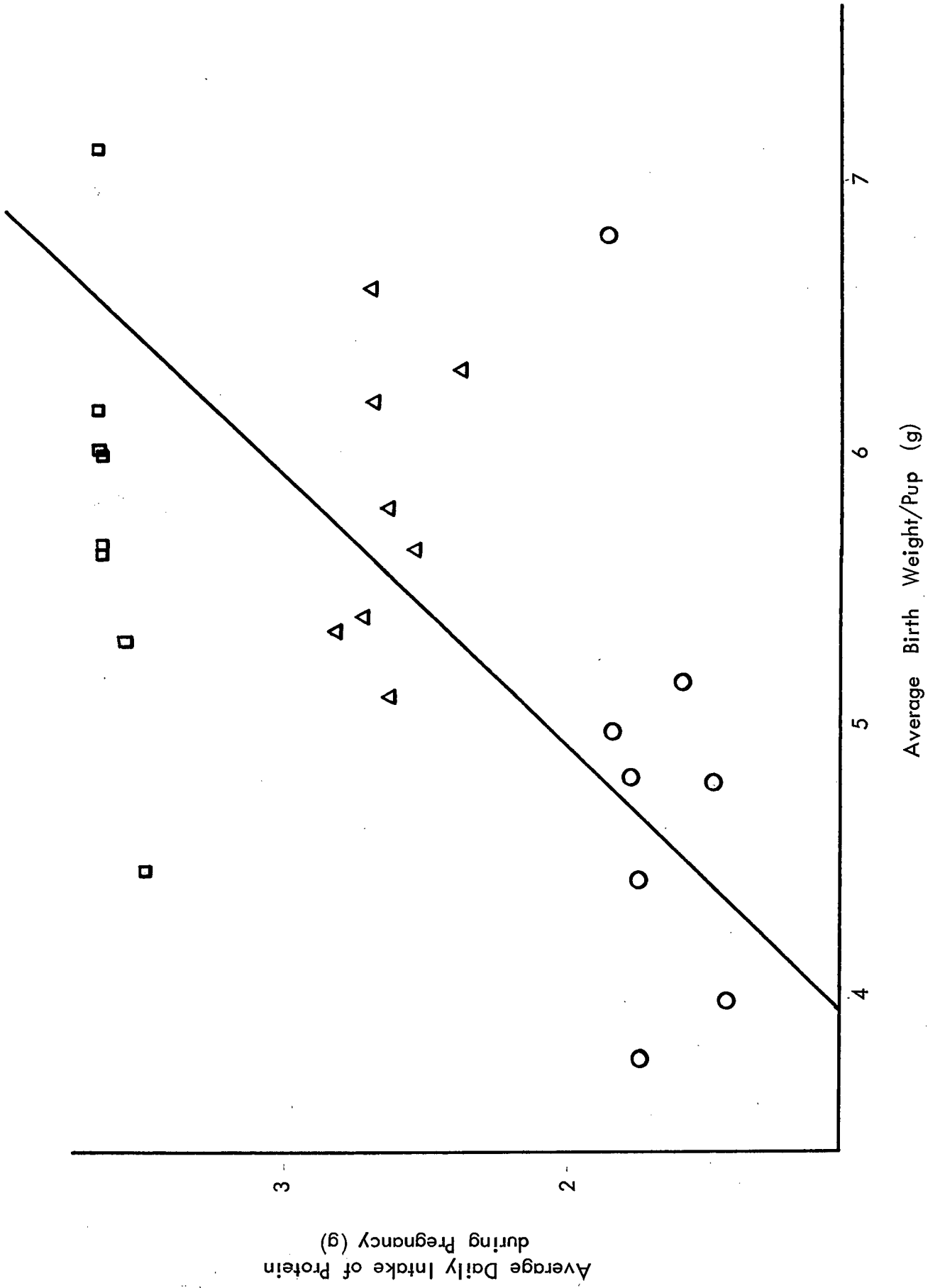Average Daily Intake of Protein
during Pregnancy (g)

TABLE X   Maternal weight, carcass weight, and fat content at the end of lactation*

Group	Weight (g.)	Carcass Weight (g.)	Carcass Fat (g.)
Low calorie (LC)	218.80 + 7.75	201.85 + 7.56	11.95 + 3.23
Non-restricted (NR)	225.16 + 9.58	204.89 + 11.17	16.23 + 3.62
Low protein (LP)	217.50 + 5.93	199.33 + 6.04	15.91 + 3.32

None of these mean values differ significantly from one another.

*Each value represents the mean and standard error for eight rats.

As can be seen from Table XI, the protein and calorie intakes of the dams during lactation varied considerably. When the protein and calorie intakes of each dam were correlated with the dam's weight change over lactation a significant correlation was found ($r=0.7326$; $p < 0.002$). Similarly, the carcass weight at the end of lactation ($r=0.7517$; $p < 0.002$) (see Figure 3) and the carcass fat content at the end of lactation ($r=0.5670$; $p < 0.003$) were significantly correlated with food intake over lactation. Neither the dam's weight gain over gestation nor her food intake during pregnancy were correlated with any of the above variables at the end of lactation.

The lactation performance of the dams, as measured by the growth, final fat content and cerebella weight of their pups is presented in Tables XII, XIII and XIV. Figure 4 shows the mean growth curves of the litters of each dietary group over the 21-day suckling period. The LP litters weighed 14.6%, 10.0% and 7.4% less than their NR controls at 7, 14 and 22 days of age respectively. Similarly, the LC animals weighed 2.5%, 0.3%, and 3.4% less at 7, 14 and 22 days respectively. These differences in weight were not significant. The growth curves of the LC and NR animals are very similar while the LP animals grew more slowly during the first fourteen days of lactation and more quickly during the last seven days. Statistical analysis of the average daily litter gain (Table XIII) showed no significant difference in the growth rates at 4, 7, 14 and 22 days. Similarly, there were no significant differences in the weight or fat content of the litters' carcasses at day 22.

TABLE XI.   Dam's average daily food intake during lactation*

Average daily food intake (g.)   24.10 ± 2.23   (15.28-33.09)

Average daily caloric intake (Kcal.)   106.78 ± 9.83   (67.69-146.59)

Average daily protein intake (g.)   3.62 ± 0.33   (2.29- 4.96)

*Each value represents the mean, standard error and range for twenty-
 four rats.

Figure 3.   The Relationship between the Dam's Average Daily
Food Intake during Lactation and her Carcass Weight
at the end of Lactation (r = 0.7517, p< 0.002 :
x = 108.27 + 3.89Y)

□ Low Calorie (LC), △ Non-restricted (NR), ○ Low Protein (LP)

Dam's Carcass Weight after Lactation (g)

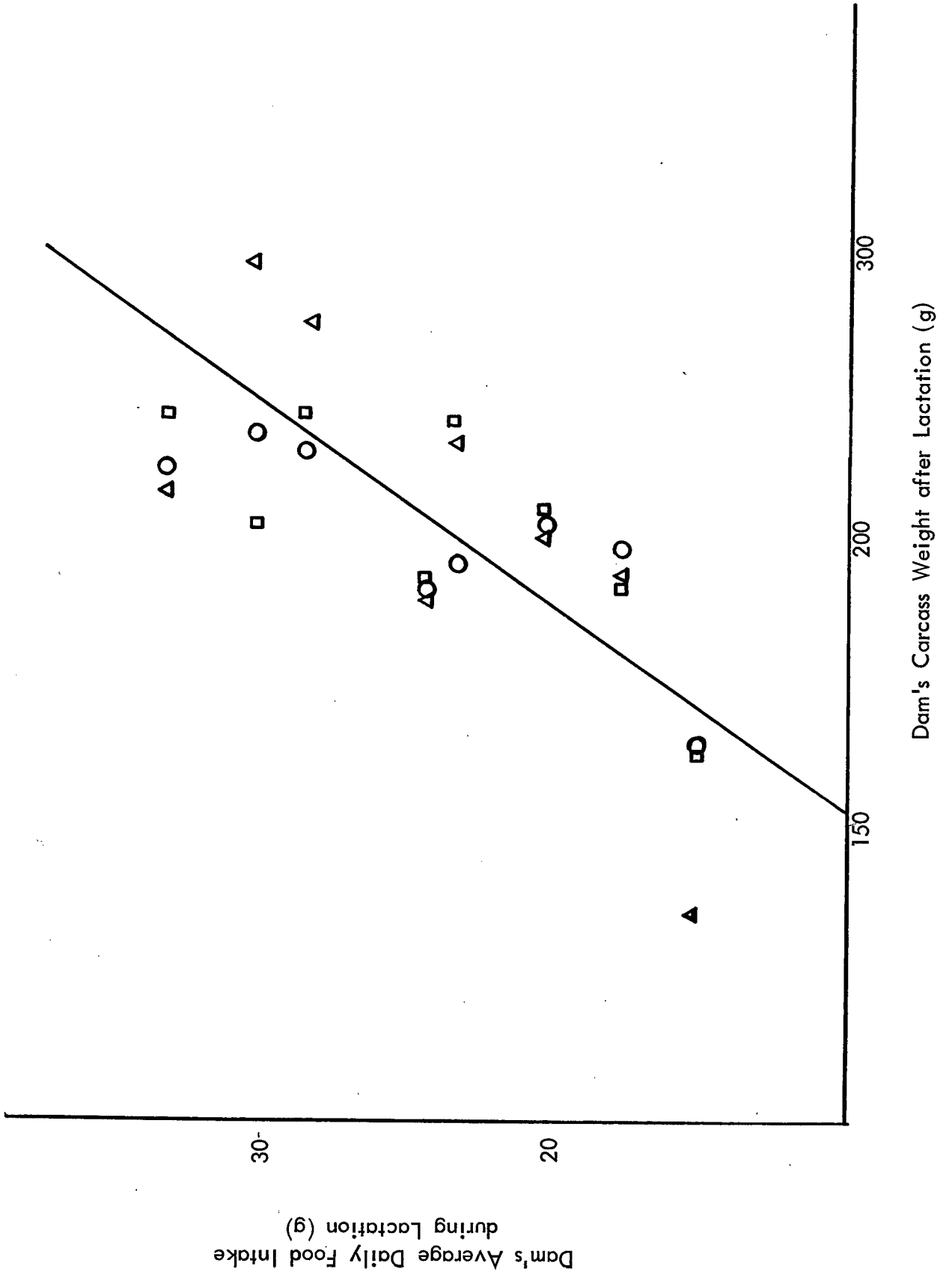Dam's Average Daily Food Intake during Lactation (g)

TABLE XII   Litter weight on days 7, 14, and 22*

Group	Weight day 7 (g.)	Weight day 14 (g.)	Weight day 22 (g.)
Low calorie (LC)	98.1 ± 4.02	171.1 ± 10.16	274.3 ± 18.26
Non-restricted (NR)	100.6 ± 4.18	171.6 ± 10.63	284.1 ± 19.39
Low protein (LP)	85.9 ± 6.84	154.4 ± 13.18	263.1 ± 21.68

None of these mean values differ significantly from one another.

*Each value represents the mean and standard error for eight litters of eight pups.

TABLE XIII   Average daily litter weight gain and total weight gain over
the suckling period*

Group	Gain for first 4 days (g/day)	Gain for first week (g/day)	Gain for second week (g/day)	Total Gain (g.)
Low calorie (LC)	7.31 ± 7.18	6.69 ± 0.54	10.43 ± 1.03	222.19 ± 18.18
Non-restricted (NR)	7.03 ± 1.05	6.37 ± 0.73	10.15 ± 1.07	226.68 ± 20.99
Low protein (LP)	4.55 ± 1.09	4.05 ± 1.40	9.79 ± 1.10	208.23 ± 22.17

None of the mean values differed significantly from
one another.

*The weight gain for the first four days represents the mean and standard
error for five litters of eight pups. The remaining values represent the
mean and standard error of eight litters of eight pups.

TABLE XIV   Total litter cerebella weight, carcass weight and fat content*

Group	Cerebella weight (g.)	Carcass weight (g.)	Fat content (g.)
Low calorie (LC)	1.63 ± 0.06	190.21 ± 12.81	14.94 ± 1.80
Non-restricted (NR)	1.68 ± 0.08	197.61 ± 14.05	15.89 ± 2.23
Low protein (LP)	1.40 ± 0.08[a]	184.34 ± 15.25	14.89 ± 2.33

Mean value differs significantly from non-restricted: (a) $p < 0.05$.

*Each value represents the mean and standard error for eight litters of eight pups at 22 days of age.

Figure 4.   The Mean Growth Curves of the Pups over
             the Twenty-one day Suckling Period.

☐ Low Calorie (LC), △ Non-restricted (NR), ○ Low Protein (LP)

Weight of Litter (g)

200

100

7 14 22

Age of Pups (days)
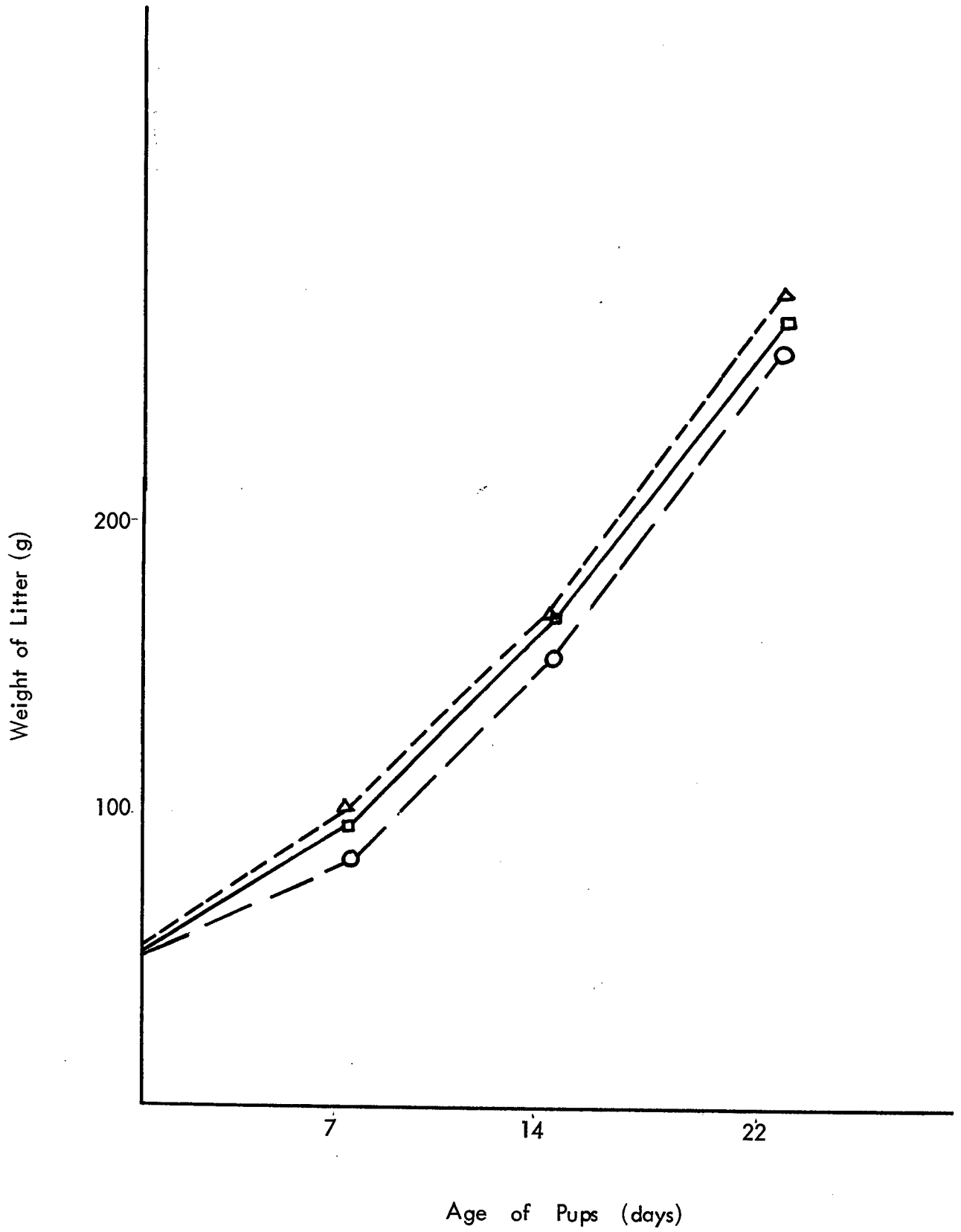
Those litters in which the mother's weight changed very little over lactation had the highest body fat content (r=0.4918; p $<$ 0.01), greatest weights (r=0.6866; p $<$ 0.002), and a greater weight gain over lactation (r=0.5445; p $<$ 0.005). The food intake of the individual dam was significantly correlated with her litter's weight gain (r=0.9477; p $<$ 0.002), total weight on day 22 (r=0.9382; p $<$ 0.002) (see Figure 5), and carcass fat content (r=0.8766; p $<$ 0.002). There appears to be a three-way relationship, as seen in Figure 6, between each animal's food intake during lactation, her weight change over lactation, and the weight gain of her pups.

The LP cerebella weight was found to be significantly lighter than that of the NR animals (p $<$ 0.005). The cerebella weight was more significantly correlated with the pups' weight on day 7 (r=0.4993; p $<$ 0.01) than it was to the pups' weight on day 22 (r=0.3634; p $<$ 0.05). Both the litter weight on day 7 and the cerebella weight were correlated with the mother's weight gain over gestation (r=0.4440; p $<$ 0.02, and r=0.4561; p $<$ 0.02 respectively), whereas there was no correlation with other variables of pup growth.

Analysis of the protein and fat composition of the dam's milk on days 7 and 14 of lactation are found in Table XV. There was no significant difference among the three groups. As can be seen from Figure 7, the LC animals appear to have the highest concentration of protein and fat on both days. The NR and LP animals had very similar concentrations of protein, with the LP animals having a slightly higher value on day 7 and a slightly lower value on day 14. The fat content of the milk ranged from 9.07 to 23.24 g./100 ml.

Figure 5.   The Relationship between the Dam's Average
             Daily Food Intake during Lactation and the
             Weight of her Litter at Twenty-two days.
             (r = 0.9382, p < 0.002 : x = -0.7 + 11.39Y)

□ Low Calorie (LC), △ Non-restricted (NR), O Low Protein (LP)

Dam's Average Daily Food Intake
During Lactation (g)

Total Litter Weight at 22 days (g)

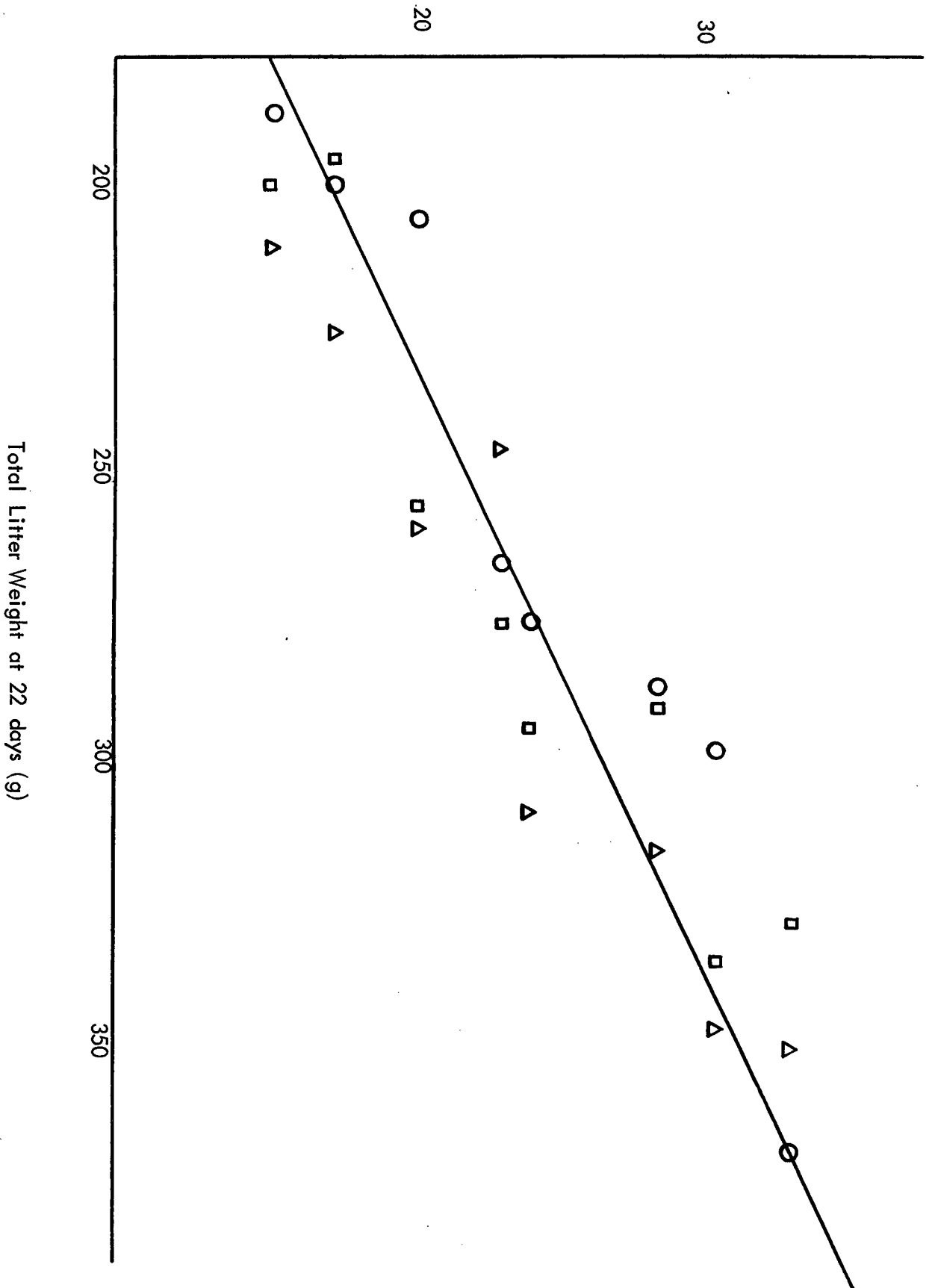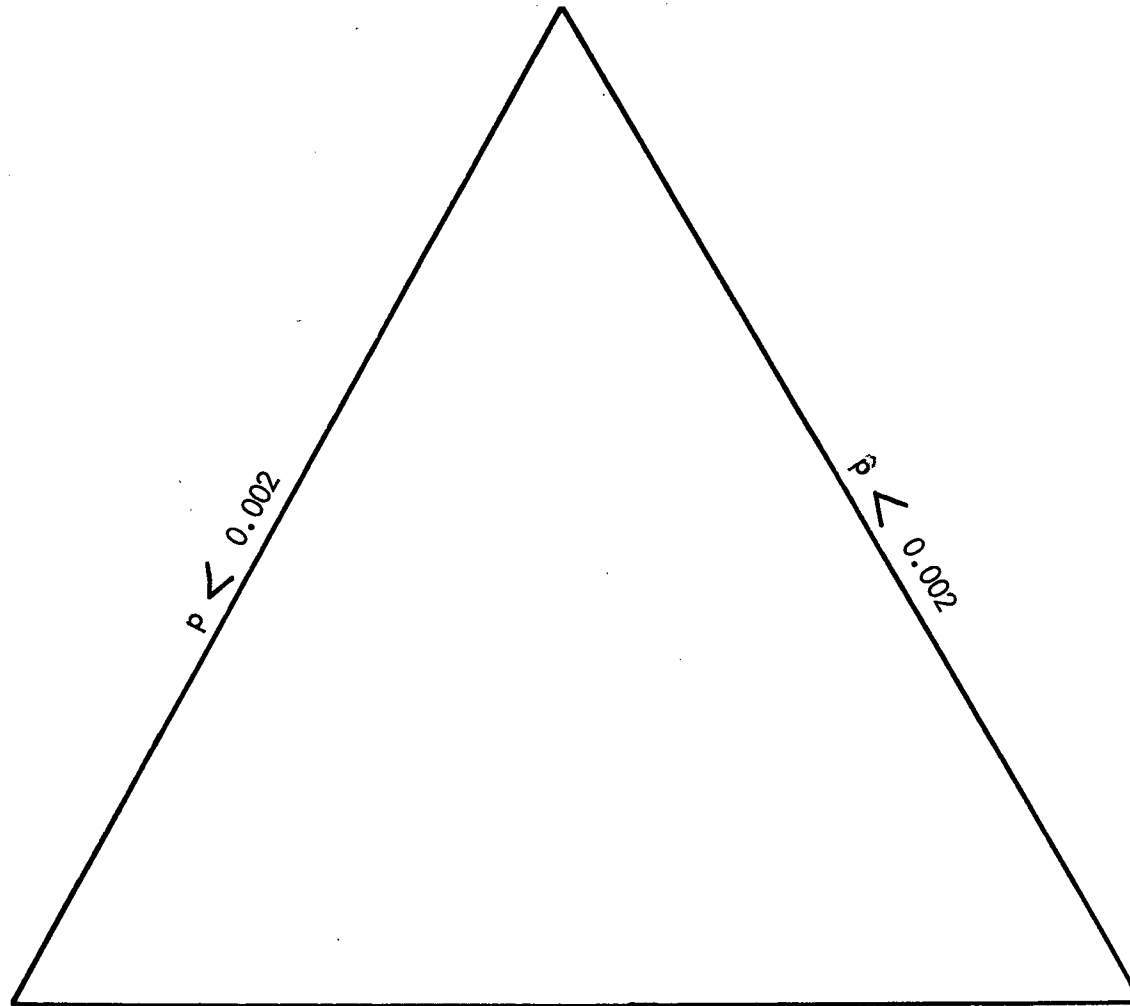Figure 6.   The Triangular Relationship between the Dam's
              Average Daily Intake during Lactation, her
              Weight Change over Lactation, and the Weight
              Gain of her Pups.

Dam's Food Intake (protein
and calories) during Lactation

$p < 0.002$

$p < 0.002$

Dam's Carcass
Weight and Fat
Content after Lactation

$p < 0.002$

Litter Weight
and Fat Content
after Weaning

TABLE XV   Protein and fat content of dam's milk on the 7th and 14th days
            of lactation*

	Low calorie (LC)	Non-restricted (NR)	Low protein (LP)
Protein content day 7 (g/100 ml)	9.27 + 0.78 (7.27 - 11.82)	8.55 + 0.55 (6.82 - 10.00)	8.82 + 0.48 (7.27 - 9.77)
Fat content day 7 (g/100 ml)	13.39 + 1.23 (10.11 - 14.57)	12.85 + 1.07 (9.92 - 15.67)	12.30 + 1.45 (9.07 - 17.04)
Protein content day 14 (g/100 ml)	10.43 + 0.50 (9.09 - 11.82)	9.41 + 0.42 (7.95 - 10.23)	9.36 + 0.78 (7.27 - 11.82)
Fat content day 14 (g/100 ml)	15.68 + 2.51 (9.73 - 23.24)	15.09 + 1.97 (10.68 - 20.95)	13.90 + 2.03 (9.71 - 20.39)

None of the mean values differ significantly from
one another.

*Each value represents the mean, standard error, and range of
 five lactating rats.

Figure 7. The Composition of the Dam's Milk at Seven and Fourteen Days.

Low Calorie (LC)

Non-restricted (NR)
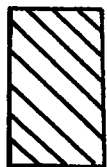
Low Protein (LP)
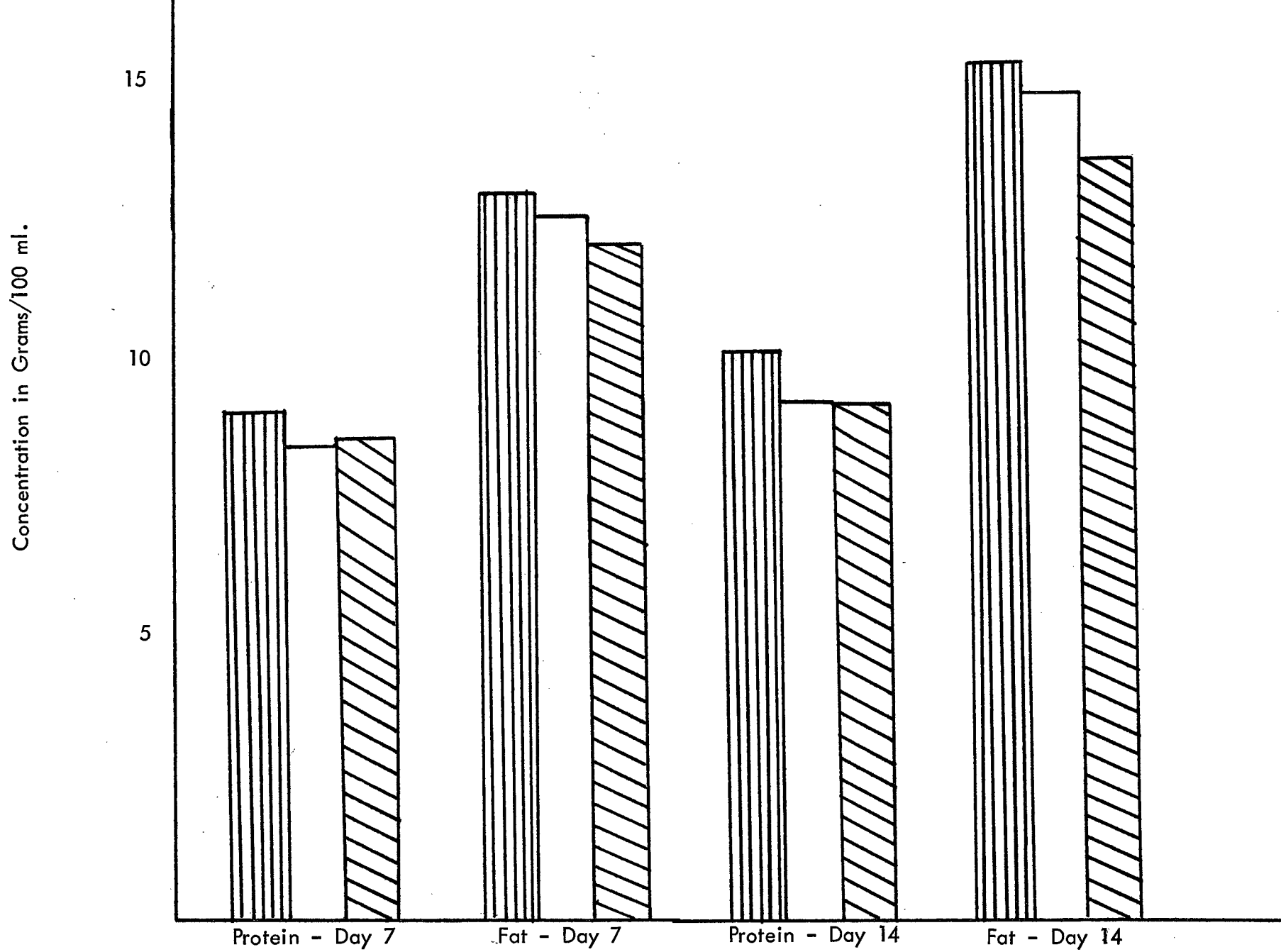
with an overall mean of 13.86 g./100 ml., while the protein content of the rat milk ranged from 7.27 to 11.82 g./100 ml.

The growth rates of the pups on days 7 and 14 were correlated with the protein content of the milk on day 7 ($r=0.3598$; $p < 0.05$) and day 14 ($r=0.5132$; $p < 0.01$) but there was no correlation between the growth rates and the fat content of the milk.

V   DISCUSSION

Interpretation of the data in this study was complicated by two findings: (1) there was a greater difference in the protein than in the calorie intake of the LC and NR animals during pregnancy in both parts of the study; and (2) the intra-litter variations due to genetic potential were greater than the inter-litter variations due to dietary manipulation during gestation.

The diet during pregnancy affects the weight gain of the mother over pregnancy, the size of her fat stores at parturition, and the birth weight of her pups.   Only the NR group increased their fat stores significantly during pregnancy.   This increase of 31.6% above the fat content of the virgin controls is slightly less than the 40% increase reported by Naismith (1971), and it is likely that part of the NR animals' stores, in Part One of this study, were used to supplement fetal needs when food intake was restricted during the third week of gestation.   It is probable that this stored energy was used to supplement the caloric requirement for milk production even though the NR group's food consumption increased by 37.7% above pregnant levels during lactation.

The LC animals ended pregnancy with no more fat in their carcasses than did their virgin controls; however, on a 25% casein diet during pregnancy, they were able to meet the protein requirement of their developing fetuses, as evidenced by normal sized pups at delivery.   This agrees with the finding of Naismith and Morgan (1973) that growth by hyperplasia is influenced by the concentration of protein in the diet even when the intake of energy approximates the maintenance level.

During lactation the LC animals were able to catabolize their small fat stores for energy, but it is likely that these became exhausted sooner than those of the NR animals. Interestingly, the growth curves (Figure 4) of the NR and LC litters show the greatest divergence during the third week of lactation. It is possible that the LC group's fat stores had been depleted at this point. If the LC diet had been continued after parturition, one would assume that the reduced "maternal stores" would be exhausted much sooner than the twelve days suggested by Malasan (in Beaton, 1966) as the length of time normal stores can subsidize lactation on a low calorie diet. Once this point is reached milk secretion ceases to maintain pup growth.

In contrast, the LP group ended pregnancy with less fat than their virgin controls, and on an 8 to 10% casein diet they were not able to meet the protein requirements of their developing fetuses. As has been reported in the literature (Zeman, 1967; Chow et al, 1968; Zamenhof et al, 1971;) and McLeod et al, 1972), the pups, at birth, were significantly lighter than those of the NR group. Pup birth weight appears to be dependent upon the protein intake of their mother during gestation.

The reduced fat stores of the LP group, on an adequate calorie intake, is quite likely the result of carbohydrates being used to supply the energy and the intermediary products for the endothermic biosynthesis of amino acids required for fetal development. Thus, the protein requirement for fetal development takes priority over the propensity for increasing "maternal stores".

Because of the extremely low fat stores of the LP animals, one would expect the energy needs for lactation to be met by the diet. However, it is likely that the lactation success of the LP group in Part Two was due to the increased weight gain (43%), and thus fat stores, above that reported for the LP group in Part One. The protein content of the LP diet was raised from 8% in Part One to 10% in Part Two.

In all dietary treatments, body fat, which was accumulated during pregnancy, was catabolized during the suckling period to provide energy for milk production. As the LC and NR animals were of similar body weight, compared with their corresponding groups in Part One, it is possible to compare the values for body fat content. After lactation, the LC rats had 73.9% and the NR rats 71.7% less fat in their carcasses than at the end of pregnancy. These values are close to those reported by Naismith (1971). He found that during lactation well-fed rats will lose 60-70% of their total body fat to subsidize the high energy costs of lactation. Since the LP animals in Parts One and Two differed so greatly in their weight gains over gestation, their fat contents can not be compared. However, since the LP animals lost the least weight over lactation it is reasonable to assume that there was less fat available for catabolism, and the energy required for milk production was supplied by the diet even though the LP and NR groups were pair-fed during lactation.

Because all animals ended lactation with essentially the same fat stores, it is likely that all animals decreased their fat stores to the same critical level. The weight loss during lactation was inversely proportional to

the fat content of the rat's body after pregnancy. In other words, the performance

of the rats during lactation in terms of body weight changes was opposite from

the trend observed in gestation: the dams that gained the most weight during

pregnancy lost the most weight during lactation, and vice versa. These findings

are similar to those reported by Buitrago et al (1974) for pigs.

Some authorities are of the opinion that the lactating rat should gain

as much weight and body fat as a non-lactating control during the same period,

since a loss in body weight indicates that maternal body tissue rather than

dietary supplies are being used for milk production (Kon and Cowie, 1961; Munro,

1964). These same authors recommend 24-30% casein should be provided for

optimal lactation in the rat. The 15% casein diet fed during lactation, in this

study, may have been deficient in protein for lactation, and thus contributed to

the finding that all three dietary treatments ended lactation with significantly

lower mean body weights than the virgin control group.

As would be anticipated, changes in body weight were dependent upon

the food intake during lactation. In this study, the only group of littermate

dams to maintain their body weights throughout lactation was the one in which

the food intake was increased by 83.4% above the NR animal's intake during

pregnancy. This is considerably higher than the mean increase of 35.7%.

Conversely, the littermates whose weights changed the most decreased the NR

food intake by 9.9% during lactation. In any case the energy requirement

for milk production was met either by catabolizing the mother's fat stores or

by increasing her food intake during lactation. Those animals with low fat

stores would not be able to subsidize milk production to the same extent as those with larger stores, and would be more dependent upon increasing their food intake during lactation.

There is a close relationship between the caloric intake during lactation and successful milk production. Those pups whose mother's caloric intake was high during lactation had the fastest growth rate, the highest fat content, and the greatest body weight at 22 days. A close inverse correlation, in successfully lactating humans, between the loss of body weight and extra energy intake, has been suggested by Whichelow (1976). She reported that although body-fat can be mobilized to subsidize milk production, the rate of mobilization is limited; milk production at any time reflects the dietary intake. Since the size of the young is proportional to the volume of milk (Mueller and Cox, 1946), it is reasonable to assume that in the rat, regardless of the diet during pregnancy, the volume of milk produced reflects the dietary intake of the mother during lactation.

When lactation performance of the three dietary treatments was evaluated by the growth, final fat content, and weaning weight of the young, there were no significant differences among treatments. There was, however, a trend towards lower body weights of the LP and LC litters at all ages. The weight of the cerebella in the LP group, which is known to be affected by undernourishment during the suckling period (Smart et al, 1974), was found to be significantly lighter than that of the NR animals. The weight of the cerebella was significantly correlated with pup weight on day 7. Thus, it could be possible that cerebellum

weight is a more sensitive indicator of dietary adequacy in early lactation than is total body weight.

Both the litter weight on day 7 and the cerebella weight were correlated with the mother's weight gain over gestation. Although not significant, the LP litter weighed 14.6% less than the NR litter at day 7. It is quite possible that during the early stage of lactation the mothers were catabolizing their body fat to subsidize milk production. During the first four days of lactation the food intake of the NR animals, and thus the LP and LC animals, was extremely low: averaging 10.3 g./day. Perhaps one explanation could be that the fall in progesterone, following parturition, is accompanied by a decrease in appetite, and under normal conditions the body has increased its fat stores sufficiently to cover the energy needs for early milk production. Those animals who were unable to increase their fat stores would thus have to increase their food intake early in lactation to avoid a fall in milk production. This is in agreement with the finding that substantial increases in nutrients offered to deficient animals immediately after parturition did not increase the milk supply quickly enough to be of full benefit to the offspring, indicating that the diet during pregnancy can affect early lactation (Thomson and Thomson, 1953).

The composition of early milk was not determined in this study and the cerebella were not analyzed for protein and DNA, to see if the decreased weight was due to a reduction in hyperplasia or hypertrophy. The hypothesis that diet during pregnancy affects early lactation remains to be fully verified. It would be interesting, in another experiment, to look at the average daily

gains over the first five days of lactation, the composition of milk on day 4, and the protein to DNA ratio of the cerebella to see if the dietary treatment during pregnancy does affect early lactation.

Lactation appears to involve an interaction between the diets during pregnancy and lactation, with a deficiency in the prenatal diet being overcome by an adequate diet during lactation, with the possibility of a transitory lag around early lactation. The findings of this study correspond with those of Broster et al (1970) who found that pre-calving and post-calving feeding contributed jointly towards the attainment of the animal's inherited yield potential. Small inadequacies in pre-partum feeding level can be offset by more generous feeding after parturition.

The possibility also exists that there may be some mechanism by which the reproducing female becomes more efficient in the utilization of nutrients during the stress situations of pregnancy and lactation. Such a mechanism has been suggested independently by Sundararaj and Pereira (1975), and by Rajalakshmi (1971). These authors reviewed the literature on the reproductive performance of poor Indian women; they found that these women survive on an apparently low plane of nutrition and yet still have a satisfactory pregnancy and excellent milk production without any noticeable changes in body weight, or in clinical or biochemical status. Perhaps those animals that have been stressed during gestation, by a low protein or a low calorie diet, develop some compensating mechanism that makes them more efficient during lactation than animals who have not been stressed.

As expected, analysis of the protein and fat composition of the dams' milk on days 7 and 14 of lactation showed no significant differences. The fat content of the milk ranged from 9.07 to 23.24 g./100 ml. with a mean of 13.86 g./100 ml. These values are slightly higher than those reported by Mueller and Cox (1946); however, they analyzed milk from the 20th day of lactation, by which time the fat content of the milk would be expected to be decreasing.

The protein content of the rat milk ranged from 7.27 to 11.82 g./100 ml., which is slightly lower than the values reported by Mueller and Cox (1946), but this variation is probably due to variations in analytical procedures. The published paper was based on values obtained using the Kjeldahl analysis. This method would measure non-protein nitrogen as well as protein nitrogen whereas the Lowry method measures only protein. Since most milks contain considerable non-protein nitrogen, this may account for the higher values previously reported.

Whereas growth is proportional to the protein intake (Miller, 1970), and the range of protein contents of the milks analyzed in this study was very small, one must assume that the wide range of growth rates among litters was mainly due to a difference in the volume of milk produced rather than the composition. The volume of milk produced would vary with the genetic potential of the animal, and with it's food intake over lactation.

In this study, only 75.0% of the animals fed a low protein diet delivered pups as opposed to 87.5% of the low calorie and 95.8% of the non-restricted dams. The fertility of the low protein mothers was similar to the value of 73.0%

reported by McLeod et al (1972); however, they reported a 93.0% and 100% fertility in low calorie and non-restricted dams respectively. These small discrepancies, from reported results, could be due in part to seasonal variations, or to the occurrence of a fire and consequent rebuilding in a laboratory across from the animal room during the mating of the rats, or to the small number of rats used.

In summary, the diet during pregnancy affects the weight gain and fat content of the mother, as well as the size of her pups at birth. In all dietary treatments, body fat, which was accumulated during pregnancy, was catabolized during the suckling period. The weight loss during lactation was proportional to the fat content of the rat's body after pregnancy.
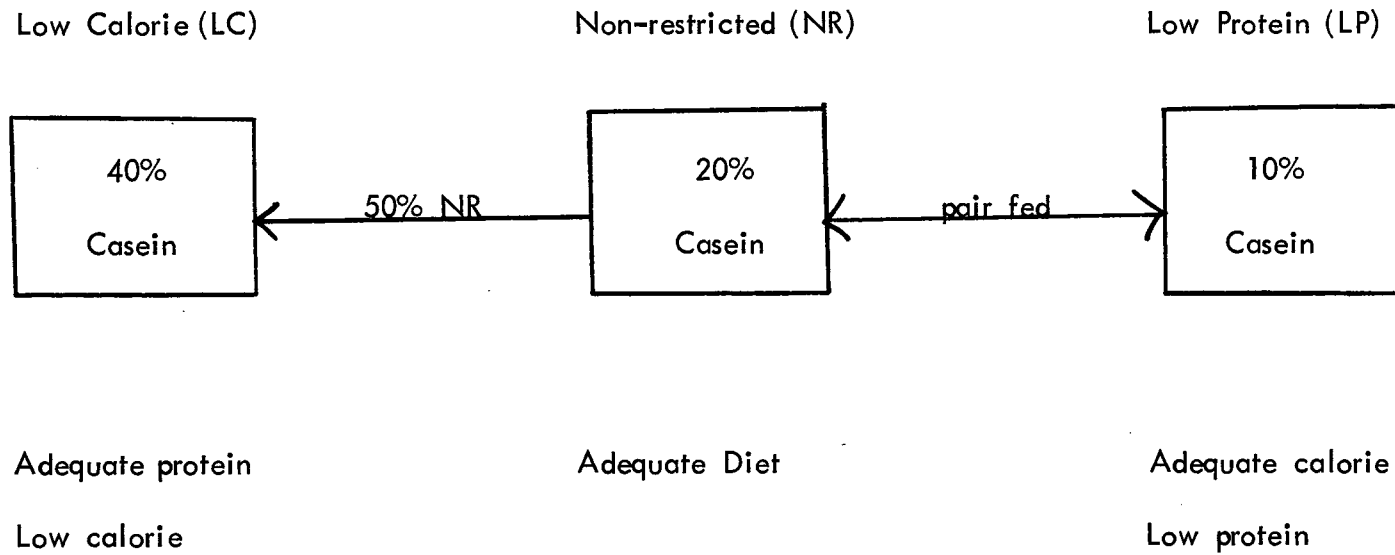
There is a synergistic effect between the diet during pregnancy and the diet during lactation on lactation performance, with a pre-partum deficiency being over come by an adequate diet after parturition; however, there is an indication of a transitory lag around early lactation. It is possible that the fat deposited during pregnancy is used to supplement early lactation.

During lactation, the diet affects the mother's weight loss and the gain of her pups by influencing the volume of milk produced. The protein and fat composition of the milk were not affected by diet during pregnancy in this study, but rather are determined by the animal's genetic potential within a normal range.

Whether or not the diet during pregnancy, through its effect on fat stores, is able to influence lactation has not been adequately determined by this study. However, this area of research definitely requires further investigation. It would be advantageous, in another study, to set the caloric level of the LC animals during pregnancy at 50% of the intake of their NR littermates, and to raise the protein content of the NR group and the LC group to 20 and 40% casein respectively. This should overcome some of the problems encountered in this study (Figure 8).

A casein intake of 25% is recommended for lactation, and the pups should be weighed daily during the first week of lactation, and at weekly intervals thereafter. If possible, it would be interesting to analyze the dams' milk much earlier in lactation; possibly at day 4. The protein to DNA ratio of the cerebella could also be determined. The above recommendations should give a much clearer indication of the efficiency of early lactation.

Figure 8.   Proposed Schematic Model for Further Study

Low Calorie (LC)   Non-restricted (NR)   Low Protein (LP)

```
+-------------+                +-------------+                +-------------+
|    40%      |  <-- 50% NR -- |    20%      |  <-- pair fed --> |    10%      |
|   Casein    |                |   Casein    |                |   Casein    |
+-------------+                +-------------+                +-------------+
```

Adequate protein   Adequate Diet   Adequate calorie

Low calorie   Low protein

LC = NR in protein

LP = NR in calories

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APPENDIX

Part One

RAW DATA - PART 1

Average daily intake (Grams)

Code	C	L.C.	N.R.	L.P.
100	16.74	16.74	19.76	19.73
101	14.43	14.43	15.29	15.26
400	14.21	14.21	16.19	16.14
110	16.40	16.40	19.23	18.33
150	15.02	15.02	17.50	17.50
300	13.33	13.33	14.45	14.50
120	14.88	14.88	16.19	15.19
Mean	15.001 ± 0.456	15.001 ± 0.456	16.944 ± 0.749	16.664 ± 0.723

Average daily caloric intake (Kcal)

Code	C	L.C.	N.R.	L.P.
100	74.16	74.16	87.54	87.54
101	63.92	63.92	67.73	67.60
400	62.95	62.95	71.72	71.50
110	72.65	72.65	85.19	81.20
150	66.56	66.56	77.53	77.53
300	59.05	59.05	64.01	64.24
120	65.92	65.92	71.72	67.29
Mean	66.46 ± 2.02	66.46 ± 2.02	75.06 ± 3.32	73.82 ± 3.20

Average daily protein intake (Grams)

Code	C	L.C.	N.R.	L.P.
100	2.51	4.19	2.96	1.58
101	2.16	3.61	2.29	1.22
400	2.13	3.55	2.43	1.29
110	2.46	4.10	2.88	1.47
150	2.25	3.75	2.63	1.40
300	2.00	3.33	2.17	1.16
120	2.23	3.72	2.43	1.22
Mean	2.249 ±0.068	3.75 ±0.114	2.541 ±0.112	1.334 ±0.058

Weight Gain over Gestation (Grams)

Code	C	L.C.	N.R.	L.P.
100	81	130	150	77
101	44	101	86	45
400	41	119	118	63
110	66	145	147	82
150	59	122	119	83
300	19	98	98	38
120	54	118	108	87
Mean	52.00 ± 7.502	119.00 ± 6.118	118.00 ± 8.984	67.857 ± 7.427

Weight of Eviscerated Carcass (Grams)

Code	C	L.C.	N.R.	L.P.
100	264.0	275.0	291.0	216.0
101	268.5	254.5	249.0	186.0
400	243.0	253.0	250.0	229.0
110	264.0	287.0	273.0	232.0
150	249.0	245.0	265.0	195.0
300	224.5	239.0	231.0	171.0
120	260.5	242.0	233.5	209.0
Mean	253.357 ± 5.908	256.5 ± 6.792	256.071 ± 8.181	205.429 ± 8.532

Fat Content of Eviscerated Carcass (Grams)

Code	C	L.C.	N.R.	L.P.
100	45.83	44.1	73.75	40.95
101	43.02	43.52	45.29	21.64
400	54.81	54.26	63.97	41.76
110	50.47	63.03	53.91	35.83
150	37.23	39.70	69.10	18.32
300	38.53	27.28	45.39	21.55
120	34.79	51.84	49.48	40.77
Mean	43.526 ± 2.769	45.819 ± 4.663	57.270 ± 4.40	31.546 ± 3.992

Number of Pups/Litter

Code	L.C.	N.R.	L.P.
100	12	11	11
101	10	5	11
400	11	14	10
110	11	11	11
150	11	8	12
300	10	12	12
120	10	13	10
Mean	10.714 ± 0.286	10.571 ± 1.172	11.000 ± 0.309

Average Weight/Pup in Grams

Code	L.C.	N.R.	L.P.
100	4.75	4.91	3.90
101	5.05	6.00	4.18
400	4.86	4.46	3.65
110	5.27	6.00	4.91
150	5.23	3.75	3.88
300	4.75	4.29	3.63
120	5.20	4.46	4.80
Mean	5.016 ±0.86	4.839 ±0.326	4.136 ±0.199

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APPENDIX

Part Two

RAW DATA - PART 2

Average Daily Intake Over Gestation (Grams)

Code	L.C.	N.R.	L.P.
130	15.00	16.95	14.54
110	15.00	17.71	14.00
180	15.00	15.92	15.52
250	15.00	17.73	17.57
200	14.04	18.38	17.71
360	15.00	18.04	17.71
300	15.00	18.04	18.11
380	14.61	19.30	17.85
Mean	14.831 ± 0.123	17.759 ± 0.352	16.626 ± 0.589

Average Daily Intake of Calories over Gestation (Kcal)

Code	L.C.	N.R.	L.P.
130	66.45	75.09	64.41
110	66.45	78.46	62.02
180	66.45	70.53	68.75
250	66.45	78.54	77.84
200	62.20	81.42	78.46
360	66.45	79.92	78.46
300	66.45	79.92	80.23
380	64.72	85.50	79.08
Mean	65.595 ± 0.544	78.673 ± 1.561	73.656 ±14.832

Average Daily Intake of Protein over Gestation (Grams)

Code	L.C.	N.R.	L.P.
130	3.75	2.54	1.45
110	3.75	2.65	1.40
180	3.75	2.38	1.55
250	3.75	2.65	1.76
200	3.51	2.75	1.71
360	3.75	2.70	1.71
300	3.75	2.70	1.81
380	3.65	2.89	1.79
Mean	3.701 ±0.033	2.657 ±0.053	1.647 ±0.056

Dam's Weight at Mating (Grams)

Code	L.C.	N.R.	L.P.
130	200	214	204
110	219	223	216
180	194	193	186
250	226	194	210
200	198	198	199
360	206	202	190
300	210	195	224
380	203	202	206
Mean	207.0± 3.85	202.6± 3.76	204.4± 4.49

Dam's Weight Gain over Gestation (Grams)

Code	L.C.	N.R.	L.P.
130	117.0	133.0	95.5
110	138.0	141.0	63.0
180	112.0	118.0	98.0
250	101.0	120.0	116.0
200	105.0	142.0	103.0
360	111.5	101.5	105.0
300	106.5	127.5	100.0
380	116.5	144.0	97.0
Mean	113.63± 4.01	129.75± 5.00	97.25± 5.39

Dam's Weight Change over Lactation (Grams)

Code	L.C.	N.R.	L.P.
130	- 49.0	- 82.0	- 39.0
110	- 35.5	- 50.5	+ 1.0
180	- 23.0	- 28.0	- 4.0
250	- 21.0	- 4.0	- 28.5
200	- 20.0	- 9.5	+ 27.0
360	+ 2.5	- 26.0	+ 12.9
300	0.0	- 3.0	- 7.0
380	- 47.0	- 51.0	- 26.0
Mean	- 24.125 ± 6.801	- 31.750 ± 9.807	- 7.937 ± 7.868

Number of Pups/Litter

Code	L.C.	N.R.	L.P.
130	10	10	11
110	14	12	10
180	8	9	10
250	11	10	13
200	11	10	11
360	11	8	13
300	13	8	8
380	14	12	9
Mean	11.5 ± 0.732	9.875 ±0.549	10.625 ± 0.625

Average Weight/Pup (Grams)

Code	L.C.	N.R.	L.P.
130	7.05	5.70	4.86
110	5.64	5.85	4.05
180	6.12	6.36	5.20
250	5.60	5.14	4.88
200	4.40	5.46	4.50
360	6.04	6.66	3.86
300	6.03	6.25	6.87
380	5.30	5.40	5.01
Mean	5.772 ±0.269	5.852 ±0.187	4.904 ±0.326

Dam's Weight after Lactation (Grams)

Code	L.C.	N.R.	L.P.
130	177.5	188.0	186.5
110	222.5	223.5	220.0
180	213.0	206.0	208.0
250	239.0	237.0	213.0
200	220.0	265.0	237.5
360	237.0	226.8	229.5
300	241.5	256.2	235.0
380	199.9	198.8	211.0
Mean	218.80± 7.75	225.16± 9.58	217.50± 5.93

Dam Carcass Weight (Grams)

Code	L.C.	N.R.	L.P.
130	164.0	147.0	164.5
110	207.8	203.8	201.8
180	193.0	189.0	191.8
250	221.0	217.9	196.5
200	203.0	248.0	218.8
360	222.0	237.0	214.5
300	223.0	209.0	211.0
380	181.0	187.4	195.7
Mean	201.850	204.887	199.325
	± 7.562	± 11.17	± 6.04

Fat Content of Dam's Carcass (Grams)

Code	L.C.	N.R.	L.P.
130	2.89	3.04	1.45
110	3.80	3.19	25.75
180	9.80	9.70	19.26
250	11.43	20.77	2.83
200	15.31	30.71	26.74
360	16.42	16.13	17.31
300	30.76	19.95	14.89
380	5.16	26.37	19.04
Mean	11.95	16.23	15.91
	± 3.23	± 3.62	± 3.32

Average Daily Intake During Lactation

Code	Diet (g)	Calories (Kcal)	Protein (g)
130	15.28	67.69	2.29
110	20.16	89.31	3.02
180	24.16	107.03	3.62
250	23.07	102.20	3.46
200	30.45	134.89	4.57
360	28.90	128.03	4.34
300	33.09	146.59	4.96
380	17.71	78.46	2.66
Mean	24.103	106.775	3.615
	± 2.23	± 9.83	±0.33

Litter's Weight on Day 7 (Grams)

Code	L.C.	N.R.	L.P.
130	100.0	100.0	90.0
110	92.0	101.5	50.0
180	101.0	92.5	88.0
250	84.0	83.5	94.5
200	113.5	107.0	84.8
360	99.5	107.5	88.0
300	111.9	121.5	118.5
380	83.0	91.0	73.0
Mean	98.1±4.02	100.6±4.18	85.9±6.84

Litter's Weight on Day 14 (Grams)

Code	L.C.	N.R.	L.P.
130	139.0	135.0	129.0
110	157.5	160.5	106.0
180	171.0	168.0	159.0
250	151.0	144.5	159.0
200	208.0	190.0	163.0
360	185.0	194.5	165.0
300	214.0	226.0	229.5
380	143.5	154.5	124.8
Mean	171.1±10.16	171.6±10.63	154.5±13.18

Litter's Weight on Day 22 (Grams)

Code	L.C.	N.R.	L.P.
130	204.5	212.0	188.5
110	257.0	258.0	209.0
180	296.0	310.0	277.0
250	278.0	247.0	267.0
200	336.0	346.0	300.0
360	292.5	315.5	289.0
300	331.0	356.0	372.0
380	199.0	228.0	202.0
Mean	274.3±18.26	284.1±19.39	263.1±21.68

Weight of Litter's Cerebellum (Grams)

Code	L.C.	N.R.	L.P.
130	1.76	1.56	1.50
110	1.64	1.82	1.24
180	1.58	1.92	1.74
250	1.65	1.70	1.67
200	1.62	1.95	1.13
360	1.50	1.49	1.30
300	1.92	1.69	1.42
380	1.36	1.34	1.19
Mean	1.629 ±0.059	1.684 ±0.075	1.399 ±0.079

Pups' Carcass Weight (Grams)

Code	L.C.	N.R.	L.P.
130	142.0	147.0	130.0
110	177.0	182.5	145.0
180	205.5	217.0	195.5
250	190.0	167.9	200.0
200	236.0	243.5	208.2
360	203.0	215.8	198.0
300	229.5	251.0	258.0
380	138.7	156.2	140.0
Mean	190.212 ± 12.810	197.612 ± 14.047	184.337 ± 15.247

Pup Carcass Fat (Grams)

Code	L.C.	N.R.	L.P.
130	8.33	8.13	7.71
110	13.74	17.52	8.62
180	17.22	19.52	20.03
250	14.56	11.79	16.69
200	21.22	23.18	18.26
360	14.75	18.79	16.06
300	21.63	21.78	25.04
380	8.04	6.44	6.71
Mean	14.94 ± 1.80	15.89 ± 2.23	14.89 ± 2.33

Average Litter Gain for First Four Days (Grams)

Code	L.C.	N.R.	L.P.
250	5.45	3.80	4.74
200	11.72	8.13	4.67
360	6.40	8.10	3.70
300	7.65	9.65	8.20
380	5.33	5.48	1.45
Mean	7.31±1.18	7.03±1.05	4.55±1.09

Daily Litter Gain Week 1 (Grams)

Code	L.C.	N.R.	L.P.
130	6.07	5.17	5.29
110	6.25	5.41	− 4.50
180	5.71	6.33	5.07
250	4.86	2.89	4.47
200	9.29	7.76	5.11
360	7.10	8.19	5.44
300	8.53	9.47	9.31
380	5.71	5.70	2.17
Mean	6.690 ± .537	6.365 ±0.729	4.045 ±1.402

Daily Litter Gain Week 2 (Grams)

Code	L.C.	N.R.	L.P.
130	5.57	5.00	5.57
110	9.36	8.43	8.00
180	10.00	10.76	10.14
250	9.57	8.71	9.21
200	13.50	11.86	11.17
360	12.21	12.43	11.00
300	14.59	14.93	15.86
380	8.64	9.07	7.40
Mean	10.430 ± 1.025	10.149 ± 1.070	9.790 ± 1.099

Lactation Gain/Litter (Grams)

Code	L.C.	N.R.	L.P.
130	147.0	143.0	135.5
110	202.5	194.4	153.5
180	235.0	255.5	220.0
250	228.0	183.7	203.8
200	287.5	293.3	251.0
360	242.7	265.8	239.1
300	278.8	300.8	318.7
380	156.0	176.9	144.2
Mean	222.187 ± 18.181	226.675 ± 20.986	208.225 ± 22.172

Protein Content of Milk on Day 7
(g/100 ml)

Code	L.C.	N.R.	L.P.
250	8.18	6.82	8.18
200	7.27	7.95	7.27
360	9.09	9.32	9.09
300	11.82	10.00	9.77
380	10.00	8.64	9.77
Mean	9.272 ±0.78	8.546 ±0.55	8.816 ±0.48

Protein Content of Milk on Day 14
(g/100 ml)

Code	L.C.	N.R.	L.P.
250	9.09	7.95	7.27
200	9.95	9.32	8.18
360	9.95	9.32	9.55
300	11.36	10.23	11.82
380	11.82	10.23	10.00
Mean	10.434 ± 0.50	9.410 ±0.42	9.364 ±0.78

Fat Content of Milk on Day 7 (g/100 ml)

Code	L.C.	N.R.	L.P.
250	10.11	15.67	17.04
200	13.24	9.92	14.09
360	16.83	12.24	10.29
300	12.19	14.91	9.07
380	14.57	11.51	11.02
Mean	13.39 ± 1.23	12.58 ± 1.07	12.30 ± 1.45

Fat Content of Milk on Day 14 (g/100 ml)

Code	L.C.	N.R.	L.P.
250	9.73	13.41	9.82
200	15.92	11.96	13.27
360	10.80	10.68	9.71
300	18.71	18.45	16.30
380	23.24	20.95	20.39
Mean	15.68 ± 2.51	15.09 ± 1.97	13.90 ± 2.03