INTRODUCTION

Undernutrition in early life of children is one of the most serious and widespread nutritional problems of the developing countries (Powell and Grantham-McGregor, 1985). The milk of underfed lactating mother is liable to be less in amount and deficient in some of its constituents compared to that of well fed mothers (Schultz, 1954; Gopalan and Belavady, 1961; Armstrong and Prescott, 1971; Elsley, 1971; Lewis, 1971; Gunther, 1976; Rasmussen and Warmen, 1983; Roberts et al., 1985; Vis, 1986; Fischbeck and Rasmussen, 1987; Briand et al., 1988; Reissland and Burghart, 1988; Kigutha et al., 1989; Koski et al., 1990; O'Connor et al., 1990). This results in some degree of nutritional deficiency and even mortality of the youngs at a very early age (Schultz, 1954; Lechtig, 1980, 1985; Bairagi et al., 1985; Boss et al., 1987; Koskiet al., 1990).

It is well established that undernutrition causes deficits in both body and organ weight (McCance and Widdowson, 1962; Hsuch et al., 1967, 1974; Srivastava et al., 1974; Scharer, 1977; Whatson et al., 1978; Goswami and Srivastava, 1978; Stephens, 1980; Rasmussen and Warmen, 1983; Hallak and Noman, 1988; Smart et al., 1987, 1989; Beck et al., 1989; Dureuil et al., 1989; Firmansyah et al., 1989b). Also, due to undernutrition, changes in body composition and metabolic processes will occur (Widdowson et al., 1960; Keys and Grande, 1973; Widdowson, 1976;
Aubert et al., 1980; Heymsfield et al., 1982; Spurr et al., 1986; Testar et al., 1988; De Luise and Harker, 1989; Tobin and Beard, 1990; Levin et al., 1990; Wittwer et al., 1991.

If neonatal undernutrition occurs for a short time then an adequate feeding is initiated early enough before the end of the suckling period, these deficits can be reversed (Winick, 1968; Winick et al., 1968) but if undernutrition continues throughout the suckling period, these deficits will be permanent and can not be reversed even with subsequent rehabilitation (Winick and Noble, 1966; Winick, 1968; Blackwell et al., 1969; Ahmed et al., 1987; Keller et al., 1990). Undernutrition affects the organs and the tissues of body differently according to their stage of maturation when the animal begins to be undernourished. Muscles mature somewhat later than some other organs like brain and skeleton, so muscles are more severely affected by undernutrition than these organs which mature early (Widdowson, 1977; Eberhardt and Halls, 1987; Cornblath and Brown, 1988; Firmansyah et al., 1989a).

Also, the effects of undernutrition on muscles is age dependent where the muscles of growing animals are more susceptible than are mature animals (Goodman et al., 1981; Kelsen, 1986). According to Dickerson and McAnulty (1975) individual muscles also mature at different rates and muscles which mature late tend to be affected more, due
Skeletal muscle is the largest single tissue in the body. The muscle tissue alone comprises 40% of the total body weight in a healthy adult man (Behnke and Wilmore, 1974) and 45% in an adult normal rat (Jackson and Lowry, 1912; Young, 1970). Moreover, skeletal muscles represent more than two-thirds of metabolically active protoplasm (Cheek et al., 1971). Muscle is primarily water (around 70%) but, protein which constitutes most of non-aqueous portion (around 22% of muscle weight) has been an important focus of the nutritionists (Heymsfield et al., 1982). The contribution of skeletal muscle to total protein content of the body varies from 20% at birth to about 45% in the adult rat (Young, 1970) and from 25% - 40% in human from infancy to maturity (Cheek et al., 1971). Protein metabolism in skeletal muscle is in a constant state of flux because of changes in the synthesis and breakdown of proteins, uptake and release of amino acids; and oxidation of amino acids (Elia et al., 1989). Since the major protein reserve of the body is found in skeletal muscle, the mobilization of amino acids from muscle protein represents an essential adaptation to food restriction (Goldberg and Chang, 1978; Zlotkin, 1986; Young and Marchini, 1990).
In man, skeletal muscle protein is the major non-fat energy store and losses in response to undernutrition can be very extensive particularly in adults. In small animals such as the rat, since the skin and visceral organs also serve as reserve energy store, losses from skeletal muscles are usually only observed as a result of prolonged total starvation or chronic dietary restriction (Millard and Waterlow, 1978; Goodman et al., 1981; Smith, 1986). This great importance of skeletal muscle to the body has drawn the attention of investigators to study the effect of undernutrition on muscle development (Elliott and Cheek, 1968; Cheek et al., 1969; Howarth and Baldwin, 1971; Goswami et al., 1974; Dickerson and Moenulty, 1975; Goldberg and Goldspink, 1975; Williams and Hughes, 1978; Miller et al., 1985; Srivastava, 1985; Glore and Layman, 1983a, b, 1987; Lewis and Belman, 1988; Bruce et al., 1989; De Luise and Harker, 1989). During undernutrition muscle mass is more severely and disproportionately reduced than the reduction in total body weight alone would indicate (Kerpe-Fronius and Frank, 1949; Winick and Brasel, 1973). The ability of skeletal muscle to grow is restricted by undernutrition (Elliott and Cheek, 1968; Williams and Hughes, 1978; Srivastava, 1985; Glore and Layman, 1983a, b and 1987) and if the muscle is growing rapidly at the onset of undernutrition it will be very retarded in relation to age (Winick and Noble, 1966; Glore and Layman, 1983b).
Undernutrition reduced DNA content in rats skeletal muscle compared with age matched control (Srivastava, 1985; Glore and Layman, 1987). In experiments with rats, undernutrition reduced the rates of both DNA and Protein accumulation (Howarth and Baldwin, 1971; Goswami et al., 1974). However, food restriction has a more severe effect on muscle DNA accumulation than protein accumulation (Elliott and Cheek, 1968). Also, protein/DNA ratio has decreased in skeletal muscle of undernourished rats indicating a loss of muscle mass (Howarth and Baldwin, 1971; Glore and Layman, 1983a; Srivastava, 1985). The protein content of skeletal muscle is determined by the balance between protein synthesis and degradation (Howarth, 1972).

In general, food restriction reduces muscle protein synthesis and markedly increases protein degradation (Millward et al., 1975; Millward and Waterlow, 1978; Millward et al., 1980, 1986). Subsequently, protein content of skeletal muscle has been reduced due to undernutrition (Winick et al., 1968; Howarth and Baldwin, 1971; Goswami et al., 1974; Dickerson and McAnulty, 1975; Srivastava, 1985; Glore and Layman, 1983a, b, 1987). However food restriction results in reduction of RNA content in skeletal muscle of underfed rats (Winick and Noble, 1966; Howarth and Baldwin, 1971; Goswami et al., 1974; Millward et al., 1974; Dickerson and McAnulty, 1975; Glore and Layman, 1983a, b; Srivastava, 1985). Dietary regimes which depress protein synthesis also lead to a fall in the RNA
content (Young and Alexis, 1968; Young et al., 1971; Howarth, 1972; Sin-Chi Chang et al. 1990). Millward et al. (1973) studied the relationship between muscle RNA content and the rate of protein synthesis. They found that reduction in protein synthesis in underfed rats is a consequence of a decreased RNA content. Howarth and Baldwin (1971) have demonstrated that food restriction inhibited RNA accumulation and RNA content per muscle decreased. The capacity of protein synthesis (RNA/protein ratio) is reduced in muscles of underfed animals (Millward et al., 1973, 1974, 1975; Millward and Waterlow, 1979). Also the protein synthetic capacity per nucleus (RNA/DNA ratio) showed a decrease in muscles of food restricted rats (Goswami et al., 1974; Glore and Layman, 1983a,b).

The influence of nutrition on the size of muscle fibres has long been recognised (Rowe, 1968). Various workers using many different animals have reported that starvation or maintenance on a low level of nutrition results in a decrease of the mean fiber diameter of the animal musculature (Robertson and Baker, 1933; Joubert, 1956; Hagan and Scow, 1957; Bogart et al., 1962; Montgomery, 1962; Staun, 1963; Goldspink, 1964, 1965; Harriman, 1965; Rowe, 1968; Sachdev et al., 1971; Dastur et al., 1975; Goldspink and Ward, 1979; Layman et al., 1981; Bedi et al., 1982; Lewis et al., 1985, 1986; Oldfors and Sourander, 1986; Sieck et al., 1989). Also,
there has been investigation on effects of early under-nutrition on muscle development and the evidence from preliminary studies is not just of deficit in muscle weight (William and Hughes, 1978; Layman et al., 1981) but of lasting alteration in the relative proportions of muscle fibre types (Bedi et al., 1982). According to Bedi et al., (1978), the previously undernourished rats (during gestation period) had deficits in extensor digitorum Longus (EDL) muscle weight which was attributed, in part, as a function of both a lower total number of fibers and the smaller cross sectional areas of their white and intermediate fibers. Similarly, the muscles from rats which had been undernourished during the gestation and suckling period undergo irreversible histological changes (Haltia et al., 1978; Howells et al., 1978; Bedi et al., 1982). Also, nutritional deprivation leads to a preponderance of small diameter fibers (Goldspink, 1965) and a reduction in the cross-sectional area in diaphragm muscle fibers of nutritionally deprived rats (Lewis et al., 1985).

Reports on the effects of malnutrition on chromosom al damage have been controversial (Sadasivan and Raghuram, 1971). While Armendares et al. (1971) found that chromosomal aberrations were six times commoner in malnourished children than in normal ones, Thorburn et al. (1972) and Khouri and McLaren (1973) reported that malnutrition per se may not influence chromosomal abnormalities. Even in the
data of Armendares et al. (1971) it was difficult to assess the extent to which malnutrition per se had contributed to the increased incidence of chromosomal anomalies, since environmental factors such as radiation, chemical agents, and viral infections are also known to produce chromosomal aberrations. However, recent studies suggest that malnutrition may also produce chromosomal damage. In advanced protein calorie malnutrition cell division would fail and chromosomes would become structurally abnormal either spontaneously or as an exaggerated response to environmental factors such as radiation, chemical agents or viral infection (Evans, 1970; Krause, 1988).

Murthy et al. (1982) showed that cell cycle duration of lymphocytes was prolonged in cultures from children suffering from kwashiorker. Also, Ortiz and Betancourt (1984) reported that malnutrition during the lactation period produced a prolongation in the time of proliferation of bone-marrow cells in the rat. However, there are few reports on the chromosomal aberrations due to malnutrition in rats and humans. In rats, the incidence of chromosomal abnormalities was higher in the embryos whose mothers had low-protein diet compared to controls (Murthy, 1984) and also in the progeny of dams fed protein-deficient diet during gestation and lactation (Sadasivan and Raghuram, 1973). Similar results have been observed in weaned rats fed a low-protein diet for 8 to 12 weeks.
(Vijayalakshmi, 1975). In humans the lymphocytes of severely malnourished children showed an increased number of chromosome abnormalities as compared to control (Armendares et al., 1971; Khouri and McLaran, 1973; Betancourt et al., 1974; Upadhyaya et al., 1975). Similar results have been observed in the study of bone marrow chromosome preparations from children suffering from protein calorie malnutrition (Tolani et al., 1978). However, the mechanism by which protein energy malnutrition can induce structural chromosomal aberrations is not clear (Murthy, 1984). It has been shown that protein calorie undernutrition before weaning in the rat causes a decrease in cellular DNA content and mitosis in rat tissues (Winick and Nobel, 1966). Defective mitosis has been implied in the etiology of structural aberrations seen in malnourished children and animals. According to Murthy (1984) the biologic significance of elevated fetal chromosomal damage seen in malnourished pregnant rats is difficult to speculate. He reported that pups born to protein restricted rats exhibited an elevated spontaneous chromosome aberrations and a decreased birth weight compared with those born to well fed animals. Also, his results suggested that severe protein energy malnutrition during pregnancy may affect the chromosomes of the fetus. However, the study of chromosome aberration in bone-marrow cells is preferable than in blood lymphocytes for two reasons (Armendares et al., 1971). First
the persistence of chromosome abnormalities in lymphocytes may reflect damage incurred in the past, for the life of a lymphocyte may be as long as 1500 days (Evans, 1970), while bone marrow proliferates rapidly and it should reflect events currently in progress. Second, megaloblastic anemia may be associated with structural chromosome damage (Kiossoglou et al., 1965; Matsaniotes et al., 1968).

The effect of meternal nutritional deficiency on rat pups has been studied at length (Schrader and Zeman, 1973; Hastings-Roberts and Zeman, 1979; Zeman, 1984; Srivastava, 1985; Gloré and Layman, 1987; Smart et al., 1987, 1989). Usually defects imposed were severe (Nelson and Evans, 1951; Warren and Bedi, 1985) and relatively short in duration often applied during gestation itself (Rasmussen and Warman, 1983; Fimansyah et al., 1989b). However, the long term effects of food restriction depend upon its severity and duration, and most important, upon the stage of development (Winick et al., 1968). One of the critical stages of development comes early in life before birth in species that are born relatively mature such as the pig and human baby or immediately after birth in others that are much less mature when they are born, e.g. the rat (Widdowson, 1977).
In the present study, rat was used as an animal model to study the effect of undernutrition imposed from birth to 30 days age combining the effect of a decrease in maternal diet and an increase in the litter size. An attempt has been made to study its effects on pup growth, total body weight and muscle weight. It is well established that the nucleic acids and protein content are the parameters which monitor the cell growth in different tissues (Winick and Nobel, 1965, 1966; Srivastava, 1985). Moreover, neonatal undernutrition has severe impact on the growth of different organs in rats (Goswami et al., 1974; Srivastava, 1985). As far as can be ascertained few specific studies had been carried out to study the effect of undernutrition on the growth of different types of muscle fibers during lactation period. Therefore, a detailed study on the effect of undernutrition on muscle growth during this critical period has been undertaken. DNA, RNA and protein content in four skeletal muscles (soleus, diaphragm, gastrocnemius and extensor digitorum longus) ranging from predominantly slow to fast twitch in character was determined.

However, relatively few quantitative histological studies have been carried out on skeletal muscle from rats previously undernourished during the early stages of life, although there is a considerable literature on the muscles of animals undernourished during adult life.
Also, according to Goldspink and Ward (1979) although a considerable amount of work has been carried out on the effect of starvation on muscle fiber size in slow and fast-twitch muscles virtually little attention has been paid to the response of the different types of fibers within mixed muscles. Thus, in addition to studying the effects of neonatal undernutrition on postnatal growth in the fast-twitch EDL and slow twitch soleus muscle this effect on the intermediate gastrocnemius and diaphragm muscles was also examined. In the present study, an attempt has been made to determine the effect of undernutrition on the mitotic index of bone marrow cells. It has been carried out in vivo from rats in which genetic and environmental variables have been controlled.