DISCUSSION
EFFECTS OF UNDERNUTRITION

Skeletal muscle:

It is known that undernutrition of shorter duration leads to the reduction in the body weight and of longer duration leads to a reduction in the height of the individual also (Vijayaraghavan, 1987). In the present study, the body weight and crown-rump length of UN rats were significantly reduced to 50.6% and 86.7% of C-UN rats respectively thus indicating that the UN rats were undernourished chronically. The muscle weights were also significantly lower in UN rats. The weight of soleus in UN rats was 57.1% of that in C-UN rats. Similarly, the weight of e.d.l. in UN rats was 59.6% of that of in C-UN rats.

The present data on the contractile functions of skeletal muscles showed significant alterations in the twitch and tetanic forces and ½ RT in UN rats. The other contractile parameters like MRR and ET were not altered in UN rats.

Twitch and tetanic forces in skeletal muscles:

The UN rats showed lower twitch force, amounting to 67.0% of the twitch force of C-UN rats in soleus and to 55.8% of C-UN rats in the case of e.d.l. This significant difference
In twitch force between UN and C-UN rats was not present when the twitch force was expressed as g/g muscle weight (Table 2) indicating that the lower muscle weights seen in UN rats may be responsible for the lower twitch forces. Similar extent of reduction in the muscle weight of diaphragm-strip due to food deprivation is reported to cause reduction in the twitch force to the extent of 50% (Lewis et al., 1986). Heymsfield et al. (1982) found decreased myofibre mass, increased connective tissue and an enlarged extracellular space in undernutrition. The wasting of skeletal muscles associated with undernutrition has been reported to be due to a reduction in the size of the muscle fibre (Heymsfield et al., 1982) and not due to decrease in the number of muscle fibres (Goldspink and Ward, 1979; Parsons et al., 1982). This is in agreement with our histological findings of a decrease in the diameter of muscle fibres of soleus and e.d.l. without a significant change in the number of the fibres in UN rats.

The tetanic force values of soleus and e.d.l. were significantly lower in UN rats at various stimulation frequencies. When expressed as g/g muscle weight the tetanic forces of UN and C-UN rats were equal (Table 2). Thus the reduction in the tetanic forces of UN rats can be attributed to lower muscle weights of UN animals. Earlier studies of short term undernutrition have shown that the muscle weight is reduced due to a greater atrophy of type II fibres than type I fibres.
(Kelsen et al, 1985; Lewis et al, 1986). This differential atrophy is associated with a shift to left of the frequency-force curve (or pattern) where the force at each frequency is expressed as percentage of the maximum tetanic force (Krishnamurthy et al, 1992c; Lopes et al, 1982; Russell et al, 1984). In the present study, there was no such shift in the frequency-force pattern of soleus (Fig.28; ANOVA: $F(1,248) = 0.815$, $P = 0.368$) as well as e.d.l. (Fig.29; ANOVA: $F(1,216) = 0.134$, $P = 0.715$). The reduction in tetanic forces without any shift of the frequency-force pattern and coupled with a reduction in the diameter of muscle fibres suggests that type I as well as type II fibres in both soleus and e.d.l. have undergone an equal amount of reduction in size. Since Kelsen et al (1985) and Lewis et al (1986) have found that undernutrition of short duration produces a greater atrophy of type II fibres, it is likely that undernutrition of longer duration produces equal atrophy of type I and type II fibres as found in the present study. The breakdown of actomyosin induced by food deprivation may be the actual cause of reduced force in the skeletal muscles of UN rats (Kadowaki et al, 1989; Li and Wassner, 1984; Preedy and Sugden, 1989).

**Half relaxation time:**

In UN rats, $\frac{1}{2}$ RT of soleus was significantly prolonged and in e.d.l. it remained unaltered. Though the mechanism
of muscle relaxation is not fully understood, calcium reuptake by calcium pump into the sarcoplasmic reticulum is the essential step which is rate limiting (Dawson et al., 1980) and requiring ATP. It has been shown that slowing of muscle relaxation during twitch is essentially related to a decrease in intracellular pH (Sahlin et al., 1981; Sahlin et al., 1987) rather than energy content (Edwards et al., 1975). However, recently Geers et al. (1991) have shown that the decreased intracellular pH may not be responsible for the prolonged \(\frac{1}{2}\) RT, where they have demonstrated that experimental metabolic and respiratory acidosis have not altered \(\frac{1}{2}\) RT. Muscle relaxation also depends on the type of muscle fibres (Stephenson and Williams, 1981). Thus, prolongation of \(\frac{1}{2}\) RT may happen wherever there is transformation of muscle fibres, namely from type II to type I fibres or whenever the number of type I fibres in a given muscle becomes greater than that of type II fibres.

The histochemical studies, the twitch/tetanus ratio and the frequency-force pattern give the information about the types of fibres in the muscle. In the present study, the twitch/tetanus ratio essentially remained the same in soleus of UN rats (UN: 0.17 ± 0.01; C-UN: 0.17 ± 0.02). The frequency-force pattern also did not show any shift (Fig.28) indicating that the proportion of type I and type II fibres has not changed in prolonged undernutrition in the present study. Thus, it is
likely that ATP deficiency or decreased intracellular pH or transformation of muscle fibres is not the probable cause for the prolonged ¼ RT in soleus of UN rats. Such a prolongation of ¼ RT was not seen in e.d.l. This may be due to a greater amount of sarcoplasmic reticulum (Flehn and Peter, 1971; Luff and Atwood, 1971) and/or faster rate of calcium uptake (Flehn and Peter, 1971; Sreter, 1970) in type II fibres.

Recently, it has been clarified that hypocaloric feeding for a short duration of one week, reduces the relaxation rate after twitch and after tetanic contraction in both soleus and e.d.l. (Nishlo and Jeejeebhoy, 1991). These authors concluded that (i) slowing of skeletal muscle relaxation after twitch and after tetanic contraction occurs after 1 week of hypocaloric feeding, (ii) slowing is independent of muscle fibre composition, (iii) there may be alterations in the calcium pump of sarcoplasmic reticulum due to undernutrition.

**Contraction time, maximum relaxation rate and endurance time:**

The other parameters like CT, MRR and ET were not significantly altered in soleus and e.d.l. of UN rats. As in the case of earlier studies (Lewis et al, 1986; Russell et al, 1984) in the present study also CT was not significantly altered. MRR gets decreased due to undernutrition (Lopes et al, 1982; Russell et al, 1984). Earlier workers have studied MRR by
using electrical stimulation of different frequencies in the range of 20-150 Hz (Brough et al, 1986; Lopes et al, 1982; Nishio and Jeejeebhoy, 1991; Russell et al, 1984). In the present study a lower (20 Hz) and a higher frequency (100 Hz) of the range were randomly chosen. It was observed that the MRR values were similar at both frequencies in a given muscle. This finding is in agreement with the earlier observations of Wiles et al (1979) who have demonstrated similar values of MRR at forces of 10% and 30% of maximum voluntary contraction, obtained by electrical stimulation.

As in the case of ½ RT, the lower values of MRR is likely due to decreased availability of ATP for the calcium sequestration (Edwards et al, 1975) and a greater proportion of type I fibres than the type II fibres in the muscle (Stephenson and Williams, 1981). In the present study, the deficiency of ATP for muscle relaxation is not indicated, because of similar ET values in UN and C-UN rats where the muscles were contracting for longer time, longer than required for MRR. Also in the present study, there was no shift of the frequency-force pattern of soleus and e.d.l. (Figs. 28, 29). The twitch/tetanus ratio of soleus remained unaltered (UN: 0.17 ± 0.01; C-UN: 0.17 ± 0.02). Similarly, the twitch/tetanus ratio of e.d.l. also remained unaltered (UN: 0.26 ± 0.02; C-UN: 0.26 ± 0.03). The unaltered frequency-force patterns and the
twitch/tetanus ratios of soleus and e.d.l. indicate that there is no change in the proportion of type I and type II fibres in the muscles of UN rats. This explains the unaltered values of MRR seen in the UN rats.

The ET of soleus and e.d.l. in the present study showed no significant changes at both 20 Hz and 100 Hz. Earlier studies have reported varying results regarding ET. Lewis et al. (1986) showed a greater endurance time of undernourished diaphragm strip in vitro. Lopes et al. (1982) showed greater fatiguability (i.e. decreased endurance time) in undernourished patients. The ET at 20 Hz is more than at 100 Hz. This can be explained by the fact that at 100 Hz, there is (i) high-frequency-induced failure of neuromuscular transmission, (ii) slowing of the muscle action potential waveform, (iii) increase in the excitation threshold of the muscle and (iv) involvement of anaerobic glycolytic energy pathway which provides limited energy supply to the contracting muscle (Barclay and Loiselle, 1992; Jones, 1981).

The biochemical factors associated with skeletal muscle fatigue include hydrogen ions (Fabiato and Fabiato, 1978), ATP, CrP (Karlsson and Saltin, 1970), blood glucose (Pruett, 1970) and glycogen (Guyton, 1991). When the muscle is contracting isometrically, its blood flow is almost absent (Barcroft, 1963;
Hussain and Magder, 1991) and the energy required for such contractions has to be from the locally available ATP, CrP and glycogen stores. For isometric contractions of brief durations lasting less than 10 s the immediate source of energy is ATP and CrP present in the contracting muscle. For intense isometric contractions of longer duration, the locally available muscle glycogen also contributes to the energy requirement. The observation that the endurance time was similar in UN and C-UN rats, probably indicates that the energy supply and hydrogen ions level in the skeletal muscles are not much altered in the present type of undernutrition.

Finally, the failure of motor nerves and neuromuscular transmission could be thought of as responsible for the changes observed in the contractile functions in UN rats. Such a failure of neuromuscular transmission has been shown to cause high-frequency fatigue (Edwards, 1981; Feldmen et al, 1991). In the present study, similar tetanic forces (expressed for 1 g muscles) were observed at all the stimulation frequencies in UN and C-UN rats both in the case of soleus as well as e.d.l. Thus, it is very likely that the nerves and the neuromuscular transmission have not been affected by the type of undernutrition involved in the present study. Besides, Boreham et al (1988) in a similar chronic semistarvation study have observed that there are no morphological
changes suggestive of denervation and reinnervation in soleus and e.d.l. of rats.

Smooth muscle:

The UN rats showed lower weight of the colon whereas its length remained similar, as compared to C-UN rats. Firmanayeh et al (1989) reported that colonic weight as well as colonic length were lower in rats after pre and postnatal undernutrition. Lack of nutrition and/or bulk may be responsible for the changes seen in colon of UN rats. Luminal nutrients contribute to 70% of energy substrates for the mucosa (Roediger, 1986) and deprivation of these substrates alters cell growth in the colon (Stragand and Hagemann, 1977a; Stragand and Hagemann, 1977b). Lack of bulk also causes reduced cellular proliferation (Morin et al, 1980; Ryan et al, 1979).

The contraction-pressures of colon by electrical field stimulation were lower in UN rats. These lower contraction-pressures may be due to lower colonic muscle weight of UN rats because when the contraction-pressures are expressed for 1 g colon these differences in contraction-pressures are nullified. The other possibility is that the release of neurotransmitter acetylcholine in the colon may be reduced in UN rats as suggested earlier (Anderson, 1981). Such possibility is unlikely because in the present study the colon of UN rats has produced
similar contraction-pressures expressed for 1 g colon at all frequencies, as compared to those in C-UN rats (Table 2). Venkataraman et al (1983) demonstrated that there was 2-3 fold increase in the sensitivity of the intestine of 21 days semi-starved rats to acetylcholine to compensate the possible deficient release of the transmitter. The contraction-pressure to topically applied acetylcholine in the present study was similar in both groups indicating again that there might not have been any reduction in the transmitter-release in the colon during electrical field stimulation. Thus, in prolonged undernutrition as in the present study, there may be some adaptive mechanisms for maintaining normal release of acetylcholine in UN rats.

EFFECTS OF 30 DAYS REHABILITATION

Skeletal muscle:

30 R rats showed similar body weight and crown-rump length as compared to C-30 R. Ad lib feeding for 30 days has rectified the functional deficiencies induced by undernutrition. The muscle weights of soleus and e.d.l. of 30 R rats in the present study were almost similar to those in C-30 R. Refeeding after starvation causes immediate increase in protein synthesis in skeletal muscles (Magnusson et al, 1990). Refeeding also increases the glucose utilization in skeletal muscles within two hours (Sugden et al, 1990).
The twitch force, CT, 1/4 RT, tetanic forces at all the stimulation frequencies, maximum tetanic force, MRR and ET of 30 R rats were similar to those of c-30 R rats. However, the tetanic forces of 30 R rats showed a tendency to be consistently lower as compared to C-30 R rats which may be due to the insignificant lower muscle weight of soleus in 30 R rats because the tetanic forces for 1 g muscle are similar in 30 R and C-30 R rats (Table 4).

The muscle fibre diameter has been reported to be significantly lower in 20 days rehabilitated rats (Gopinath et al, 1983). This may explain the decrease in muscle weight and tetanic forces seen in 30 R rats. Since the frequency-force patterns of soleus and e.d.l. of 30 R rats are similar to those of C-30 R rats (Soleus: Fig.30, ANOVA, $F_{(1,144)} = 3.311$, $P = 0.071$; e.d.l.: Fig.31, ANOVA, $F_{(1,136)} = 3.310$, $P = 0.071$) recovery to equal extent in type I and type II fibres is indicated in both the muscles.

Smooth muscle:

In colon, the contraction-pressures by electrical field stimulation of 30 R rats were similar to those of C-30 R rats. However, the pressure at all the frequencies in 30 R rats showed a tendency to be consistently lower than those of C-30 R rats. The weight of the colonic segment was similar in both
groups. The response to acetylcholine was also similar in both
groups. Thus the consistent lower contraction-pressures in 30 R
rats may be due to the small but negligible difference in the
weights of the colonic segment of 30 R rats.

**EFFECTS OF 60 DAYS REHABILITATION**

**Skeletal muscle:**

60 R rats showed similar body weight and crown-rump
length as compared to C-60 R. *Ad lib* feeding of the under-
nourished rats for 60 days showed improved results in the
tetanic forces at all frequencies, contractile functions. The twitch force, CT, 1/2 RT, 1/2 maximum
tetanic force, MRR and ET of 60 R rats were similar to those
of C-60 R rats. However, the tetanic forces of e.d.l. in 60 R
rats still showed a tendency for lower values. Such a tendency
for lower forces may be due to the still existing deficiency
in the muscle weight of 60 R rats because the tetanic forces
for 1 g muscle are found to be similar in 60 R and C-60 R
(Table 6). The frequency-force patterns of soleus and e.d.l.
of 60 R are similar to those in C-60 R (Soleus: Fig.32, ANOVA,
$F_{(1,125)} = 0.578, P = 0.449$; e.d.l.: Fig.33, ANOVA, $F_{(1,104)} = 0.184, P = 0.669$) suggesting an equal extent of further recovery
in type I and type II fibres of soleus and e.d.l.
Smooth muscle:

The colonic functions of 60 R rats were similar to C-60 R rats indicating full recovery of colonic contractile response. Such possibility for the complete recovery of structure and function has been reported in jejunum and ileum of rats (Buts et al, 1990).

EFFECTS OF UNDERNUTRITION PLUS SWIMMING

As it was felt more appropriate to discuss the comparison of UNS vs C-UN which would bring out the combined effects of undernutrition and swimming on muscle contractile functions, subsequent discussion will be limited to comparison of UNS vs C-UN.

The body weight and the crown-rump length of UNS rats were significantly lower as compared to C-UN rats. The body weight of UNS rats was 52.0% of C-UN rats. The crown-rump length was 86.7% of C-UN rats. The muscle weights were also significantly lower in UNS rats. The weight of soleus in UNS rats was 56.1% of that in C-UN rats. Similarly, the weight of e.d.1. in UNS rats was 48.2% of that in C-UN rats. When these changes in body weight, crown-rump length and the muscle weights of UNS are compared with those seen in UN rats it is obvious that the superimposed forced physical activity, namely
swimming has not significantly modified the effects of undernutrition.

**Skeletal muscle:**

It has been demonstrated by several workers that exercise or functional overload spares skeletal muscle atrophy (Czerwinski et al, 1987; Hickson et al, 1984; Hickson et al, 1986). Further, it has been shown that exercise reduces the breakdown of myosin heavy chain (Czerwinski et al, 1989) and endurance training does not increase myofibrillar content or concentration (Booth et al, 1985). The protective effects of exercise are seen in the contractile functions of soleus, e.d.l. and colon in the present study also. Similar beneficial effects of exercise have been shown during intermittent fasting (Sakamoto and Grunewald, 1987) in the contractile functions. Treadmill exercise in fed as well as starved rats substantially increases the insulin induced stimulation of glucose utilization by the hind quarter and alpha amino isobutyric acid transport into red muscles (Zorzano et al, 1986). It is also seen that there is a marked increase in plasma epinephrine level in fasted exercising rats stimulating glycogenolysis and maintaining the normal plasma glucose level (Winder et al, 1985). Similarly, Favier and Koubi (1988) observed that swimming (2 h) exercise caused hypoglycemia in control rats and normoglycemia in 10 weeks chronically (intermittent) fasted rats.
The CT and ½ RT of soleus and e.d.l. of UNS rats were significantly prolonged as compared to those in C-UN rats. This can be explained on the basis of transformation of type II fibres to type I fibres. Histochemical studies have shown that normal soleus and e.d.l. contain about 15% and 97% type II fibres respectively (Armstrong and Phelps, 1984). The prolongation of CT and ½ RT suggests that these type II fibres might have become type I fibres in the present study due to swimming as reported earlier (Saltin et al., 1976). The shift to left of the frequency-force pattern of soleus in the present study (see below) indicates such transformation of muscle fibres to be occurring in UNS rats.

The twitch and the maximum tetanic forces of soleus and e.d.l. in UNS rats were not significantly different as compared to those of soleus and e.d.l. in C-UN rats. Similarly, MRR and ET of UNS rats were not significantly different as compared to those of C-UN. The tetanic forces of soleus and e.d.l. at various stimulation frequencies in UNS rats were similar to those in C-UN rats. The apparent lower values of tetanic forces in UNS rats may be due to lower muscle weights in them because the tetanic forces for 1 g muscle are similar (even more in the case of soleus) in UNS and C-UN (Table 8). When the tetanic forces are normalised and expressed as percentage of maximum tetanic force of each animal it is observed that there is a significant shift
to left in soleus but not in e.d.l. (Soleus: Fig.34, ANOVA, $F(1,176) = 18.220, P < 0.001$; e.d.l.: Fig.35, ANOVA, $F(1,152) = 0.420, P = 0.518$). This indicates a transformation of type II fibres to type I fibres in soleus. But histologically, there was no reduction in the diameter of the muscle fibres in soleus and e.d.l. The undernutrition alone caused a reduction in the diameter of the muscle fibres in the present study as well as in the earlier studies (Lewis et al., 1986; Lewis and Sieck, 1992). Exercise is reported to prevent the atrophy of muscle fibres (Czerwinski et al., 1989). Moreover, endurance exercise increases the cross-sectional area of type II fibres (Saltin et al., 1976). When exercise was combined with undernutrition in the present study, the diameter of muscle fibres was not altered. Thus, undernutrition plus swimming might have caused transformation of type II fibres to type I fibres without significant reduction in the diameter of the fibres. Histochemical studies are thus warranted to clarify and confirm the actual changes in the muscle fibres.

Smooth muscle:

The combination of undernutrition and swimming has reduced the length and weight of colon in UNS rats as compared to C-UN rats. Histologically, the muscle layer thickness was significantly more in UNS as compared to C-UN. However, when the actual weight of the muscle layer per cm was calculated (utilising the percentage of muscle layer with respect to the total
colon wall thickness; UNS: 38.7%; C-UN: 41.1%) the weight of the muscle layer in UNS rats was 78.6% that of C-UN (UNS: 11.4; C-UN: 14.4 mg). Such reduction in colonic length and colonic weight has been reported in rats subjected to severe undernutrition (Firmansyah et al., 1989). Despite such structural reduction in the colon, the contraction-pressures by electrical field stimulation were similar in UNS as compared to C-UN rats. Even the apparent lower values seen in the contraction-pressures of UNS rats were nullified when the contraction-pressures were expressed for 1 g colon, indicating that the lower (though not significantly so) colon weight may be responsible for the lower contraction-pressures in UNS rats. The contraction-pressure to acetylcholine in UNS was similar to that seen in C-UN rats indicating that the sensitivity of colon for acetylcholine was not altered in UNS rats. Similar unaltered acetylcholine sensitivity was also observed in UN rats in the present study.

**OTHER INTERESTING COMPARISONS**

The following comparisons are also available though they are not directly related to the aims of the present study:

**Comparison of C-UN vs C-UNS:**

In both soleus and e.d.l. all the parameters of muscle contractile function were similar in these two groups except the CT of soleus in C-UNS which was significantly longer than that in C-UN.
In colon of C-UNS the contraction-pressures due to electrical field stimulation and exogenous acetylcholine were lower as compared to those in C-UN. The stress component of swimming (Tierney et al, 1991) may be causing the release of various hormones like norepinephrine and endogenous opioids (Tierney et al, 1991) which are known to inhibit intestinal motility (Kromer, 1988).

Thus, in control rats, swimming slowed soleus and profoundly decreased colonic contractions.

Comparison of UN vs UNS:

All the contractile functions studied in soleus and e.d.l. were similar in both groups, namely UN and UNS, except that CT and ET of soleus and ½ RT of e.d.l. which were significantly longer in UNS as compared to those in UN.

In colon, the contraction-pressures due to electrical field stimulation were similar in both groups. Acetylcholine produced greater contraction-pressure in the colon of UNS than UN.

Thus, in undernourished rats, swimming slowed soleus and e.d.l. Swimming did not cause any reduction in colonic contractions which may be due to simultaneous stresses, namely undernutrition and swimming.