Arab Gulf Journal of Scientific Research, 19 (2), pp 78-83 (2001)

ORIGINAL PAPER

M M Al-Rawi

Alterations Produced in the Spinal Cord and Testis of Mice during Starvation

Abstract: The effect of starvation on the content and distribution of RNA, proteins and glycogen in the spinal cord and testis of mice was studied. The spinal cord and testis were taken from mice both, nonstarved and after one, two and three days of total starvation. In the neurons of the spinal cord, starvation decreased RNA and proteins early in the fast; decreases in glycogen occurred more slowly, late in the fast. In the testis there was no obvious decrease in RNA and proteins content and a very slight decrease of the glycogen granules; i.e. the mice conserved testis RNA, proteins and glycogen during prolonged starvation. Moreover, it has been found that the effect of starvation on RNA, protein and glycogen of the testis and spinal cord was variable.

Keywords: Testis, RNA, spinal cord, starvation, glycogen

التغيرات الناتجة في النخاع الشوكي والخصية في الفئران خلال التجويع

ميساء محمد الراوي

المستخلص: لأظهرت الدراسات الكيمياخلوية بالمجهر الضوئي تأثير التجويع على محتوى وتوزيع الحامض النووي (RNA) والبروتين والجليكوجين في كل من نسيجي النخاع الشوكي والخصية في الفأر. لدراسة تأثير الصوم على الفأر، أخذت عينات نسيجية من النخاع الشوكي والخصية لكل من المجموعات التجريبية وهي كاللاتي: المجموعة الأولى كمجموعة ضابطة غير صائمة، المجموعة الثانية بعد يوم من الصوم، المجموعة الثالثة بعد يومين من الصوم، المجموعة الرابعة بعد 3 أيام من الصوم. أظهرت الخلايا العصبية في النخاع الشوكي للفئران الصائمة انخفاض في محتوى الحامض النووي (RNA) والبروتين مبكراً خلال الصوم، بينما كان الانخفاض في محتوى الجليكوجين متأخراً. في الخصية كان هناك انخفاض غير واضح في الحامض النووي (RNA) والبروتين وكان الانخفاض في حبيبات الجليكوجين قليل جداً، أي أن نسيج الخصية يحتفظ بمحتوى الحامض النووي (RNA) والبروتين والجليكوجين خلال فترات الصوم الطويلة، وقد وجد أن تأثير الصوم على الحامض النووي (RNA) والبروتين والجليكوجين يختلف في كل من نسيجي النخاع الشوكي والخصية.

كلمات مدخلية: الفئران-تجويع. تغيرات-النخاع الشوكي-الخصية.توزيع-الجليكوجين-البروتين-الحامض النووي

Introduction

Over 80 years ago, Benedict (1915) published his classic report on the response of a normal man (60 kg) to 31 days of total caloric deprivation. This study established that carbohydrate stores (mainly glycogen) provide a significant component of the body's fuel only during the first few days of a fast and that thereafter fat accounts for 85% of its fuel needs and protein the remainder. Benedict's data also suggested that protein is conserved during prolonged starvation.

Although many studies suggested a close linkage between changes in fuel metabolism, hormonal adaptations and protein conservation during prolonged starvation, the precise interrelationships between these events is not clear (Goodman and Ruderman, 1980).

The hormonal and metabolic alternations in the fasting man have been studied in obese subjects by Cahill (1976); Cahill *et al.* (1968) and Saudek and Felig (1976). These investigators established that during prolonged starvation, glucose is initially replaced as a fuel in many tissues by fatty acids and ketone bodies and that eventually ketone bodies partially replace glucose as a fuel for the brain (Cahill, 1976; Saudek and Felig, 1976).

Many authors have studied the effect of starvation on the different tissues. Wernerman *et al.* (1985) investigated the changes of ribosomes in muscles during starvation and found that the percentage of polyribosomes was significantly lower on days 2 or 3 of starvation than on day 0 (nonstarved); Banhawy and Riad (1970) and Goodman and Ruderman (1980) found that fasting reduced the amount of RNA and protein in muscle, heart and liver cells.

Maisaa M Al-Rawi
Science Department, Girls College,
P. O . Box 10122, Makkah, Saudi Arabia
Tel: 025445823, 055544854
Fax: 026871037
Email: alrawi_maisaa@hotmail.com

A number of studies have been conducted on the ultrastructural changes in the parathyroid gland (Isono *et al.,* 1985), and muscles (Yousif and Sorour, 1992).

Glycogen changes associated with refeeding after a fast have been described for rat myocardium, liver and skeletal muscle (Poland *et al.,* 1981 and Hirose et al., 1986). However, as far as the author knows, none of the previous investigations dealt with the effect of starvation on the RNA, protein, and glycogen content in the nervous tissue and the testis, which is the aim of the present study.

Material and Methods

Twenty adult male mice 8-wk-old (weighing 22 grams) were divided into four experimental groups, each of five animals. One group had free access to food and water (control animals) the other three groups were given water only for 1, 2 and 3 days respectively (starved animals). Experiments of starvation for 4 days or more were not carried out because the majority of the animals died at 4 days of starvation. All animals were killed by cervical dislocation and the spinal cord and the testis were quickly removed and fixed in 5% neutral formalin and carnoy's fluid. Sections 5 um thick were stained by the Borret's methylene blue method to demonstrate RNA basophilia. Total proteins were demonstrated by mercury bromophenol blue (Hg-BOB) method. Glycogen content was demonstrated by Best's carmine method (Pearse, 1968; Gatenby and Beams, 1950).

The diameter and germinal epithelial height of the seminiferous tubules in the testes of control and starved animals were measured with an ocular micrometer. For statistical analysis of the data, the Student's test was used.

Results

The Spinal Cord:

The nerve cells, or neurons, have a cell body consisting of a nucleus and the surrounding cytoplasm, which is called perikaryon. The cytoplasm is drawn out into several short-radiating processes called dendrites (Fig. 1). The cell bodies of neurons are scattered throughout the gray matter of the spinal cord; the nucleus is large, pale, spherical or slightly ovoid, and usually centrally placed within the perikaryon. In most cases there is a single conspicuous nucleolus as well as very fine chromatin particles. Because of uniform dispersion of the chromatin, the nuclei of nerve cells, stained with basic dyes, appeared empty and pale (vesicular). The grey matter contained also the non-neural supporting cells (neuroglia cells).

Data in Table (1) show the loss in body weight during the experiment. Extensive changes were observed in the histology of the spinal cord of all groups of starved mice. The empty spaces observed in the gray matter of normal spinal cord were not observed in the spinal cord of starved mice (compare Fig. 1 and Fig. 2) The cell membranes of the neurons were ill defined after starvation. Vesiculation of the nuclei of the neurons was darkly stained, indicating an increase of the dense chromatin (heterochromatin) and an increase in size of nuclei.

Table (1): Effect of starvation on body weight

Group	Mean body weight g	The percentage of body weight loss
Control		
1 day fasting	22.00g	-
2 days fasting	20.80 g	5.42%
3 days fasting	16.52 g	7.83%

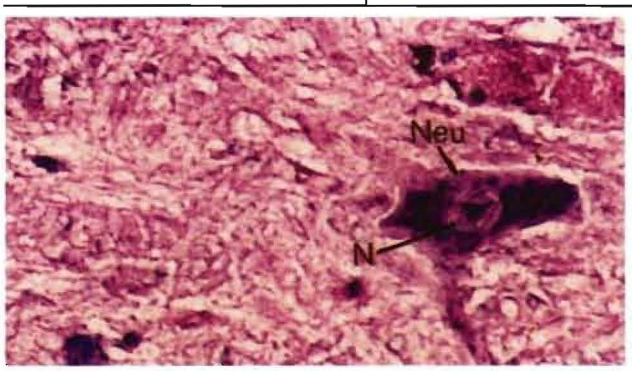

Fig. 1: Transverse section (T.S.) of spinal cord of normal mouse. Note the neurons (Neu) with large nucleus (N) and large nucleolus; the cytoplasm contains large amounts of RNA, the nucleoplasm lightly stained. (x 100).

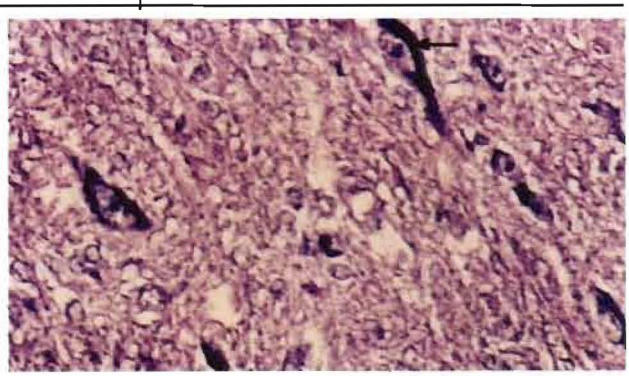

Fig. 2: T.S. spinal cord of starved mouse (3 days) Borret's methylene blue stain. Note the neurons contain dense accumulation of a basophilic substance (RNA) (arrow) (x 100)

Localization of basophilic substances (RNA) was demonstrated by Borret's methylene blue. The ribonucleic acid (RNA) (basophilic substance) of the nerve cells of normal mice gave positive strong homogenous reaction to methylene blue, indicating their richness in RNA in the nucleus (Fig. 1). After 1 and 2 days starvation, RNA was concentrated in the nuclei at the periphery. The remaining part of the nuclei was negatively stained and looked clear. Small clumps in the nucleoplasm were observed. The nucleolus was strongly basophilic and the cytoplasm of the neurons was weakly stained by methylene blue; this indicated a marked loss of RNA material from such cells. The content of RNA decreased early in starvation (after 1 day of starvation). After 3 days of starvation the cytoplasm of the neurons contained small accumulations of basophilic substance (RNA) (Fig.2).

Proteins of neurons of control spinal cord have strong positive affinity to bromophenol blue staining; homogenous dense blue coloration occupies the whole cytoplasm and the neurons are characterized by a high concentration of total proteins (Fig. 3). The nuclei were relatively pale in color and the nucleolus were strongly stained. In the neurons of starved mice, the cytoplasm was weakly stained, indicating marked reduction of total proteins. The cytoplasm contained numerous spaces that would have been occupied with proteinic material in normal cases. Protein content in the neurons diminished early in the starvation, after 3 days starvation proteins appeared in the form of coagulation masses (Fig. 4).

The cytoplasm of the neurons gave positive reactions with Best's carmine, indicating its glycogen content (Fig.5). Decreases in the glycogen content in the neurons were late in starvation. At day 1 and day 2 of starvation, there was a slight decrease, which was more pronounced at day 3 of starvation (Fig. 6).

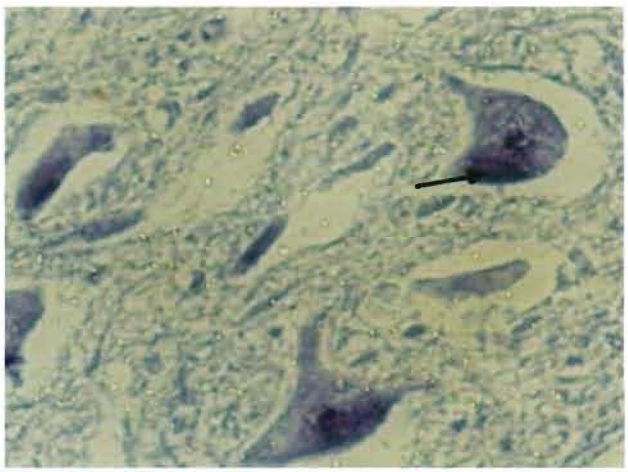

Fig. 3: T.S. spinal cord of normal mouse, Bromophenol blue stain (Hg-BPB). The cytoplasm of the neuron contains a large amount of protein (arrow) (x 40).

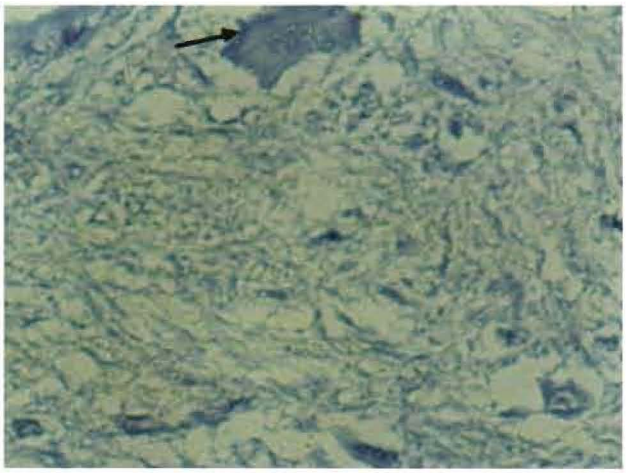

Fig. 4: T.S. spinal cord of starved mouse (3 days), Bromophenol blue stain. Note small accumulations of protein (arrow) (x 40).

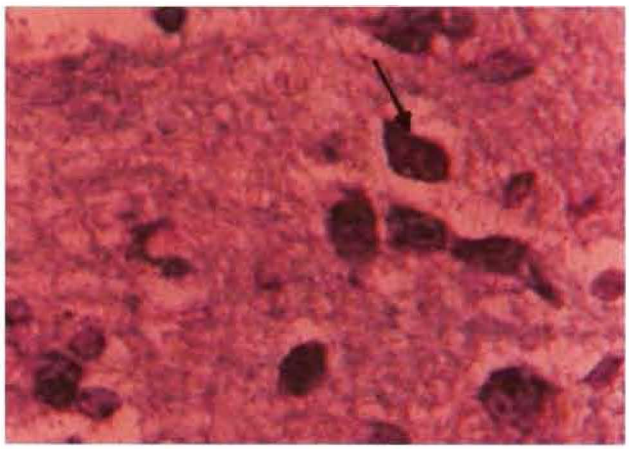

Fig. 5: T.S. spinal cord of normal mouse. Best's carmine stain showing the amount of glycogen in the neurons (arrow) (x 40).

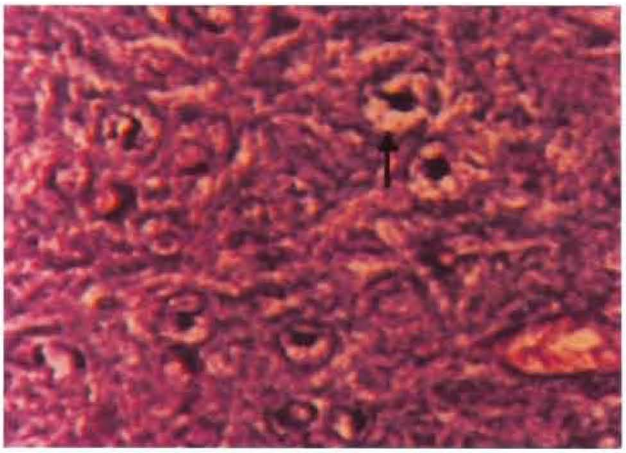

Fig. 6: T.S. spinal cord of starved mouse (3 days). Best's carmine stain showing obvious residue on the glycogen content (arrow) (x 40).

The Testis:

Table (2) shows changes in the diameter and epithelial height of the seminiferous tubules during starvation, indicating a significant decrease in the diameter of the seminiferous tubules ($p < 0.05$) after two days of fasting. This decrease became highly significant ($p < 0.01$) in the testes of animals after three days of fasting. There was likewise a significant decrease in the epithelial height of the seminiferous tubules in the starved mice.

Histological examination in a transverse section in the testis of normal mice showed a large number of seminiferous tubules. The seminiferous tubules were equipped with germ cells, many layers respectively at different spermatogenic stages. The interstitial spaces of normal testis were occupied by connective tissue containing cells (Leyding cells) embedded between tubules.

In the testis of starved mice, the germinal elements representing different phases of spermatogenesis appeared normal with some seminiferous tubules showing a slight decrease in spermatogenesis and a slight disarrangement of the regular succession of the cells. Spermatozoa were not arranged in normal position and were generally decreased in their number. The sperms were broader than those of normal animals (compare Fig 7 and Fig. 8). The seminiferous tubules of starved mice showed a gradual decrease in their diameter and this decrease was found in the central and peripheral regions of the testis. Clear spaces appeared between the seminiferous tubules as the starvation was extended (Figs. 7 and 8).

The cytoplasm of all spermatogenic cells and the tubular lumen in normal testis gave a positive reaction with methylene blue (Fig. 7). It has been observed that the RNA was found in a great amount in the case of starved animals (1,2 and 3 days). The content of the basophilic substance (RNA) in the spermatogenic cells showed no detectable change and the cells contained abundant RNA (Fig. 8). It was found that the spermatogenic cells as well as the tubular lumen was characterized by a high concentration of total proteins, in the cytoplasm and also in the nuclear membrane, nucleolus and chromatin. Judging from the bromophenol blue method, there was no significant decrease in protein content in the testis of all groups of starved mice.

The cytoplasm of all the spermatogenic cells as well as the seminiferous lumen gave a positive reaction with Best's carmine (Fig. 9). Glycogen granules were numerous in the cells and in the seminiferous lumen. A slight decrease in the glycogen content was observed in all starved groups (Fig. 10).

Table (2): Effect of starvation on the diameter and epithelial height of the seminiferous tubules in mice testes.

Group	Mean diameter in μm ± S.D.	Mean epithelial height in μm ± S.D.
Control	1.85 ± 0.5	0.71 ± 0.3
1 day fasting	1.56 ± 0.4	0.65 ± 0.5
2 days fasting	0.82 ± 0.2*	0.43 ± 0.6*
3 days fasting	0.61 ± 0.3**	0.24 ± 0.5**

(*): Significant at $P < 0.05$ in comparison with control.
(**): Highly significant at $P < 0.01$

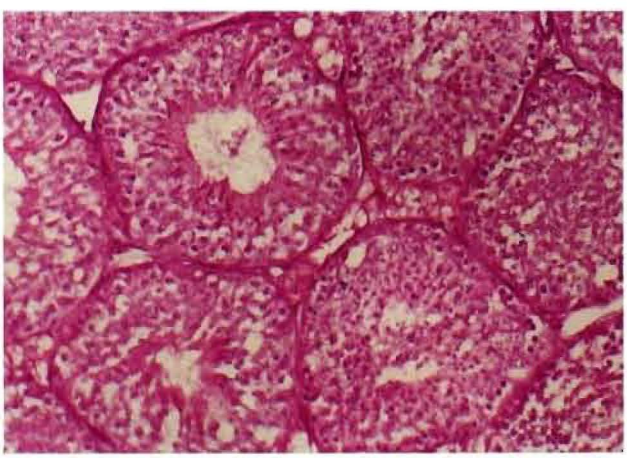

Fig. 7: T.S. spinal cord of normal mouse. Booret's methylene blue stain, showing seminiferous tubles (st) (x 10).

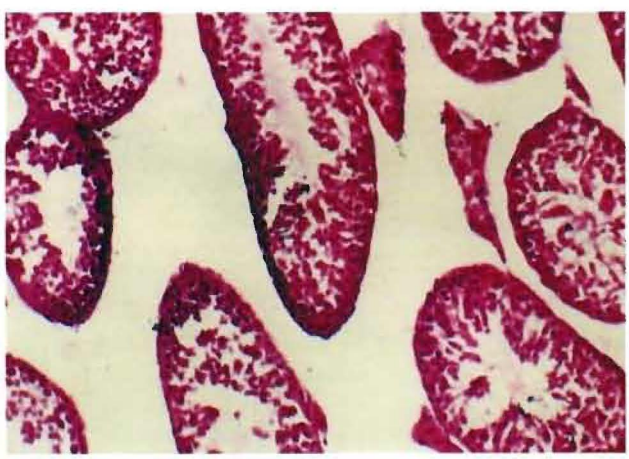

Fig. 8: T.S. spinal cord of starved mouse (3 days), Booret's methylene blue stain. Note the clear wide spaces between the seminiferous tubles (x 10).

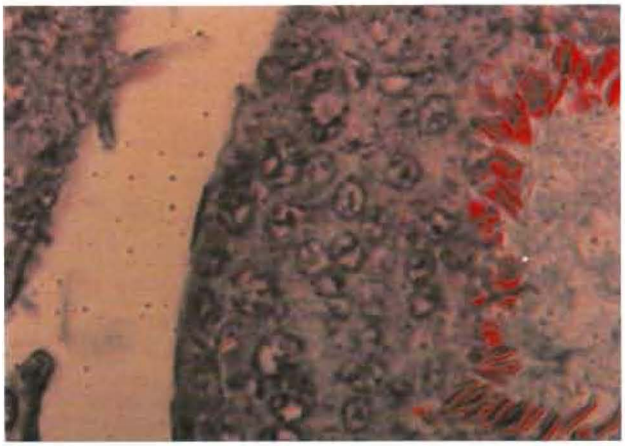

Fig. 9: T.S. spinal cord of normal mouse. Best's carmine stain. Note the glycogen granules in germ cells and in tubular lumen (x 40).

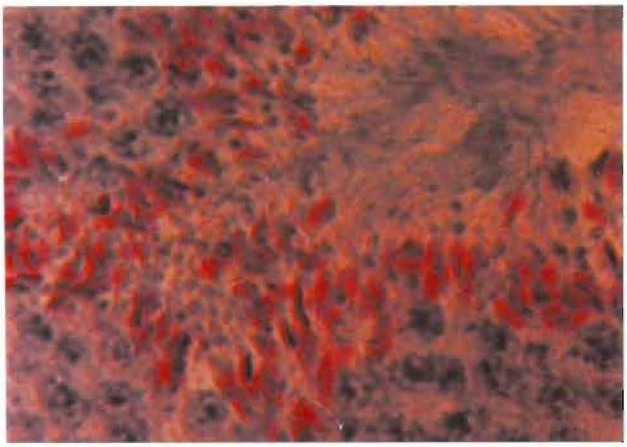

Fig. 10: T.S. spinal cord of starved mouse (3 days). Best's carmine stain, showing a slight decrease in glycogen content (x 40).

Discussion

The chromophilic, basophilic or Nissl substance stand out clearly in the cytoplasm of neurons stained with basic dyes and showed important changes in some pathologic conditions (Bloom and Fawscett, 1976).

Proteinic substances are essential constituents of the general structure of animal cells, and are essential substances in the maintenance of the different vital activities (De Robertis, 1960; Myron and Noble, 1965). A high capacity for protein synthesis is correlated with a high concentration of polyribosomes in the cells, demonstrating the association of ribosomes with messenger RNA (Von der dacken, 1983; and Bergen, 1974). Monoribosomes accumulate when messenger RNA becomes limited or when initiation or polypeptide synthesis is suppressed. Both conditions are established during total starvation when the synthesis of proteins takes place at a reduced rate (Henshaw *et al.,* 1973). The ratio of poly-to mono-ribosomes is then shifted towards single ribosomes (Lied *et al.,* 1982). Eventually these became degraded and the total ribosome content in the cells is reduced (Henshaw *et al.*, 1973).

Prolonged starvation has been studied previously in the rodent by Cuendet *et al.* (1975). They reported that genetically obese mice (ob/ob) survive for a much longer period of time than their nonobese littermates and their excretion of urea nitrogen decreases gradually throughout the fast. Likewise, Goodman and Ruderman (1980) have noted that an obese zucker rat weighing 700 g is able to conserve proteins and survive total starvation for 72 days; in contrast 8-wk-old 200 g. rats do not survive starvation for more than a week, and as judged from the excretion of urinary nitrogen, they fail to conserve total body protein (Goodman *et al.*, 1980 and Parilla, 1978). In the present study, it has been noted that 8-wk mice weighing 22-24 g. were not able to survive starvation for more than three days.

In the studies reported here, of interest was the observation that the RNA and protein content of the spinal cord decreased earlier and more markedly than did glycogen.

Goodman and Ruderman (1980) stated that the concentration of RNA and protein could easily be obtained using the values for the organ weight provided.

In agreement with the present study, Goodman and Ruderman (1980) stated that the effect of starvation on the weight, RNA and protein of individual organs was quite variable. Whereas most organs lost weight at some time during the fast, the brain, adrenal, testis and soleus muscles were spared. Some other organs, such as the liver, tended to lose weight, predominantly during the first few days of the fast, whereas others, such as muscle, tended to lose weight later. The basis for the relatively protected status of the brain, testis and soleus muscle has not been well defined. Possibly, it is related to the fact that protein synthesis is not impaired in these organs by short periods of starvation (Henshaw *et al.*, 1971). The liver, kidney and heart generally maintained their weight as the fast was extended.

Also, Goodman and Ruderman (1980) stated that starvation decreased hepatic RNA and protein early in the fasting 8-wk-old and 16-wk-old groups. In heart and muscles a decrease in RNA occurred in the 8-wk-old group. A similar observation was made of the spinal cord of 8-wk-old mice.

On the other hand, some authors have studied the

ultrastructural changes of some organs after starvation. Isono *et al.* (1985) examined the parathyroid gland of starved mice and found a decrease in the volume of storage granules, cisternae of granular endoplasmic reticulum, secretory granules and the number of prosecretory granules. They concluded that starvation exerts an inhibitory influence not only on the synthesis but also on the release of parathyroid hormones. Yosif and Sorrour (1992) examined the sartorius muscle of starved mice and found a decrease in the sarcoplasmic reticulum and ribosomes, an increase in the heterochromatin at the periphery of the nucleus and an increase of the nucleolus. We confirm the above observations.

Many authors demonstrated changes of glycogen content in muscular tissue. Mong (1982) stated that glycogen in muscle grafts of rats decreases with exercise and fasting. Hirose *et al.* (1986) found a marked decrease in muscle glycogen. In the present study the glycogen content in the spinal cord decreased late in the fast; in contrast the glycogen in the testis was approximately conserved during the fast.

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(Received 22/12/2000, in revised form 28/04/2001)