PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 491-493 (1975)

Effect of Fasting, Phenobarbital, or Hydrocortisone on Serum Creatine-Phosphokinase And Aldolase Activity in Myopathic Syrian Hamsters (38834)

B. SOLYMOSS AND G. JASMIN

Départment de Pathologie, Université de Montréal, Montréal, Qué, Canada

Hereditary polymyopathy and cardiomyopathy are characteristic of the UM × 7.1 inbred strain of Syrian hamsters developed in this laboratory from a dystrophic strain, discovered earlier by Homburger *et al.* (1). The disease is transmitted on an autosomal recessive basis and resembles, in many of its phases, certain forms of human muscular dystrophy (2). As in the Duchenne type of myopathy, these hamsters show a marked increase in serum creatine-phosphokinase (CPK) and aldolase activity, at an early age, when the morphologic changes are still inconspicuous or absent, indicating that plasma membrane alterations may be important in the pathogenesis of the disease.

While always elevated in comparison to controls, the serum CPK and aldolase levels of these animals fluctuate, sometimes inexplicably, raising the possibility that experimental conditions (e.g., number of hamsters per cage, diet) or other unknown factors also influence the results. Therefore, as a first step, we studied the effect of overnight fasting on the two enzymes in myopathic hamsters; for control purposes, we used normal hamsters as well as their first generation (F_1) offspring produced by crossbreeding with the myopathic strain. We also tried to prevent the fasting-induced changes by the administration of phenobarbital or hydrocortisone.

Materials and Methods. The normal hamsters were supplied by the Lakeview Farm (Lakeview, NJ), and the myopathic UM × 7.1 animals—as mentioned earlier—were bred in our laboratory; F_1 controls were obtained by crossbreeding the two lines. The age of the hamsters at the beginning of the experiments was 45–50 days, and the average body wt was 110 g (100–120 g) for the controls and 65 g (60–70 g) for the myopathic animals. Except during the periods of fasting, the animals were maintained ad lib on Purina Lab Chow (Ralston Purina Company of Canada) and tap water.

Tables I to III outline the experimental arrangements. Each group contained at least eight hamsters, kept in two separate cages for males and females. During the fasting periods, the animals were deprived of food and water from 5 PM until 9 AM. Phenobarbital sodium (Merck, Sharpe & Dohme, Dorval Que., Canada) was injected once ip at 5 PM, in doses of 2.5 mg/100 g body wt in 0.5 ml H_2O. Hydrocortisone or "Solucortef" (Upjohn, Pointe Claire, Que., Canada) was given sc at a dose level of 10 mg/100 g body wt in 0.1 ml solvent, also at 5 PM.

Blood was taken from the jugular vein under light ether anesthesia between 9 and 10 AM. Serum CPK [expressed as micromoles of pyruvate (equivalent to creatine phosphate) produced per liter of serum per minute, at 37°C] was determined according to the modified method of Nuttal and Wedin (3), using the reagents manufactured by the Dade Division of the American Hospital Supply Corporation (Miami, Fl.). The technique of Sibley and Lehninger (4), modified by the reagent supplier (Sigma Chemical Co., St. Louis, MO), was employed to measure serum aldolase (expressed as micromoles of alkali-soluble phosphate produced per liter of serum per minute, at 37°C). Serum glucose levels were determined according to the orthotoluidine method of Dubowski (5), using the reagents of Harleco (Philadelphia, PA).

The results were statistically evaluated by Student's t test, and $P < 0.05$ values were regarded as significantly different (6).

Results. The serum CPK and aldolase levels of normal and F_1 (heterozygote) hamsters were approximately in the same range but those of the myopathic (homozygote) strain were many times higher (Table I). Under the influence of overnight fasting, there were no significant changes in the serum enzyme values of normal and F_1

TABLE I. INCREASED SERUM CREATINE PHOSPHOKINASE (CPK) AND ALDOLASE LEVELS (IU) CAUSED BY FASTING IN MYOPATHIC HAMSTERS (MEANS ± SE).

Group	CPK (3 days before fasting)	Aldolase (3 days before fasting)	CPK (immediately after fasting)	Aldolase (immediately after fasting)	CPK (3 days after fasting)	Aldolase (3 days after fasting)
Normal hamsters	142 ± 17	57 ± 3	185 ± 29	65 ± 3	121 ± 34	50 ± 4
Myopathic hamsters	7620 ± 1644[a]	1251 ± 165[a]	44500 ± 3400[a,b]	2738 ± 417[a,b]	9275 ± 1892[a]	1157 ± 132[a]
F_1 offspring	193 ± 40	65 ± 6	129 ± 15	60 ± 2	106 ± 32	67 ± 11

[a] Significantly different ($P < 0.05$) in comparison to normal hamsters or F_1 offspring.
[b] Significantly different ($P < 0.05$) in comparison to prefasting levels.

animals, but a striking further increase was noted for the myopathic hamsters. These alterations were reversible and returned to prefasting levels, according to measurements taken 3 days later.

Even when the myopathic animals were rendered somnolent by phenobarbital administration, the overnight food and water deprivation induced a significant elevation of serum CPK and aldolase activity (Table II).

A single dose of hydrocortisone, given at 5 PM, caused a significant rise in the serum enzyme levels of the myopathic hamsters, as measured the next morning (Table III). These changes were more pronounced than those produced by fasting. The alterations observed in hydrocortisone-treated animals after fasting were of the same order as those elicited by hydrocortisone alone. These serum enzyme increases were not related to changes in serum glucose, since hydrocortisone induced an elevation of the serum enzyme levels unassociated with hypoglycemia, as seen in fasted animals.

Discussion. The mechanism responsible for the rise of the serum CPK and aldolase levels in fasting myopathic hamsters is still unknown. Theoretically, these changes could be due to increased synthesis and/or liberation of the enzymes from tissues, to their decreased metabolic degradation or to variations in the level of hitherto undefined activators or inactivators. Since fasting did not induce similar changes in normal or F_1 animals, it seems that the serum enzyme rises were related to subtle preexisting alterations in the plasma membranes of myocytes, resulting in further enzyme liberation through these defective structures. This view is supported by: (a) the lack of a significant increase in the number, or size, of necrotic foci after overnight fasting, and (b) by the reversibility of the changes.

TABLE II. FAILURE OF PHENOBARBITAL TO AFFECT THE INCREASES IN SERUM CREATINE PHOSPHOKINASE (CPK) AND ALDOLASE PRODUCED IN MYOPATHIC HAMSTERS BY OVERNIGHT FASTING (Means ± SE).

Group	CPK (IU)	Aldolase (IU)
Myopathic controls	13,550 ± 1,383	1,070 ± 42
Fasting	22,275 ± 2,600[a]	1,952 ± 111[a]
Fasting + phenobarbital	21,075 ± 1,456[a]	1,940 ± 178[a]

[a] Significantly different ($P < 0.05$) in comparison to myopathic controls.

Hamsters are nocturnal eaters, and food and water deprivation might induce nervous tension, associated with a discharge of catecholamines or other substances, which in turn might influence serum CPK and aldolase levels. If so, mild somnolence caused by phenobarbital failed to prevent the changes.

Since corticosteroids are known to diminish membrane permeability under certain conditions, we tried to influence the alterations due to fasting by administration of hydrocortisone. Contrary to our expectations, this corticosteroid provoked a further increase in serum enzyme levels. It is possible that the dose was relatively high and that the changes observed were similar to some manifestations of steroid myopathy (7, 8). Fasting can induce nonspecific stress, associated with an enhanced endogenous secretion of steroids. At present, we cannot exclude the possibility that a similar action was responsible for the changes, which would explain the

TABLE III. ELEVATION OF SERUM CREATINE PHOSPHOKINASE (CPK) AND ALDOLASE LEVELS BY HYDROCORTISONE IN MYOPATHIC HAMSTERS (MEANS ± SE).

Group	CPK (IU)	Aldolase (IU)	Glucose (mg/dl)
Myopathic controls	8,762 ± 1,600	798 ± 97	143 ± 11
Fasting	18,628 ± 3,000[a]	1,343 ± 233[a]	73 ± 7[a]
Hydrocortisone	31,925 ± 4,420[a]	2,071 ± 380[a]	162 ± 6[b]
Fasting + hydrocortisone	39,200 ± 6,350[a]	2,715 ± 416[a]	158 ± 7[b]

[a] Significantly different ($P < 0.05$) in comparison to nontreated myopathic controls.
[b] Significantly different ($P < 0.05$) in comparison to fasting levels.

rises noted in both experiments (Tables I and III); however, it is unlikely that fasting could result in an enormous increase of corticosteroid production, equivalent to the administered dose of hydrocortisone. It is noteworthy in this respect that restraint at 2°C induces a marked elevation of the serum CPK level in rats, and changes of similar magnitude are also elicited in adrenalectomized animals (9).

We measured also the serum glucose level at the end of the hydrocortisone experiment. As seen in Table III, the mean value of the control group was 143 mg/dl, which could be explained by the fact that the hamsters ate during the night, before blood was collected. Fasting induced a decrease in glucose concentrations, but it is unlikely that this relative hypoglycemia was responsible in itself for the augmented liberation of muscle enzymes, since, in hydrocortisone-treated animals, there was no similar association between the glucose and serum enzyme levels.

Total serum CPK activity represents the sum of the activities of its isoenzymes, originating mainly from the heart, skeletal muscles and brain. Experiments are now under way to detect which isoenzymes show changes in myopathic animals in comparison to controls, and whether the isoenzymes of the brain, heart, and skeletal muscles are increasingly liberated from tissues and appear in the blood under the influence of fasting or hydrocortisone. We are presently also studying the effect of agents capable of decreasing membrane permeability upon the serum and tissue levels of CPK isoenzymes in myopathic hamsters.

Summary. The hereditary polymyopathy and cardiomyopathy of inbred Syrian hamsters (UM × 7.1) are associated with a marked elevation of serum creatine phosphokinase (CPK) and aldolase levels. Fasting (overnight) further increases (+100–500%) the serum concentration of these enzymes in myopathic hamsters; however, no such effect is demonstrable in normal hamsters, or in first generation offspring produced by crossbreeding the two strains (myopathic and normal). The changes are reversible, and the enzyme values return to previous levels within 72 hr. Neither phenobarbital nor hydrocortisone prevents the rises, as shown by CPK determinations; on the contrary, hydrocortisone elicits even greater serum enzyme increases.

This work was supported in part by the Medical Research Council of Canada (MA-4669).

The authors thank the companies listed in the Materials and Methods section for the compounds used in this study.

The competent technical assistance of Mrs. C. Jubert and Miss L. Robitaille, the secretarial help of Miss L. Faulkner, and the editing by Mr. O. M. Da Silva are also gratefully acknowledged.

1. Homburger, F., Baker, J. R., Nixon, C. W., and Withey, R., Med. Exp. **6,** 339 (1962).
2. Jasmin, G., and Bajusz, E., Ann. Anat. Pathol. **18,** 49 (1973).
3. Nuttal, F. Q., and Wedin, D. S., J. Lab. Clin. Med. **68,** 324 (1966).
4. Sibley, J. A., and Lehninger, A. L., J. Biol. Chem. **177,** 859 (1949).
5. Dubowski, K. M., Clin. Chem. **8,** 215 (1962).
6. Snedecor, G. W., and Cochran, W. G., "Statistical Methods," 6th Ed., Iowa State Univ. Press, Ames, Iowa (1967).
7. Coomes, E. N., Ann. Rheum. Dis. **24,** 465 (1965).
8. Afifi, A. K., Bergman, R. A., and Harvey, J. C., Johns Hopkins Med. J. **123,** 158 (1968).
9. Meltzer, H. Y., Amer. J. Physiol. **221,** 896 (1971).

Received Feb. 11, 1975. P.S.E.B.M., 1975, Vol. 149.