18. Osterhout, L. E., *J. Nutrition,* 1960, v70, 226.
19. Steele, B. F., Sauberlich, H. E., Reynolds, M. S., Baumann, C. A., *J. Biol. Chem.,* 1949, v177, 533.
20. Rosenberg, H., Ennor, A. H., *J. Biochem.* (Tokyo) 1961, v50, 81.

Received May 13, 1963. P.S.E.B.M., 1963, v113.

ATPase, Myokinase, 5'Adenylic Acid Deaminase Activity and Syneresis of the Myofibrills of the Dystrophic Mouse.* (28495)

GEORGE KALDOR AND JOSEPH GITLIN
(Introduced by B. W. Volk)
Department of Biochemistry, Isaac Albert Research Institute, Jewish Chronic Disease Hospital, Brooklyn, N. Y.

Previous work of Fischer(1), Aloisi *et al* (2,3,4) and Feuer and Frigyes(5) has shown qualitative and quantitative differences in the contractile proteins of the rabbit in denervation, E-avitaminosis and nutritional atrophy. Sandow and Burst(6) found pronounced changes in the contractility and Zymaris *et al* (7) in the nucleotide composition of the muscle of the dystrophic mouse(8). Such findings led us to investigate the activity of certain enzymes immediately involved in the ATP† metabolism of the dystrophic mouse. Syneresis (superprecipitation) of the myofibrills was used to study contraction. This model appeared to be most suitable because of its high actomyosin content and its structural organization(9).

Materials and methods. Myofibrillar suspension was prepared as described previously (10). Myokinase was prepared according to Colowick(11). The preparation of myosin-B was carried out as described by Mommaerts (12). ATP, ADP, AMP and ITP were purchased from the Sigma Co. of St. Louis, Mo. All other reagents were of reagent grade.

ATPase determinations were carried out at 37°C for 5 minutes and were assayed as previously published(10). The composition of the individual incubation mixtures is described in detail in Table I. Myokinase activity was determined according to Colowick (11), using rabbit myosin-B as the ATPase. Deaminase activity of the myofibrills was assayed with the Conway microdiffusion method(13) as described earlier(14). The mean deviation (an average of deviations from the mean) between replicate analysis of the same batch was within 5% of all enzymes studied. The mean deviation between batches is listed in the respective tables. The syneresis of the myofibrills(15) was investigated at 23°C in a 0.05 M, pH 7.5 buffer. The results were evaluated 3 minutes after addition of ATP by judging the amount of the shrunken, clumped myofibrills precipitated at the bottom of the test tube. Noncollagenous nitrogen was extracted according to Lilienthal *et al*(16). This was used as a correction factor in calculation of the enzyme activity. Protein concentration was determined by a micro-Kjeldhal method.

Results. The ATPase and ITPase activity of the myofibrills of the dystrophic mouse showed no significant difference from the normal control, irrespective of the kind of activator and the ionic strength and pH of the incubation mixture employed (Table I). The ATPase activity of the dystrophic myofibrills, however, showed a more rapid decrease during storage at plus 1-3°C than did the normal

* Aided by Nat. Inst. Health grant.

† In this paper the following abbreviations have been used. ATP: adenosinetriphosphate, ADP: adenosinediphosphate, AMP-5': adenosine-5-monophosphate, AMP 2'3': a mixture of adenosine-2-monophosphate and adenosine 3 monophosphate, ITP: inosinetriphosphate, ATPase: adenosine triphosphatase, ITPase: inosinetriphosphatase μM P_i/mg/min: micromoles of inorganic phosphate split by 1 mg of protein in 1 minute, BAL: British anti-lewisite.

We wish to thank Dr. A. Saifer and Dr. Bruno W. Volk for their interest during this work.

TABLE I. ATPase and ITPase Activity of Normal and Dystrophic Myofibrills.

No.	Added	pH	Normal		Dystrophic			
			Avg splitting rate (μM P_i/mg/min)	Mean deviation (%)	Avg splitting rate (μM P_i/mg/min)	Mean deviation (%)	No. exp	No. mice/ batch
1	50.0 μM tris + 5.0 μM Mg^{++} + 5.0 μM ATP	7.5	.360	12	.320	16	7	10
2	*Idem* + 5.0 μM Ca^{++} *Idem*	7.5	.325	10	.290	18	5	10
3	" + 5.0 μM Mg^{++} " + 150.0 μM KCl	7.5	.140	10	.120	15	5	10
4	" + " *Idem* + 500 μM KCl	7.5	.050	15	.030	15	5	10
5	Like No. 1 except for 90' preincubation at 37°C	7.5	.280	12	.240	13	4	10
6	" " " " 150' " "	7.5	.140	9	.110	15	4	10
7	50.0 μM histidine buffer + 5.0 μM Mg^{++} + 5.0 μM ATP	6.0	.340	12	.250	18	6	10
8	50.0 μM tris + 5.0 μM Mg^{++} + 5.0 μM ATP	9.0	.360	9	.300	14	6	10
9	*Idem* *Idem* + 5.0 μM ITP	7.5	.180	11	.135	17	7	10
10	" " + 500 μM KCl	7.5	.120	15	.100	15	4	10
11	" + 5.0 μM Ca^{++} *Idem* + 500 μM KCl	7.5	.080	15	.050	20	3	10
12	" + " *Idem* + " + 500 μM KCl	7.5	.360	6	.360	10	3	10

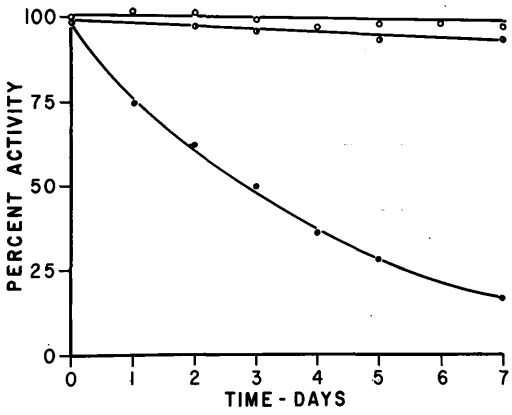

FIG. 1. *Change of activity during storage.* Myofibrillar suspensions contained about 20-30 mg/ml proteins and were stored at 1-3°C temp. Experimental points on this figure represent average of 5 experiments. The main deviation between batches was similar to No. 1 incubation mixture in Table I.
○ Myofibrillar suspension of control mice.
◑ Myofibrillar suspension of dystrophic mice after addition of 5.0 mM BAL.
● Myofibrillar suspension of dystrophic mice.

control (Fig. 1). This finding led us to investigate the sensitivity of the myofibrills toward heat denaturation which again showed no difference between dystrophic and control. Five mM BAL, however, restored the ATPase activity of the dystrophic myofibrills, which was lost during the storage.

The results of the myokinase and deaminase determinations are compiled in Table II. No difference was found in the myokinase content and a small decrease was revealed in the deaminase activity of the dystrophic myofibrills.

The results of the syneresis experiments are summarized in Table III. The myofibrills of the dystrophic mouse behaved differently than did the normal controls. No syneresis was shown in absence of added Mg++ and a poor response was obtained with the dystrophic myofibrills if 5.0 mM Mg++ was added to the incubation mixture. If 0.15-0.25 M KCl was added to the buffer, 1.0 mM or higher concentration of ATP did not produce syneresis and precipitation of the myofibrills. The sedimentation of such myofibrills was usually slower than that of the control. This phenomenon, which may be related to the gelation response of the actomyosin(17), became more pronounced if a 4-10 mg/ml

TABLE II. Myokinase and 5′ Adenylic Acid Deaminase Activity of the Normal and Dystrophic Mouse.

Enzyme	Normal: Avg activity	Normal: mean deviation (%)	Dystrophic: Avg activity	Dystrophic: Mean deviation (%)	No. exp	No. mice/ batch
Myokinase	3.2	5	3.0	5	4	10
Deaminase	1.6	4	1.1	6	5	10

TABLE III. Syneresis of the Myofibrills of the Normal and Dystrophic Mouse. G? = Gelation-like effect.

Added	Syneresis: Normal	Syneresis: Dystrophic
.5 μM ATP	++++	0
1.0 " "	++++	0
2.5 " "	++++	0
.5 " " + 5.0 μM Mg^{++}	++++	0
1.0 " " + 5.0 " "	++++	+
2.5 " " + 5.0 " "	++++	+
100.0 μM KCl + 1.0 " "	++++	0
150.0 " " + 1.0 " "	+++	0
200.0 " " + 1.0 " "	G?	G?
100.0 " " + 1.0 " " + 5.0 μM Mg^{++}	++++	+
150.0 " " + 1.0 " " + 5.0 " "	G?	0
200.0 " " + 1.0 " " + 5.0 " "	G?	G?

myofibrillar protein solution was used for the test instead of the usual 2.0 mg/ml. The dystrophic myofibrills showed this effect to a lesser extent and at a slightly higher KCl concentration than did the controls.

Discussion. Enzyme studies on the muscle of mice with the genetic type of muscular dystrophy failed to elicit an explanation for the diminished ATP concentration, which has been reported repeatedly(5,7) in the extracts of such tissues. The only difference found in this respect was the more rapid deterioration of the ATPase activity of the dystrophic myofibrills during cold storage. This was not caused by a heat denaturation of the protein, but rather by the oxidation of the SH groups of the dystrophic enzyme. Since restoration of enzyme activity is caused by introduction of a sulfhydryl reducing agent (BAL), a chemically active factor may be present in the dystrophic but absent from the control myofibrills. A major defect was also shown in the syneresis of the dystrophic myofibrills. This alteration, which was absent in a case of incipient dystrophy from E avitaminosis (18) of the rabbit, may lend support to the findings of Sandow and Burst(6) in regard to the alterations they found in the contraction and relaxation of the muscle of the dystrophic mouse.

Summary. No pronounced differences were found in the ATPase 5′adenylic acid deaminase activity of the myofibrills and in the myokinase activity of the dystrophic mouse. A significant defect was revealed in the syneresis of the dystrophic myofibrills.

1. Fischer, E., *Proc. of Symposium: Le Muscle,* Schapira, G., Ed., *L'Expansion scient. francaise,* Paris, 1950.

2. Aloisi, M., Ascensi, A., Bonetti, E., *Experientia,* 1952, v8, 266.

3. ———, *J. Path. Bact.,* 1952, v64, 321.

4. ———, *Biochim. Biophys. Acta,* 1953, v18, 455.

5. Feuer, C., Frigyes, A., *Acta Physiol. Hung.,* 1953, v3, 1.

6. Sandow, A., Burst, M., *Am. J. Physiol.,* 1958, v194, 557.

7. Zymaris, M. C., Epstein, N., Saifer, A., Aronson, S. M., Volk, B. W., *ibid.,* 1959 v196, 1093.

8. Michaelson, A. M., Russel, E. S., Harman, P. J., *Proc. Nat. Acad. Sci.,* 1955, v41, 1079.

9. Perry, S. V., *Biochem. J.,* 1953, v55, 114.

10. Gergely, J., Kaldor, G., Briggs, F. N., *Biochim. Biophys. Acta,* 1959, v34, 218.

11. Colowick, S. P., *Methods in Enzymology,* Vol.

II, Colowick, S. P., Kaplan, N. O., Ed., Academic Press, N. Y., 1955.

12. Mommaerts, W. F. H. M.: *Methods in Medical Research,* Vol. 7, Warren, J. V., Ed., Yearbook Publishers, Chicago, 1958.

13. Conway, E. J., *Microdiffusion Analysis and Volumetric Error,* Macmillan Co., New York, 1951.

14. Kaldor, G., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 21.

15. Kaldor, G., Gergely, J., *Arch. Biochem. Biophys.,* 1959, v80, 393.

16. Lilienthal, J. L., Zierler, K. L., Folk, B. P., Buka, R., Riley, M. J., *J. Biol. Chem.,* 1950, v182, 501.

17. Spicer, S. S., *ibid.,* 1951, v190, 161.

18. Azzone, G. F., Aloisi, M., *Biochem. J.,* 1958, v69, 161.

Received March 1, 1963. P.S.E.B.M., 1963, v113.

Glucose Tolerance in Mildly and Moderately Diabetic Rats Exposed to Cold. (28496)

CARL E. MONDON*† (Introduced by D. R. Drury)

Department of Physiology, University of Southern California School of Medicine, Los Angeles

Normal rats are able to acclimate and survive prolonged exposure to cold by maintaining sustained elevations in metabolic rate. This is accompanied by an increased consumption and utilization of their caloric intake. It has been noted that severely alloxan diabetic rats are able to survive extended cold exposure when sustained on a high fat regimen(1). When they were placed on a high carbohydrate diet, the elevation in metabolic rate after cold exposure was severely depressed and duration of survival sharply curtailed. However, administration of insulin to these rats was found to increase their metabolic utilization of the ingested carbohydrate and thereby extend their length of survival at 5°C.

In view of the demands for sustained increases in metabolic rates initiated by cold exposure, an attempt was made to evaluate the influence that chronic exposure to 5°C might have on the development of diabetes in rats with subnormal carbohydrate tolerance. Under conditions where the major caloric food is carbohydrate, it was anticipated that the metabolic demands of acclimation to cold would accelerate the progression of diabetes in such animals. To test this, rats in varying degrees of abnormal carbohydrate tolerance were placed on a moderately high carbohydrate diet and subjected to periodic evaluation of the progression of their diabetic state during cold confinement.

Methods. Male Sprague-Dawley rats ranging from 125-150 g in weight were made mildly to moderately diabetic by intravenous injection of alloxan monohydrate (30 mg/kg of 2% solution in physiological saline). Assessment of the diabetic state was determined by the response to periodically administered glucose tolerance tests (GTT) and expressed in terms of a diabetic index (Id) Coupland *et al*(2). Glucose was administered intraperitoneally as a sterile 7% solution in dosage of 3.5 g/kg to animals following a 16-17 hour fast. Blood sugars were determined in duplicate from blood obtained from the tip of the tail at one and 2 hours after administration of the glucose solution. The extent of deviation of experimental blood sugars from the response obtained in normal animals was calculated as follows:

$$\text{Id} = \frac{\text{1 hr exp blood sugar (mg \%)}}{\text{1 hr normal blood sugar (mg \%)}} \times \frac{\text{2 hr exp blood sugar (mg \%)}}{\text{2 hr normal blood sugar (mg \%)}}.$$

The variability of Id assessment in 12 normal rats acclimated to room temperature was observed to range from 0.75-1.40. The associ-

* This investigation represents a portion of a dissertation submitted in partial fulfillment of Ph.D. requirements, University of Southern California. U.S.P.H.S. Predoctoral Fellow, General Medical Division, P.H.S.

† Present address: NIAMD, Bethesda, Md.