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Research Article

CHRONIC PROGRESSIVE MYOPATHY WITH MYOGLOBINURIA: DEMONSTRATION OF A GLYCOGENOLYTIC DEFECT IN THE MUSCLE *

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Although little is known of the metabolic disturbances in the various myopathies of man and animals (1), in 1956, one of us (R. S.) observed a patient with a bizarre muscle disorder, associated with paroxysmal myoglobinuria, whose unusual clinical manifestations suggested a possible lead as to the nature of the metabolic defect involved. In this patient, the most striking feature was his inability to perform moderately severe muscular work even over short periods of time, whereas muscular activity of a *minimal* degree was tolerated almost without limitations. For example, moderate exercise, such as climbing 10 steps of a stairway, produced severe and painful cramps in the involved muscle groups, lasting for approximately an hour, and was followed by transient myoglobinuria. Contrariwise, slow walking on level ground was tolerated for hours without developing cramps or even undue fatigue. Moreover, it was found that during and following moderate exercise, which was sufficient to produce a cramp, the expected rise in blood lactate concentration (2–4) did not occur. These observations suggested that the patient may suffer from a defect in glycogenolysis, limiting the energy available for muscular contraction to sources other than anaerobic breakdown of glycogen. This possibility was studied *in vivo* and *in vitro* (5).

CLINICAL DATA

Investigations on this patient were initiated in 1957 at the National Institutes of Health [1] and were continued in the summer of 1958 at the Thorndike Memorial Laboratory, Boston City Hospital.

The patient was a single, 52 year old male of Portuguese extraction, who had lived all his life in California. Since childhood he had become easily fatigued and had been unable to keep pace with other children. At the age of 20 he began to notice that following exercise, cramping pain and weakness developed in the muscles of his limbs; such episodes were usually followed by transient excretion of dark urine. For instance, stooping or crouching for a few seconds induced painful contractures of the muscles of his thighs and calves, the cramps lasting for one to two hours. After gradual relaxation, the involved muscle groups exhibited a putty-like consistency and extreme weakness which subsided slowly over the next two weeks. Following such an acute episode, the urine regularly turned dark brown, but usually reverted to a normal color within 12 hours.

These manifestations, which occurred with varying degrees of severity, were most pronounced in the proximal muscles of the four extremities, but at times involved the calves and the strap muscles of the back. His disability forced him to take a job as a doorman, in which occupation little demand was made on his physical performance. Over the years he observed a slow loss of strength in his arms and legs, and in 1947 he was admitted to the University of California Hospital for study and possible treatment.

A report of these investigations (6) revealed no obvious abnormalities, except for weakness of the upper part of the arms and thighs together with slight wasting of the proximal limb muscles, which suggested the diagnosis of progressive muscular dystrophy. The dark urinary pigment, excreted after episodes of cramps, was identified as myoglobin; in addition, such urine specimens contained large amounts of creatine. A series of other laboratory tests was performed, the results of which were believed to be essentially normal. On re-examination of the published data (6), however, it is apparent that following exercise the concentration of lactic acid in the blood showed a distinct decrease, instead of the expected rise. The significance of this finding was not appreciated at that time.

Since this report in 1948 (6), the patient's condition has slowly deteriorated. His musculature has become progressively weaker and atrophic, involving particularly the thighs, the upper arms and the shoulder girdle. Se-

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† Eli Lilly Traveling Fellow of the Medical Research Council of Great Britain.

[1] The authors are grateful to Dr. R. H. Rosenman, San Francisco, California, for the referral of this patient and for his continued interest and cooperation.

vere attacks of cramps and myoglobinuria have occurred less frequently. This may, in part, have been due to the patient's having learned to avoid situations prone to induce such episodes. In spite of his semi-invalid condition, he stayed on his job until summer 1957, when because of a fall and injury in descending a stairway, he was forced to retire.

Since that time, he has been taking daily walks of 10 to 20 blocks on level ground at slow pace to keep up his physical exercise. He has great difficulty, however, in entering or leaving a bus or taxicab, or climbing even a few steps of a stairway. Over the past year he has noticed the onset of difficulties in chewing, resulting from cramp-like pain in his muscles of mastication.

His past history included pulmonary tuberculosis at age 20, gonorrhea at 27, and syphilis at 30. At the time of the present studies, repeated X-ray and laboratory tests failed to reveal any evidence for continued activity of these conditions.

The patient has nine living siblings, none of whom has noticed abnormalities in muscle function or excretion of dark urine. One sister died at the age of 20 of pulmonary tuberculosis. His father died at 80 from carcinoma of the lung, while his mother is still living and well at 82.

For all of his adult life the patient has smoked approximately one package of cigarettes per day and has taken alcohol only sparingly. He denies the use of drugs, or known exposure to toxins.

Physical examination revealed a temperature of 93.8° F., blood pressure 124/96, regular pulse rate of 76 and respiratory rate of 18. He was a pyknic, slightly obese white male in no apparent distress (Figure 1). Abnormal physical findings were limited to the muscular system. There was general wasting of the proximal muscles of the extremities and the shoulder girdle. In the upper limbs the muscle groups most severely affected included biceps, triceps, infraspinatus, pectoralis, trapezius and latissimus dorsi, as well as the sternomastoids. The muscles of the forearms and the hands were fairly well preserved and so were the muscles of the trunk and spine. In the lower extremities, atrophy and weakness were most severe in the quadriceps and adductors, but the calves appeared to be actually hypertrophic. No atrophy was noticed in the muscles supplied by cranial nerves. There was no percussion myotonia or fasciculation. The tendon reflexes were equal on both sides, but were quite hypoactive. Pathological reflexes were absent. The cranial nerves and the sensory system were intact.

X-ray examinations of the chest and the gastrointestinal tract were normal. The heart was not enlarged and two electrocardiograms failed to reveal significant abnormalities.

The following laboratory examinations, all giving results within the normal range, were performed:

Blood. Hemoglobin level was 15.6 Gm. per cent; RBC, 5.6 millions; hematocrit, 50 per cent; platelets, 370,000; leukocytes, 6,300; granulocytes, 61 per cent; lymphocytes, 28 per cent; monocytes, 9 per cent; eosinophiles, 2 per cent; Na, 138 mEq.; K, 4.0 mEq.; CO_2, 31 mEq.; Cl, 104 mEq.; BUN, 10 mg. per cent, creatinine, 0.7 mg. per cent; fasting blood sugar, 72 mg. per cent; total protein, 6.3 Gm. per cent; globulin, 3.0 Gm. per cent (7); albumin, 3.3 Gm. per cent; bilirubin, 0.3 mg. per cent (8); serum iron, 96 μg. per cent; total serum iron-binding capacity, 124 μg. per cent; protein-bound iodine, 5 μg. per cent; prothrombin time, 11 seconds (control 11 seconds); cephalin flocculation negative; alkaline phosphatase, 1.9 Bessey-Lowry units; Kolmer, negative; VDRL, less than 1 unit.

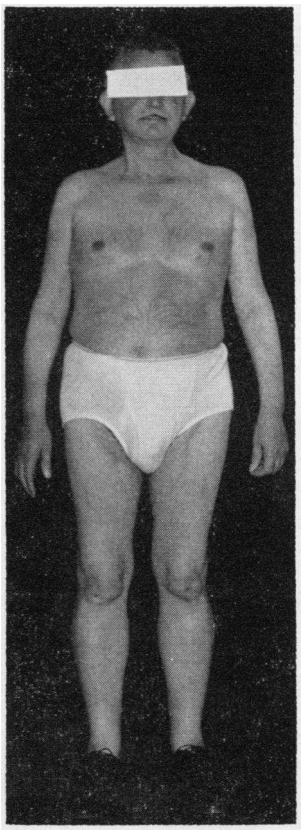

FIG. 1. APPEARANCE OF THE PATIENT IN SUMMER OF 1958, DEMONSTRATING THE MUSCULAR WASTING IN THE PROXIMAL PARTS OF THE EXTREMITIES AND THE SHOULDER GIRDLE

The calves show muscular hypertrophy.

Urine. The urinary pH was 4.8 to 6.8; concentration was 1.001 to 1.032; reducing substance, protein and bile pigments were absent; formed elements were absent; xanthurenic acid (9) was not demonstrable; 5-hydroxyindolacetic acid (10) excretion was 6.0 mg. per 24 hours; PSP excretion was 44 per cent in 15 minutes and 61 per cent in 30 minutes; myoglobin (11, 12) was absent before exercise, but present in small amounts after exercise.

Feces. The feces were negative for blood; urobilinogen (13) excretion was 71.6 mg. per 24 hours.

Radioactive iodine uptake was 24.8 per cent in 24 hours.

TABLE I

Effect of epinephrine and of glucagon on blood sugar, lactate, pyruvate, NEFA, phosphorus and potassium*

Time	Glucose	Lactate	Pyruvate	P	K	Glucose	Lactate	Pyruvate	P	K	NEFA*
min.	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mEq./L.*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mEq./L.*	*μEq./L.*
Control	65	4.4	1.4	2.9	4.6	78	5.6	1.2	2.8	4.2	740
0	0.5 mg. Epinephrine, (s.c.)					Glucagon, 1 mg. (i.v.)					
10						125	5.6	1.2	1.7	4.3	745
15	77	6.0	1.4	2.7	4.4						
20						123	7.0	1.4	1.6	3.8	690
30	91	7.2	1.3			113	7.0	1.4	1.8	3.4	515
45	108	8.0	1.3			155	6.8	1.4	1.6	3.5	375
60	113	7.2	1.3	2.7	3.7	116	7.4	1.4		3.9	340
90	105	8.4	1.4			91	7.6	1.5	2.5	3.8	290
120	91	7.4	1.4								

* Nonesterified fatty acids.

METHODS

All studies *in vivo* were performed with the patient and the control under fasting and resting condition. A healthy, 36 year old physician served as the control subject. For the oral glucose tolerance test, the patient was given 100 ml. of a 50 per cent glucose solution and the blood concentrations of glucose (14), lactate (15), pyruvate (16), phosphorus (17) and potassium (18) were determined at intervals of 30 minutes for the following three hours.

Epinephrine was injected subcutaneously in a dose of 0.5 ml. of a 1:1000 solution and glucagon intravenously in a dose of 1 mg. (Table I). Nonesterified fatty acids (NEFA) were determined by the method of Dole (19).

The combined glucose and insulin test (Table II) was carried out as follows. The fasting patient was given a rapid intravenous injection of 11 Gm. of glucose in water and was then connected with a Bowman constant infusion pump which delivered 20 Gm. of glucose in water per hour. After 30 minutes on the pump, 10 units glucagon-free insulin [2] was rapidly injected through a separate vein and blood samples were withdrawn at the time indicated (Table II).

TABLE II

*Effect of intravenous glucose and insulin on blood glucose, lactate, pyruvate, phosphorus and NEFA**

Time	Glucose	Lactate	Pyruvate	P	NEFA*
min.	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*μEq./L.*
Control	69	4.6	1.2	2.8	940
0	Glucose, i.v. (11 Gm. during first 5 min., then 20 Gm./hr. at constant rate)				
30	170	7.2	1.1	1.9	605
35	(10 Units glucagon-free insulin, i.v.)				
45	161	9.5	1.4	2.0	515
55	128	9.9	1.2	1.8	350
65	120	9.5	1.5	1.5	370
75	109	10.0	1.4	1.5	320
95	111	11.9	1.5	1.7	330

* Nonesterified fatty acids.

[2] Obtained from Eli Lilly Co., through courtesy of Dr. W. R. Kirtley.

For the exercise tolerance test, the patient walked on a treadmill at an angle of 2° and a speed of two miles per hour, and the time which elapsed until cramps in the legs occurred was determined. After a rest of 30 minutes, the experiment was repeated with the patient connected to the Bowman pump, which delivered 0.9 per cent saline at a constant rate of 200 ml. per hour. In order to minimize subjective factors, the patient was made to believe that he was getting glucose and insulin

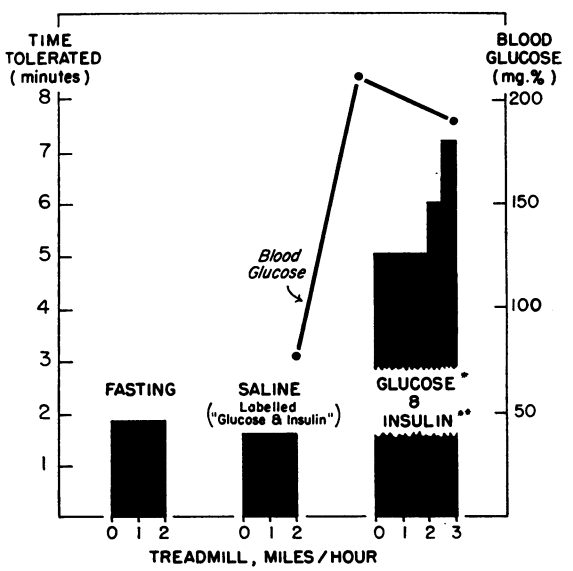

FIG. 2. EXERCISE TOLERANCE (TREADMILL) IN FASTING STATE, WITH SALINE INFUSION AND WITH INFUSION OF GLUCOSE AND INSULIN

The treadmill was inclined at an angle of 2°. Exercise was terminated when cramps developed (fasting and saline) or when the patient became dyspneic (glucose and insulin).

TABLE III

Plasma and urinary findings before and after exercise

	Venous plasma				Urine, 24 hr. collection				
	pH	K	O_2	CO_2	Volume	Creatine	Creatinine	K	Myoglobin
		mEq./L.	*vol. %*	*vol. %*	*ml.*	*mg.*	*mg.*	*mEq.*	
Before exercise	7.40	3.9	11.8	51.1	1,020	460	768	73	Absent
5 Min. after exercise*	7.38	3.8	6.8	52.6					
After exercise					2,470	776	970	161	Present

* Exercise consisted in assuming a crouched position for 20 seconds, which resulted in severe cramps in both thighs and calves.

instead of saline. After a second rest period of 30 minutes, he was given a rapid infusion of 11 Gm. of glucose, followed by constant infusion of glucose at a rate of 20 Gm. per hour. Thirty minutes before the third exercise test, an intravenous injection of 10 units glucagon-free insulin was given. During the test, the speed of the treadmill was increased to 2.5 and finally to 3.0 miles per hour (Figure 2).

Other exercise tests were performed as follows: with the patient at rest, urine was collected over a 24 hour period for quantitative determination of creatine, creatinine and potassium excretion (18, 20) as well as for the demonstration of myoglobin (11, 12). The patient was then asked to assume a crouching position for 20 seconds, at which time severe cramps developed in both thighs and calves. Venous blood specimens were drawn from the femoral vein before and five minutes after the excercise for estimation of pH, CO_2, O_2 and potassium, and the urine was collected for 24 hours following the exercise test (Table III).

Another type of exercise test was performed by having the patient climb briskly 20 steps of a stairway. This resulted in severe cramps in the legs, making it necessary to carry the patient to his bed. Blood samples were withdrawn before and at various time intervals after exercise for determination of lactate, pyruvate and NEFA.

Ischemic exercise in the forearm was studied by the following method. After a rest period of 30 minutes, a cuff inflated to 200 mm. Hg was applied around the wrist to prevent admixture of arterial blood from the hand, and venous blood was then withdrawn without

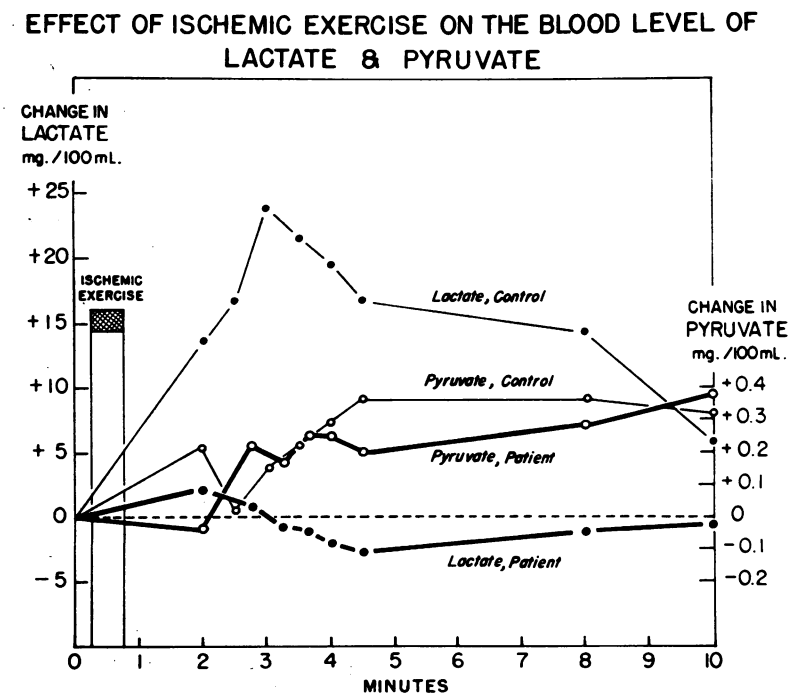

FIG. 3. THE ISCHEMIC EXERCISE CONSISTED IN PUMPING WATER WITH A SPHYGMOMANOMETER BULB WHILE ARTERIAL AND VENOUS BLOOD FLOW WERE OCCLUDED

TABLE IV

Lactate produced by muscle homogenate without added substrate and with addition of glycogen or phosphorylase in vitro

Source of tissue	Addition	Lactate formed	
		μMoles/ Gm.	*μMoles/mg. protein-N*
Patient, trapezius		2.2	0.06
Patient, trapezius, same	epinephrine*	2.0	0.06
Patient, trapezius, same	glycogen R†	1.9	0.05
Patient, trapezius, same	phosphorylase‡	6.8	0.19
Patient, lat. dorsi		2.8	0.07
Patient, lat. dorsi, same	epinephrine*	3.4	0.08
Patient, lat. dorsi, same	glycogen R†	2.9	0.07
Patient, lat. dorsi, same	glycogen H†	2.9	0.07
Control, quadriceps		13.0	0.32
Control, quadriceps, same	phosphorylase‡	15.0	0.37
Control, sartorius		9.5	0.24
Control, rectus abd.		13.2	0.30
Control, quadriceps		11.7	0.29
Control, quadriceps (3 weeks immobilized)		8.4	0.20

* Tissue slices preincubated with epinephrine for 10 minutes prior to preparation of homogenate.

† Addition of 3 mg. glycogen prepared from human (H) or rat (R) muscle.

‡ Addition of 2 mg. crystalline animal phosphorylase.

stasis from the antecubital vein. A second cuff around the upper arm was then inflated to 200 mm. Hg and the muscles of the forearm were worked ischemically for 45 seconds by pumping water against a resistance with a sphygmomanometer bulb. One minute after the end of the ischemic exercise, the cuff around the upper arm was deflated and blood samples were rapidly withdrawn from the antecubital vein (Figure 3). A healthy individual served as control, performing the same amount of exercise over the same length of time. In the latter, the exercise was accomplished without undue fatigue, whereas the patient developed severe cramping pain in the muscles of the ischemic forearm and hand. In both experimental subjects the test was repeated on a separate day.

Blood flow before and after exercise was studied by occlusion plethysmography of the whole leg (21).[3]

The glycogen content of leukocytes was determined by the method of Seifter, Dayton, Novie and Muntwyler (22).[4] The rate of glycolysis of shed blood on aerobic incubation was estimated by the method described by Bird (23).

For incubation, muscle specimens were obtained by biopsy of various muscle groups under local or general anesthesia.[5] The anesthesia employed did not appear to interfere with the assay of the enzyme systems studied. Five individual biopsies of different muscles were performed on the patient and five biopsy specimens were obtained from control subjects who did not suffer from musculo-skeletal diseases. In addition, one biopsy was performed on the quadriceps of a 70 year old woman who had been totally immoblized for the preceeding three weeks because of a comminuted fracture of the hip. The tissue specimens were immediately put into ice cold 1.5 per cent KCl and processed at once.

For estimation of anaerobic glycolysis, approximately 1 Gm. of muscle was blotted dry, weighed on a torsion balance and thoroughly homogenized with four volumes of ice cold 0.3 M Tris (hydroxymethyl aminomethane) buffer, pH 7.5, containing 4×10^{-3} M $MgCl_2$. A loose-fitting hand homogenizer was used and the homogenate was spun for 1 minute at 200 rpm to remove large particles of connective tissue. All operations were carried out in the cold.

For incubation, the mixture contained 0.5 ml. homogenate, 1×10^{-3} M adenosine triphosphate (ATP), 5×10^{-4} M diphosphopyridine nucleotide (DPN), 4×10^{-2} M nicotinamide, 1×10^{-3} M cysteine, substrate where applicable and Tris buffer to make a final volume of 2 ml. In those experiments where substrate was added, 20 μMoles glucose-1-phosphate, glucose-6-phosphate, or fructose-1, 6-diphosphate or 3 mg. of human or animal glycogen was present per flask. In some instances, 2 mg. muscle phosphorylase[6] in microcrystalline suspension was added.

Incubations were carried out in an atmosphere of 95 per cent N_2, 5 per cent CO_2 for thirty minutes at 38° C. in a Dubonoff shaking water bath. At the end of the incubation, the reaction was terminated by the addition of 3 ml. 10 per cent trichloracetic acid. In some instances (Table IV), muscle slices of approximately 100 mg. were preincubated for 10 minutes at 38° C. with Tris buffer, containing 2 μg. per ml. of epinephrine to assure complete conversion of phosphorylase B to A (24–26). The tissue was then homogenized and incubated as indicated above. Glycogen was freshly prepared from human muscle obtained at surgery or from rat muscle (27).

Lactate was estimated by the method of Barker and Summerson (15), all assays being done in duplicate. The amount of lactate formed during incubation was calculated as the difference between lactate concentration in the incubated flask and that present in the original muscle homogenate. The latter was measured by adding 0.5 ml. of chilled homogenate to 4.5 ml. of 10 per cent trichloracetic acid.

Phosphorylase activity was estimated by the method of Sutherland and Wosilait (25) using glucose-1-phosphate as substrate. The results were expressed in milligrams of inorganic phosphorus liberated in 30 minutes per gram of tissue used. In some instances (Table V), the reaction mixture contained 0.02 M adenylic acid or 1×10^{-6} M epinephrine, or prior to homogenization, tissue slices of approximately 100 mg. were preincubated for 10 min-

[3] This determination was kindly performed by Dr. W. H. Abelmann.

[4] This determination was kindly performed by Dr. Jane Shohl.

[5] The biopsies were kindly performed by the staff of the Fifth (Harvard) Surgical Service of the Boston City Hospital.

[6] Sigma Co., St. Louis, Mo.; Lot No. P38-98, 1,200 units per mg.

utes at 38° C., in 0.1 M NaF, containing 2 μg. per ml. of epinephrine.

Muscle glycogen was determined by the method of Good, Kramer and Somogyi (28) and expressed in per cent of muscle wet weight. Protein nitrogen was determined by the micro-Kjeldahl method (29).

For histological examination, muscle tissue was fixed in Zenker's solution and sections were stained with hematoxylin and eosin, periodic acid-Schiff or with Best-carmine. In some experiments, Zenker-fixed tissue sections were washed and then incubated for three hours at 38° C. in 0.9 per cent saline containing 50 mg. per ml. animal diastase.[7] Similar sections cut from the same paraffin block were incubated with heat-inactivated enzyme. At the end of the incubation period the tissues were washed, stained with Best-carmine and the amount of red staining material was estimated.

RESULTS

A. In vivo studies of carbohydrate metabolism in the resting patient

The oral glucose tolerance test revealed a normal response (30), with blood glucose concentrations rising from a fasting level of 81 mg. per cent to 140 mg. per cent in 30 minutes and returning to 65 mg. per cent after 90 minutes. Concomitantly, there was a transient rise in blood lactate concentration, the respective values being 7.2 mg. per cent fasting, 11.6 mg. per cent after 30 minutes and 7.2 mg. per cent after 90 minutes. Changes in phosphorus and potassium concentration were insignificant.

The data obtained after administration of epinephrine or glucagon are summarized in Table I. Both compounds produced a transient hyperglycemia, which was comparable in degree to that seen in normal individuals (31, 32), suggesting adequate glucose mobilization in the liver. There was a simultaneous rise in lactate concentration, whereas pyruvate remained essentially unchanged. Phosphorus levels were low in the fasting state and both epinephrine and particularly glucagon produced a further fall to values which were distinctly below normal (32, 33). The decrease in potassium concentration was similar to that found in normal individuals (34). Glucagon administration was followed by the expected fall in NEFA concentration (35).

The effect of intravenous injection of glucose and insulin on the level of lactate, pyruvate, phosphorus and NEFA is summarized in Table II. Administration of glucose alone produced an increase in lactate and a fall in NEFA concentration; the addition of insulin further enhanced this effect (9, 31, 36–38). Similar to the findings in Table I, the hyperglycemia was accompanied by a low phosphorus level and the values were further depressed after the administration of insulin (33, 39).

B. In vivo studies before and after exercise

The results obtained in the exercise test which involved climbing 20 steps of a stairway are given in Figure 4. In the control subject there was the expected rise in lactate, pyruvate and NEFA concentrations (2–4, 40), with pyruvate increasing from 1.05 mg. per cent to 1.9 mg. per cent six minutes after exercise. Contrariwise, in the patient, lactate and pyruvate did not increase, but remained at or slightly below the control values obtained at the onset of the experiment. For pyruvate, the fasting level was 1.32 mg. per cent and the values 1, 5, 15 and 35 minutes after exercise were 0.92, 1.18, 1.06 and 1.16 mg. per cent, respectively. In contrast to these results, the rise in NEFA concentration was com-

TABLE V

Phosphorylase activity in muscle homogenate

Source of tissue	Addition	P_{in}* liberated
		mg./Gm.
Patient, trapezius		0.1 †
Patient, lat. dorsi		1.9
Patient, gastrocnemius		0
Patient, gastrocnemius, same	epinephrine, 1×10^{-6} M	0
Patient, gastrocnemius, same	epinephrine‡	0
Patient, gastrocnemius, same	AMP, 0.02 M§	0
Control, quadriceps		54
Control, sartorius		58
Control, rectus abd.		17
Control, rectus abd.		25
Control, rectus abd., same	epinephrine‡	42
Control, quadriceps (3 weeks immobilized)		1.9
Control, quadriceps (3 weeks immobilized), same	AMP, 0.02 M§	13
Control, rectus abd.		38
Equal parts of above and patient's muscle		18

* P_{in} = inorganic phosphorus.
† Average of duplicate determinations.
‡ Tissue slices preincubated with epinephrine for 10 minutes prior to preparation of homogenate.
§ AMP = adenylic acid.

[7] Nutritional Biochemicals Corporation.

parable to that found in the control subject. Blood glucose concentration did not change significantly in either of the experimental subjects.

In the ischemic exercise test (Figure 3), the difference between patient and control subject was even more striking. Here, the return of blood from the exercised limb to the liver was obstructed, permitting a closer evaluation of the metabolic events during muscular contraction. As seen in Figure 3, ischemic exercise of the control subject resulted in a prompt and manifold increase in lactate concentration, with a lesser rise in pyruvate. In the patient, on the other hand, there was a minimal increase in lactate concentration during the initial postexercise period, with a subsequent fall below the control values which were obtained prior to the exercise. The initial small rise, observed immediately after releasing the pressure cuff around the upper arm, probably reflected stasis of blood (41), rather than lactate production in the exercised muscle. In contrast to the values for lactate, pyruvate concentration showed a slight increase, similar in extent to that observed in the control subject. In neither of the two experimental subjects was there a significant change in potassium concentration.

Ischemic exercise tests were also performed in five other patients with neuromuscular disorders, including three patients with familial muscular dystrophy, and one patient each with amyotrophic lateral sclerosis and with myositis. In all instances, the results were similar to those obtained in the normal subjects, in that after exercise, lactate concentrations rose from 18.7 to 63.1 mg. per cent over the respective control values.

The plethysmographic determination of blood flow showed a mean resting blood flow of 0.5 ml. per 100 ml. leg volume per minute, which rose to 1.2 ml. during exercise. These values are within normal limits (21). Unfortunately, the status of the patient's peripheral vessels in all four extremities did not permit more extensive studies of blood flow at rest or during exercise.

As seen in Table III, exercise resulted in a slight decrease in venous pH and in increased oxygen extraction by the exercised limb. Carbon dioxide and potassium concentrations did not show significant changes. Urinary excretion of creatinine was decreased, while excretion of creatine was greatly increased at rest (1, 30), and may have risen further after exercise. Many attempts to demonstrate myoglobin in urine at rest were unsuccessful, but after exercise, small amounts of myoglobin could at times be identified. It should be noted, however, that after exercise, the urine did not change color and the amounts of myoglobin present appeared to be very much smaller than those found in a previous study of this patient reported in 1948 (6).

C. Studies of muscle tissue in vitro

In view of the finding that, on exercise, the patient's muscles failed to produce lactic acid, anaerobic glycolysis of muscle tissue was studied *in vitro*. In preliminary experiments, it was observed that in spite of a high glycogen content, incubation of muscle homogenates, *without* added substrate, produced little lactate, whereas with fructose-1, 6-diphosphate, glucose-6-phosphate or glucose-1-phosphate as substrates, lactate formation proceeded at a normal rate. This indicated the presence of a defect in glycolysis involving, most likely, the conversion of glycogen to glucose-1-phosphate. In subsequent experiments, these enzymatic steps were studied in more detail.

The glycogen content of the patient's muscles was found to be greatly increased (42), ranging from 2.4 to 3.0 per cent wet weight (three separate determinations). In spite of this, the amount of lactate formed on incubation was approximately 25 per cent of that, produced by similar preparations of normal muscles (Table IV). Addition of human or rat muscle glycogen to the patient's homogenate or preincubation of the tissue with epinephrine failed to increase lactate formation. On the other hand, addition of crystalline phosphorylase resulted in a more than threefold increase in the amount of lactate produced.

Among the homogenates prepared from control individuals, the least amount of lactate was formed by the muscle, which had been totally immobilized for three weeks (Table IV). Even in this preparation, however, glycolysis was significantly higher than in any of the patient's muscles. Addition of phosphorylase to homogenate of control muscle resulted only in an insignificant increase in lactate production. In contrast to these results, lactate formation with glucose-1-phosphate as substrate was the same in the patient's muscles and in

homogenates prepared from control individuals (Table VI). The lowest value was obtained with the immobilized muscle.

These findings indicated that the defect in glycogenolysis probably involved the phosphorylase system. Determination of phosphorylase activity in the patient's muscles revealed the absence of significant enzymatic activity (Table V). Addition of epinephrine (26, 43) or adenylic acid (25, 26) to the incubation mixture or preincubation of tissue with epinephrine failed to activate the enzyme system. On the other hand, muscle preparations obtained from control individuals showed phosphorylase activity comparable in magnitude to that reported in earlier studies (44). Preincubation of normal muscle with epinephrine or addition of adenylic acid to the homogenate, increased the enzymatic activity. This was particularly striking in the normal muscle, which had been immobilized for three weeks prior to biopsy.

The glycogen content of circulating leukocytes was found to be 71 and 72 mg. per 10^{10} cells, which is within the normal range (45), but differs from the increased value found in a case of hepatic glycogen storage disease (46). Incubation of shed blood revealed a normal rate of glycolysis (47).

TABLE VI

Lactate produced by muscle homogenate from glucose-1-phosphate in vitro

Source of tissue	Lactate formed	
	μMoles/ Gm.	*μMoles/mg. protein-N*
Patient, trapezius	55	1.5
Patient, lat. dorsi	35	0.9
Control, quadriceps	43	1.1
Control, sartorius	40	1.0
Control, rectus abd.	44	0.9
Control, rectus abd.	51	1.0
Control, quadriceps (3 weeks immobilized)	38	0.8

D. Exercise tolerance studies with infusion of glucose and insulin

The findings *in vivo* and *in vitro* indicated a muscular defect in glycogenolysis, making glycogen unavailable as a source of energy for muscular contraction. On the other hand, the effect of administration of glucagon, epinephrine and particularly of glucose and insulin in lowering the blood level of phosphorus and NEFA and increasing the lactate concentration suggested that muscular utilization of glucose may not be impaired. In support of this concept, it was noted that during and after infusion of glucose and in-

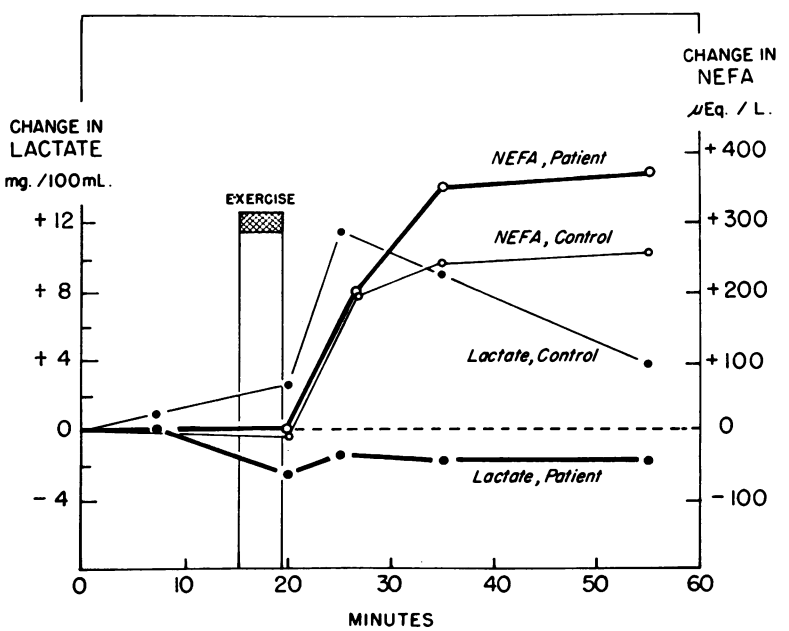

FIG. 4. EFFECT OF EXERCISE ON THE BLOOD LEVEL OF LACTATE AND OF NONESTERIFIED FATTY ACIDS (NEFA)

The exercise consisted in climbing briskly 20 steps of a stairway.

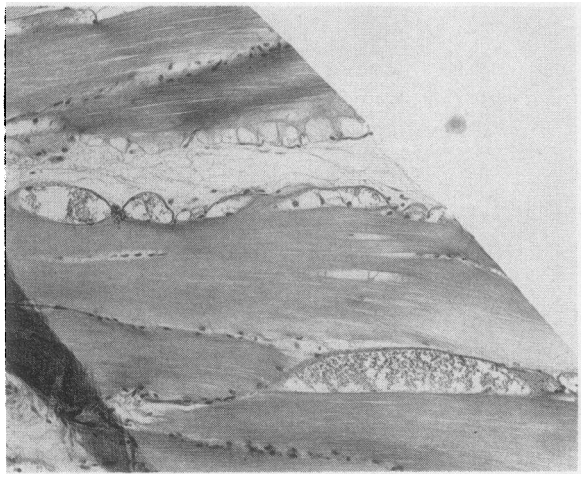

FIG. 5. LONGITUDINAL SECTION OF GASTROCNEMIUS STAINED WITH BEST-CARMINE

Hypertrophied muscle fibers and blebs of raised sarcolemma, containing granular glycogen, can be recognized.

sulin, the patient exhibited a noticeable increase in muscular strength. An attempt was made to quantify this improvement on the treadmill (Figure 2).

In the fasting state and during infusion of saline, the exercise test had to be terminated after a short period of time because of the development of cramps in the lower extremities (Figure 2). Contrariwise, during the infusion of glucose and insulin, the patient was able to walk for a much longer period and at increased speed, without the occurrence of cramps. After approximately seven minutes on the treadmill, the experiment had to be interrupted because of increased dyspnea, but the patient was able to resume walking after a short period of rest.

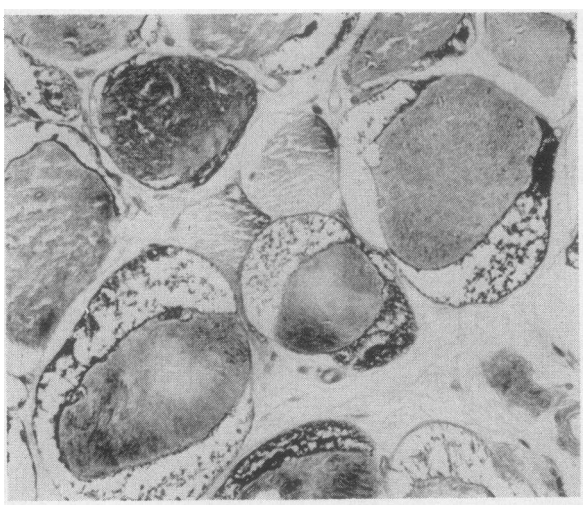

FIG. 6. CROSS SECTION OF QUADRICEPS STAINED WITH PARA-AMINOSALICYLIC ACID (PAS)

Large amounts of PAS-positive glycogen are present under the raised sarcolemma and within the muscle fibers (courtesy Dr. Thomas, National Institutes of Health).

E. Histological studies of muscle

The histological appearance of the muscle is illustrated in Figures 5 through 7. All muscle specimens were found to contain large amounts of a granular material which stained red with para-aminosalicylic acid (PAS) and Best-carmine. This material disappeared completely after incubating the tissue sections with diastase, whereas

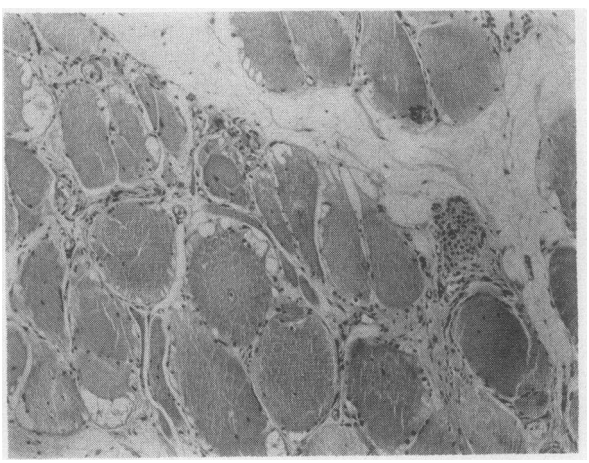

FIG. 7. CROSS SECTION OF GASTROCNEMIUS STAINED WITH HEMATOXYLIN AND EOSIN

A necrotic fiber is present on the right and a remnant of another fiber is seen in the upper left corner.

in specimens incubated with heat-inactivated enzyme, the amount of PAS and Best-carmine-positive material was similar to that found in control section. These observations indicated that the muscle contained large amounts of glycogen. In addition some sections showed muscle fibers in various states of degeneration (Figure 7) or scattered fibers exhibiting acute necrosis.

Figure 5 illustrates a longitudinal section of gastrocnemius stained with Best-carmine demonstrating hypertrophied fibers with internal chains of nuclei, and with blebs of raised sarcolemma. The latter contained granular material which stained bright red with the Best-carmine method.

In the upper two fibers, the granular material has partly disappeared and histiocytic nuclei are present within the blebs. Figure 6 is a cross section of quadriceps, stained with PAS. There is a similar accumulation of PAS-positive material under the raised sarcolemma and, in some fibers, infiltrating diffusely the sarcoplasm. Figure 7 is a cross section of gastrocnemius stained with hematoxylin and eosin. The granular material was largely washed out by the staining procedure. On the right is a necrotic fiber with histiocytic invasion and on the upper left a remnant of another damaged fiber.

DISCUSSION

Current physiological concepts indicate that adenosine triphosphate (ATP) plays a central role as the immediate source of energy which is required for muscular contraction as well as for the basic metabolism of muscle at rest (1, 42, 48). In resting muscle, the relatively small amount of high energy phosphate needed is largely generated through oxidation of noncarbohydrate materials (49), while oxidation of glucose to CO_2 and water, and anaerobic dissimilation of glucose to lactate appear to be of minor importance. However, even resting muscle which actively takes up O_2 produces a small amount of lactate which is almost all derived from degradation of glucose (49). Approximately 60 per cent of the glucose uptake by forearm muscles can be accounted for by lactate production (49), while anaerobic breakdown of glycogen appears to be of little importance as a source of energy at rest.

In contracting muscle, on the other hand, the increased energy requirements cannot be met by lipid and carbohydrate nutrients which diffuse from the blood into the muscle cells, nor is the oxygen supply adequate to sustain a predominantly aerobic oxidation (1, 42). Maximal activity of frog muscle, for instance, increases the rate of ATP utilization almost 1,000-fold above the resting rate (42). The energy for this muscular work must be largely supplied through anaerobic mechanisms and from substrates which are present within the muscle cells.

The amounts of ATP and of phosphocreatine stored in the muscle are insufficient to sustain anaerobic contraction for more than a very short period of time (42). For continued activity, the energy demand must be met by anaerobic glycolysis of glycogen to lactic acid. The latter diffuses into the interstitial fluid and is carried by the circulation to the liver, where it is in part oxidized to CO_2 and water and in part utilized for resynthesis of glycogen (42). The rise in lactate concentration, which is always observed after exercise, is a reflection of the greatly increased rate of anaerobic glycolysis in contracting muscles (2, 3).

In the patient studied, a moderate degree of exercise for a very short period of time resulted in severe cramps which prevented further use of the involved muscle groups for several days. Associated with this process was histological evidence of muscle necrosis (Figure 7) and transient myoglobinuria. These observations suggested that the patient may be unable to mobilize glycogen for contraction of his muscles and may depend solely on whatever other fuels the muscles are able to abstract from the blood. This concept was borne out by both the findings *in vivo* and the studies of muscle metabolism *in vitro*.

In vivo, exercise and ischemic exercise failed to result in increased lactate concentration in venous blood (Figures 2 and 3), whereas in the normal individual and in five patients with other forms of myopathies, comparable amounts of exercise resulted in marked elevation of venous lactate levels. The finding that exercising the patient's muscle under ischemic conditions did not lead to a rise in venous lactate concentration indicated that the lack of lactate response was independent of changes in muscular blood flow. *In vitro,* lactate production by the patient's muscle was greatly reduced in spite of the presence of increased amounts of glycogen. This defect was not corrected by preincubation of muscle tissue with epinephrine (26, 50), nor by addition of glycogen prepared from normal human muscle or from rat muscle. On the other hand, addition of crystalline phosphorylase greatly increased lactate formation (Table IV). This finding and the demonstrated absence of phosphorylase activity, even in the presence of epinephrine or adenylic acid (26, 50) (Table V), indicated that the primary defect involved the phosphorylase system.

In normal muscle that has been exercised (26, 50) or totally immobilized (Table V), phosphorylase may be largely present in its metabolically

inactive form "b," but conversion to the enzymatically active form "a" can be achieved by epinephrine (26, 50). This is illustrated in Table V where preincubation of normal rectus muscle with epinephrine resulted in significantly increased phosphorylase activity. Moreover, addition of adenylic acid to homogenate prepared from previously immobilized muscle greatly increased phosphorylase activity (Table V). With the patient's muscle, on the other hand, these compounds did not increase enzymatic activity. Furthermore, the patient's muscle did not appear to contain phosphorylase inhibitors or increased activity of the phosphorylase-rupturing enzyme (50, 51), since the muscle homogenate from the patient did not reduce the enzymatic activity of normal muscle preparations (Table V).

In contrast to this defect in the phosphorylase system, lactate formation from phosphorylated hexoses was not impaired *in vitro* (Table VI). Likewise, *in vivo,* oral and intravenous administration of glucose and intravenous injection of insulin (Table II) resulted in a significant increase in venous lactate concentration; this was associated with a concomitant reduction in serum phosphorus and NEFA. Moreover, the hyperglycemia following injection of glucagon was accompanied by similar changes in lactate, phosphorus and NEFA levels (Table I). These observations indicated active utilization of glucose in the periphery. It should be noted that the fall in serum phosphorus, following injection of glucose and insulin (Table II) and of glucagon (Table I), exceeded that which is commonly observed in normal individuals under similar conditions; whether this reflected participation of an ancillary mechanism of phosphorus metabolism could not be assessed on the basis of the available data.

Additional evidence for active utilization of blood glucose by the patient's muscles was provided by the observation that, on the treadmill, infusion of glucose and insulin prevented the occurrence of cramps and greatly increased the patient's work performance (Figure 2). It could not be determined, however, whether under these circumstances, peripheral uptake of glucose in the patient was comparable to that which would be expected in normal individuals subjected to similar treatment. Similarly, no information was obtained to indicate whether, in the fasting patient, the assimilative capacity of muscle for blood glucose differed from that of normal muscles. The rapid onset of cramps in the patient's muscles on moderate exertion may suggest that in addition to impaired glycogenolysis, uptake and/or utilization of blood nutrients may have been reduced. One may consider the possibility that, in contracting muscle, glycogenolysis may exert a priming effect in initiating increased assimilation of glucose and other nutrients from the blood. This could be achieved in various ways, perhaps by increasing blood flow through the muscle, by facilitating transport of nutrients across the cell membrane, or by modifying their metabolic disposition in the muscle cell. A possible approach to long term management of this patient may be maintenance of a hyperglycemic state in the presence of adequate insulin.

The defect in the phosphorylase system apparently did not involve the liver, as administration of epinephrine and glucagon evoked a significant degree of hyperglycemia, indicating adequate breakdown of glycogen and output of glucose by the liver (26). Moreover, the liver was not enlarged and hepatic function, including glucose tolerance, appeared to be within normal limits. The glycogen content of circulating leukocytes was not increased and there was no demonstrable abnormality of glycolysis in peripheral blood (47).

In agreement with the chemical analysis, the histological findings in the muscle demonstrated the presence of increased concentrations of glycogen. The latter was predominantly located directly under the sarcolemma, but to a lesser degree was present diffusely throughout the muscle fibers. The presence of a high glycogen content in the patient's muscle suggested that only the breakdown but not the formation of glycogen may be impaired. This is consistent with recent reports by Leloir and Cardini (52), Villar-Palasi and Larner (53) and Robbins, Traut and Lipmann (54), that phosphorylase is involved only in glycogenolysis, while synthesis of glycogen occurs via the uridine nucleotide pathway. The formation of glycogen in the patient's muscle is presently under investigation; the results of this study are reported in a separate communication (55).

The disturbance described in this patient appears to be a very rare disorder of muscle metabolism. In 1951, McArdle (56) reported the find-

ings in a 30 year old patient, who exhibited a similar type of muscle dysfunction, although excretion of myoglobin was not observed. Exercise and ischemic exercise failed to produce increased lactate concentrations, whereas epinephrine resulted in both hyperglycemia and elevation of lactate levels. From this, it was concluded that the patient "suffered from a disorder of carbohydrate metabolism, affecting chiefly if not entirely, the skeletal muscles and preventing the breakdown of glycogen to lactic acid." Biochemical or histological investigations of muscle tissue were not performed.

In a recent abstract, Pearson, Rimer and Mommaerts reported studies on another case with similar features (57). This patient was 19 years old and "had a life-long history of progressive weakness upon usage of exercised muscles and severe cramps if exertion was intense or prolonged." Muscle wasting was not present and myoglobinuria was not observed. Venous lactate concentration did not rise following exercise, and biochemical studies of muscle tissue showed virtual absence of phosphorylase activity.

In addition to these reported cases, one of us (R. M.) has observed five other patients with similar clinical and laboratory findings, but biochemical studies of muscle tissue have not yet been performed. Two of the patients were sisters, but this is the only evidence to suggest a hereditary nature of the disorder. The patient presently reported has nine living siblings and one sister, who died from tuberculosis, none of whom had any evidence of disturbed muscle function.

The history of the patient suggests that his clinical course may be somewhat arbitrarily divided into three distinct periods. The first included childhood and adolescence when the patient's only complaint was increased fatigability. The period from age 20 to approximately 40 was characterized by severe cramps on exertion associated with transient myoglobinuria, but persistent weakness and wasting of muscles was not prominent. During the third period, beginning at approximately 40 years of age, cramps and myoglobinuria became gradually less conspicuous, but weakness and wasting of individual muscle groups began to appear with increasing severity. Associated with this loss of muscle mass was a marked elevation of creatine excretion in the urine (Table III). It is apparent that the features of the two patients reported in the literature resembled those of our patient during the second clinical period. McArdle's case (56) was 30 years of age and had severe cramps on exertion, but persistent weakness, wasting of muscles and creatinuria were absent. Pearson's patient (57) was 19, and although intense or prolonged exercise produced cramps, exercise tolerance in the fasting state was much higher than in our patient. In neither of the two reported cases was myoglobinuria observed, but unless specifically sought, this may have passed unnoticed.

Several features of the present patient remain unexplained. It is not clear why the pattern of muscle cramps on exertion did not develop until the beginning of the third decade; this may perhaps suggest that the energy metabolism of growing muscle may quantitatively differ from that of adult muscle. Moreover, it is not understood why wasting of muscles did not occur until later in life and why some muscle groups were more affected than others, while the calf muscles showed actual hypertrophy. Finally, it was of interest that no evidence was found to suggest impairment of myocardial function.

SUMMARY AND CONCLUSIONS

1. Clinical manifestations and laboratory investigations of a 54 year old male patient, who had suffered for the past 35 years from chronic, progressive myopathy, are reported. The outstanding feature of this patient was his inability to perform moderate degrees of muscular exercise, even over short periods of time, whereas minimal muscular work was tolerated almost without limitation. Moderate exercise of a few seconds' duration resulted in prolonged, painful cramps of the involved muscle groups, associated with tissue necrosis and transient myoglobinuria.

2. Exercise and ischemic exercise, sufficient in extent to produce a cramp, failed to result in the expected rise in venous lactate concentration. This finding suggested a defect involving the glycolytic mechanisms in the muscle.

3. Glycogen content of the muscle was found to be approximately five times increased; in spite of this, incubation of muscle homogenate produced four times less lactate than in control preparations.

This defect could be corrected by addition of crystalline phosphorylase.

4. With glucose-1-phosphate as substrate, lactate formation in the patient's muscle was comparable to that of control preparations. These observations suggested that the disturbance involved the phosphorylase system, which catalyzes the breakdown of glycogen to glucose-1-phosphate.

5. In the patient's muscle, phosphorylase activity was found to be absent, even in the presence of adenylic acid or after preincubation of tissue with epinephrine.

6. Treatment of the patient with epinephrine or glucagon resulted in hyperglycemia and increased serum lactate levels, associated with marked reduction in serum phosphorus.

7. Infusion of glucose and insulin produced increased venous lactate concentration and reduced serum levels of phosphorus and nonesterified fatty acids. Associated with this was a marked increase in exercise tolerance.

8. These findings indicate a defect in the phosphorylase system of the muscle, which precludes breakdown of glycogen and eliminates anaerobic glycogenolysis as a source of energy for muscular contraction. Muscular work is limited by the energy which can be derived from nutrients diffusing from the blood into the muscle cell. Contraction beyond these limits results in cramps, muscle necrosis and myoglobinuria.

9. A disturbance of similar nature has been described in two other patients; in addition, five patients with myopathy and similar clinical findings were observed, but details have not yet been reported. The possible hereditary nature of this rare syndrome remains to be investigated.

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ADDENDUM

For the last four months, the patient has been treated with intramuscular injections of 1 mg. glucagon three times daily. Glucagon is given simultaneously with or shortly following meals and results in sustained hyperglycemia, lasting from 90 to 120 minutes. During this time, the patient notices a significant improvement in his ability to perform muscular work. For example, one hour after injection of glucagon, he was able to walk on the treadmill for 17 minutes (angle 2°, speed two miles per hour), while after injection of saline, cramps occurred in both legs in less than two minutes, necessitating termination of the exercise.

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