same as those occurring during muscular contraction.

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*A FUNCTIONAL DISORDER OF MUSCLE ASSOCIATED WITH THE ABSENCE OF PHOSPHORYLASE**,†

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Introduction.—The present report deals with the biochemical identification of the defect in a recently encountered muscle disorder, characterized by a rapid exhaustion of otherwise mechanically and physiologically normal muscle, which exhaustion could be prevented by the intravenous administration of lactate, glucose or fructose. The case, which has some resemblance to the one reported by McArdle (1951), will be described extensively elsewhere (Pearson, Rimer and Mommaerts, 1959).

The observed peculiarities gave the impression that, while the capacity for resting or low-activity metabolism is sufficient, a block exists preventing the transition from rest to higher rates of activity metabolism. As long as only the preventive effect of lactate was known, we thought, as McArdle had concluded for other reasons, that this restriction might resemble that in iodoacetate poisoning and consist of a defect in phosphoglyceraldehyde dehydrogenase. The subsequent observation that glucose (and also fructose, Pearson, 1959), can likewise alleviate the symptoms pointed to a block closer to the phosphorolysis of glycogen.

The studies reported herein show that the disease is characterized by a lack of phosphorylase combined with a considerable storage of glycogen.

Materials and Methods.—Tissue material was obtained from the patient (D. G.), a 19-year-old male, in the fasting state by biopsy from the lateral aspect of the right upper thigh under local anesthesia. Immediately from the operating table, separate tissue fragments were immersed in a large Dewar flask with isopentane at −180°C. They were then transferred to liquid nitrogen, coarsely powdered, and stored in a −70°C cryostat. Samples taken for analysis were finely powdered at −180°C, and extracted at 0°C with 0.001 *M* aqueous ethylenediaminetetraacetate (with or without fluoride) for the enzyme assays; with 0.3 *N* perchloric acid for the analysis of soluble metabolites; or with 3 volumes of 30 per cent KOH at 100° for the determination of glycogen. The further methodology for each of these studies will be described under the respective headings.

Results.—Glycogen: Determinations of glycogen were performed by the method of Good *et al.* (1933), using the anthrone reagent as described by Hassid and Abraham (1957). The results are shown in Table 1. It is seen that the resting

TABLE 1

DETERMINATION OF GLYCOGEN IN DISEASED (D.G.) AND NORMAL HUMAN MUSCLE

Mgs. Glycogen Per Gram Tissue	
D.G.	Normal
41.2	9.2
37.5	9.4

glycogen value is in marked excess over the accepted standard values, 10 to 20 mg glycogen per gram. A sample taken from normal human thigh muscle gave a figure in the expected range. In keeping with the analytical results, it was found that histochemical staining (cf. Pearson, Rimer and Mommaerts, 1959) applied to a biopsy sample sectioned in the frozen state, showed excessive amounts of glycogen in a diffuse granular form.

Glycolysis in tissue extracts: In minced muscle suspended or extracted in buffer, glycolysis occurs at a fairly reproducible rate which provides some measure of the glycolytic capacity. Frozen muscle powder was homogenized in 0.001 *M* phosphate buffer of pH 7.4. The centrifuged extracts were incubated anaerobically and analyzed for lactate by the method of Barker and Summerson (1941) after deproteinization with trichloroacetic acid. Typical results are those of Figure 1 obtained on extracts to which nicotinamide was added. Similar results have been observed without this addition and in whole homogenates. It is seen that in the diseased muscle glycolysis from endogenous glycogen is negligible. Added glycogen is not glycolyzed either, but glucose-1-phosphate and glucose-6-phosphate raise the activity to quite normal levels. This would indicate that, but for the initial step of glycogenolysis, the glycolytic enzyme system is sufficient to sustain significant rates of metabolism. The glycolytic rate in diseased muscle on addition of hexosephosphates is actually two or three times as high as comparable rates obtained with several biopsy samples of normal muscle. However, in view of the many factors which influence the rate of glycolysis in extracts (Neufach and Melnikova, 1958), it is not justified to say without further investigation that the diseased muscle is richer than normal in some rate limiting enzyme. The major result is qualitative:

there is no glycolysis with glycogen, but seemingly normal magnitudes of activity with glucose-1-phosphate and other intermediates.

These experiments suggest that glycolysis in the diseased muscle is limited either by absence of phosphorylase, or by an inadequate transformation of phosphorylase *b* to *a*.

Determination of phosphorylase a and b: Direct tests for the presence or absence of phosphorylase were performed by extracting powdered frozen muscle with 4 volumes of water containing 0.001 *M* ethylenediaminetetraacetate (EDTA) (cf. Krebs and Fischer, 1955a) with or without 0.1 *M* fluoride for 20 min. Assays for

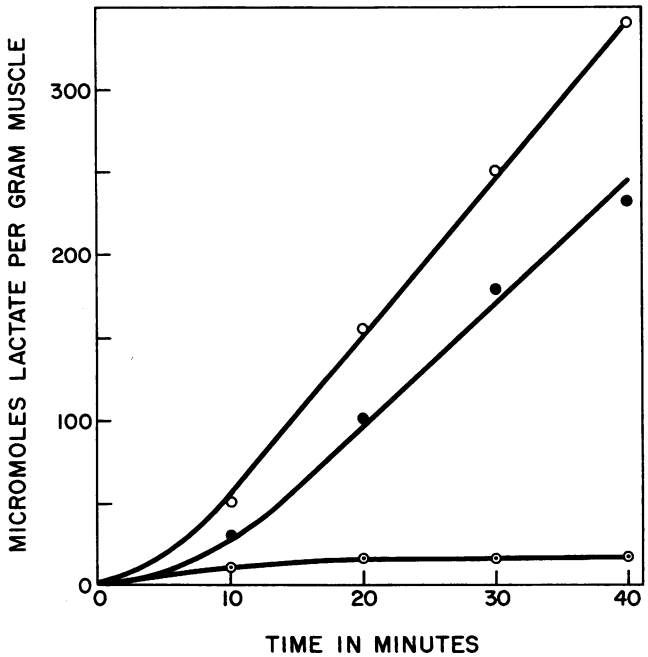

FIG. 1.—Glycolysis in extracts of diseased muscle, with no addition (⊙), glucose-6-phosphate (○) or glucose-1-phosphate (●). Composition of medium: 0.3 ml 0.024 *M* K-phosphate buffer pH 7.6; 0.3 ml 0.5 *M* K HCO_3; 0.1 ml 0.01 *M* Na-K-ATP; 0.2 ml 0.2 per cent DPN; 0.2 ml 0.1 *M* Mg Cl_2; 30 μ moles of substrates; 0.4 ml 0.025 *M* nicotinamide; water to 3 ml final volume, including 0.5 ml tissue extract, corresponding to 50 mg muscle. Temperature 37°C.

phosphorylase *a* and *b* were done according to Illingworth and Cori (1953). As shown in Table 2, the pathological muscle contained no detectable amounts of either phosphorylase *a* or *b*. Nor could pyridoxal-5-phosphate (cf. Illingworth *et al.* 1958), 3.8 μ moles added during extraction and sometimes also during the pre-incubation, induce such activity. Histochemical phosphorylase tests according to Takeuchi (1958) were also completely negative, contrary to comparably treated samples from normal human muscle (cf. Pearson, Rimer and Mommaerts, 1959). The absence of detectable phosphorylase *b* could not be ascribed to abnormally rapid deamination of the adenylic acid added to the assay, since no excessive adenylate deaminase content was demonstrable. Disappearance of a sizable fraction

TABLE 2

ANALYTICAL DATA ON ASSAY FOR PHOSPHORYLASE *b* AND *a* IN DISEASED (D.G.) AND NORMAL MUSCLE

Muscle samples (162.6 and 161.3 mg, respectively) were extracted with 0.4 ml 0.001 *M* EDTA; of the extracts, 0.1 ml samples were diluted 20-fold with 0.04 *M* Na-glycerol phosphate-0.03 *M* cysteine at pH 6.0; 0.4 ml of this solution was incubated with 0.2 ml of 4 per cent glycogen at 30°C for 20 min. At zero time, 0.2 ml of 0.064 *M* glucose-1-phosphate was added, with or without 0.004 *M* AMP. Aliquots of 0.2 ml were taken at intervals for analysis. For the phosphoglucomutase correction test, a 0.1-ml aliquot was used.

	D.G.		Normal	
Phosphorylase Assay	Without AMP	With AMP	Without AMP	With AMP
γ P at zero time in aliquot	6.6	7.3	6.5	6.9
γ P per aliquot formed				
5 min	+0.3	−0.1	+0.1	+13.2
10 min	+0.4	+0.1	+0.1	+15.1
15 min	...	...	+0.9	+16.8
Phosphoglucomutase Correction Test				
γ P at 10.5 min	26.2	26.3	24.1	25.2
Expected from G-1-P hydrolysis		26.3; 26.2		

of the added AMP would be possible during the course of the test, but the activity was not raised by fluoride (cf. Krebs and Fischer, 1955b). Phosphorylase assays were likewise negative when performed at lesser dilution of the enzyme, and at incubation times as long as 1 hour at pH 6.2. Addition of crystalline phosphorylase *a* to the reaction mixture resulted in complete recovery of its activity.

Phosphorylase kinase and PR enzyme: The absence of phosphorylase might be unique, or might be associated with an absence of its activating and deactivating enzymes. Hence, determinations of phosphorylase kinase and of phosphorylase phosphatose (PR enzyme) were performed. The former enzyme was assayed following the procedure of Krebs and Fischer (1956), and an activity of 683 units per gram of muscle was found. The latter was assayed according to Keller and Cori (1955) with a yield of 3870 units per gram of muscle. These amounts are significant, illustrating that the absence of phosphorylase is sharp and specific, and is not associated with generalized defects of biologically related enzymes.

Determination of UDPG-glycogen transferase: For reasons set forth in the *Discussion*, it was apparent that the formation of glycogen could not take place by reversal of the phosphorylase reaction, but must occur by the UDPG pathway first suggested by Leloir and Cardini.

UDPG-glycogen transferase was determined by the method of Hauk *et al.* (1959), such that a sample of frozen muscle was homogenized with nine volumes of 0.02 *M* Tris-0.001 *M* EDTA buffer at pH 7.5 for 3 min at top speed in a Virtis homogenizer, and an aliquot of the uncentrifuged homogenate was assayed by 15 min incubation at 30°C in a reaction mixture containing 0.001 *M* UDPG, 1 per cent glycogen, 0.003 *M* $MgCl_2$ and 0.04 *M* Tris buffer, pH 7.2. An activity of 6 transferase units (Hauk *et al.*, 1959) per gram of tissue was found, which is in the normal range previously found in well preserved tissues.

Chemical nature of deposited glycogen: A sample of glycogen was isolated and subjected to the combined action of phosphorylase and amylo-1,6-glucosidase (Illingworth, Larner and Cori, 1952). The glycogen was completely digested and had a normal endgroup percentage of 7 per cent. When treated with phosphorylase free of amylo-1,6-glucosidase, 41 per cent of the glucose residues present in the

glycogen were liberated, showing that the length of the outer chains was likewise within the normal range (Illingworth and Cori, 1952). Thus, the glycogen formed in the absence of phosphorylase, but presumably in the presence of branching enzyme, had in every respect a normal structure.

Determination of some phosphorus compounds and other metabolites: Analyses were performed for some of the major phosphate-containing metabolites on perchloric acid extracts, using the standard methods of this laboratory (Mommaerts, 1957). None of the comparisons between the pathological samples and normal muscle are accurately valid, since during the biopsy procedures no precautions could be taken to have all specimens in the same physiological condition. Also, not enough information is available about the spread of normal values. With these restrictions in mind, it can be said that the results (Table 3) are not indicative of

TABLE 3

DETERMINATIONS OF SOME METABOLITES IN DISEASED AND NORMAL HUMAN MUSCLE

	Micromoles per gram of tissue	
	D.G.	Normal
Free creatine[1]	6.4	9.2
Phosphocreatine[2]	16.0	13.3
Inorganic P[3]	7.4	7.0
Hexosemonophosphate[4]	0.22	0.35
Hexosediphosphate[5]	0.25; 0.22	0.48; 0.30
Lactate[6]	1.1; 1.2	2.7; 2.9
ATP[7]	5.55	5.36
Total nucleotide[8]	6.15	6.17

[1] Methods (cf. Mommaerts, 1957): Diacetyl method; [2] *Ibid.*, increase upon hydrolysis; [3] Isobutanol extraction method; [4] Slater's method B; also separate determination of glucose-6-phosphate with glucose-6-phosphate dehydrogenase and TPN after acid hydrolysis of ATP; [5] Including triosephosphates, according to Slater's method A; [6] Barker and Summerson's method; [7] Reduction of TPN with glucose-6-phosphate dehydrogenase and hexokinase; [8] Ultraviolet absorption calculated for adenine nucleotides.

any significant change in the diseased muscle. However, one might regard the hexose monophosphate, hexose diphosphate and lactate contents as low, in keeping with low glycogenolytic activity.

Discussion.—The case, the clinical features of which will be described in full (Pearson, Rimer and Mommaerts, 1959), is of interest as another example of a metabolic disorder which is referable to the absence of a single enzyme. That the biochemical lesion is specific is shown by the fact that other enzymes associated with phosphorylase are present in normal amounts, particularly phosphorylase kinase and PR enzyme which regulate the level of active phosphorylase. The fact that the accumulated glycogen is normal in structure points to the presence of the branching enzyme. Among the various types of glycogen storage disease, each characterized by the absence of a single enzyme, none has so far been associated with an absence of phosphorylase (G. T. Cori, 1953, 1957). A special feature of the present case seems to be that the enzymatic lesion is apparently confined to skeletal muscle. This is suggested by the fact that there are no cardiac disturbances, that the fasting blood sugar is not abnormally low, and that injection of epinephrine evokes a normal hyperglycemic response.

The observation that the muscles contained 4 per cent glycogen in the virtual absence of phosphorylase makes it clear that there must be another pathway for glycogen synthesis. It has long been suspected that phosphorylase alone cannot account for the synthesis of glycogen since any factor known to activate phosphory-

lase always leads to glycogenolysis rather than synthesis. The original observation by Leloir and Cardini (1957) that there is an enzyme present in liver which can synthesize glycogen from glucose-1-phosphate via uridine diphosphate glucose (UDPG) has now been extended to muscle (Villar-Palasi and Larner, 1958; Robbins, Traut, and Lipmann, 1959; Hauk and Brown, 1959; Hauk, Illingworth, Brown, and Cori, 1959). This synthetic process, unlike that catalyzed by phosphorylase, is not influenced by the concentration of inorganic phosphate and has an equilibrium far to the side of glycogen synthesis. The presence of considerable amounts of glycogen and of the UDPG-glycogen transferase in the muscles of the patient, in the absence of phosphorylase, provides strong support for the operation of the UDPG mechanism for the synthesis of glycogen.

It may be envisaged that normally glycogen is being synthesized at a certain rate by this pathway, but that it is kept at a moderate level by periodic decreases whenever there occurs an increased glycogenolysis associated with exercise, when phosphorylase *b* is activated to phosphorylase *a* (C. F. Cori, 1956; Krebs and Fischer, 1955a; Guillory, Mommaerts, Schottelius and Schottelius, 1959). The elimination of these recurrent decreases would lead to an increased glycogen level, as encountered in the present case. It is of interest, however, that in cases of generalized glycogen storage disease the glycogen content of skeletal muscle may rise as high as 12 per cent, in spite of the fact that phosphorylase is present. The fact that in the present case the glycogen content does not rise to these high values when degradation by phosphorylase seems excluded would indicate that other regulatory factors are in operation which limit the amount of glycogen deposited in muscle.

Summary.—Biopsy specimens of skeletal muscle from a patient with a functional disorder similar to that described by McArdle were analyzed for various enzymes. A homogenate of muscle showed no lactic acid formation from endogenous glycogen, whereas added hexosephosphates were glycolyzed at a normal rate. The enzyme defect was shown to consist in a virtual absence of phosphorylase. Other enzymes related directly or indirectly to glycogen metabolism (phosphoglucomutase, PR enzyme, phosphorylase kinase) were present, and the glycogen had a normal branched structure. The glycogen content (4 per cent) was higher than normal. The presence of the UDPG-glycogen transferase of Leloir and Cardini was demonstrated. Glycogen synthesis in the absence of phosphorylase is ascribed to the action of this enzyme.

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*ACTION SPECTRA FOR THE POSITIVE AND NEGATIVE PHOTOTROPISM OF PHYCOMYCES SPORANGIOPHORES**

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One of the most effective approaches to the study of the mechanism of phototropic perception is by means of the action spectrum. In the case of the phototropism of *Phycomyces* sporangiophores in visible light, the action spectrum has been studied by Blaauw,[2] Castle,[8] Wiechulla,[18] and Bünning,[6] and an analysis of Wiechulla's results was published by Buder.[3] These investigators obtained single-peaked action spectra with a maximum phototropic effectiveness in the blue region, which, according to Wiechulla, is also the most effective region in promoting straight growth. Working with *Pilobolus*, a genus related to *Phycomyces*, Bünning found two maxima of phototropic effectiveness in the blue for sporangiophores with subsporangial vesicles,[4] and only one maximum for those without these vesicles.[6] However, the results obtained by these earlier workers are not sufficient to clarify the details of the action spectra because filters with broad transmission bands were