# Energy Metabolites in Red and White Striated Muscles of the Pig

G. R. Beecher,[1] L. L. Kastenschmidt,[2] W. G. Hoekstra,[2] R. G. Cassens,[2] and E. J. Briskey[2]

The concentrations of G-6-P, F-6-P, F-1,6-diP, pyruvate, ATP, and citrate were determined in two red and two white muscles from six pigs at several periods post-exsanguination. The *rectus femoris* (red) showed an increase in citrate during the first 60 minutes post-mortem, during which time glycogen was being metabolized. The *gluteus medius* (white), *longissimus dorsi* (white), and *serratus ventralis* (red) showed decreasing citrate levels post-exsanguination. Even though the initial citrate level was high in the *serratus ventralis* (red), the disappearance of F-6-P did not appear to be inhibited during early post-mortem periods.

Muscles containing a majority of red fibers (red muscles) have high oxidative enzyme activity, whereas muscles low in red fiber content (white muscles) have especially high glycolytic enzyme activity (Beatty *et al.*, 1963, 1966; Needham, 1926; Ogata, 1960; Slater, 1960). Since these muscles differ markedly in myoglobin content (Lawrie, 1952), thereby having different oxygen tensions, they could have different rates of transition to anaerobiosis upon cessation of blood flow. Metabolic variations (*in situ*) in red and white muscles under apparently anaerobic conditions (curtailment or removal of blood supply) have not been examined. One might expect a greater accumulation of Krebs' cycle intermediates in red muscle than in white muscle, which could be important in view of the recently suggested role for Krebs' cycle intermediates, particularly citric acid, in limiting glycolysis via phosphofructokinase inhibition (Garland *et al.*, 1963; Parmeggiani and Bowman, 1963; Williamson and Jones, 1964; Williamson, 1965). Additionally, it has been postulated that Krebs' cycle intermediates may activate particular isozymes of lactic dehydrogenase (Fritz, 1965) and thereby influence the type and rate of metabolism in the tissue (Vessell and Pool, 1966).

In view of these relationships, studies were made to determine the concentration of several energy metabolites in red and white striated muscles, to establish the association of *in situ* metabolite concentration with post-mortem metabolism, and to study lactic dehydrogenase isozymes in regard to post-mortem metabolism in red and white muscle.

## EXPERIMENTAL

**Sample Isolation.** Fifty- to 100-gram samples were excised from the *rectus femoris*, *gluteus medius*, *longissimus dorsi*, and *serratus ventralis* (*in situ*) of six pigs immediately after exsanguination (1 minute after exsanguination) and at 15, 30, 60, 120, 180, 240, and 1440 minutes post-mortem (post-exsanguination). Carcasses were stored at 5° C. within 45 minutes post-exsanguination. Muscle pH was determined immediately after excision (homogenized in 10 volumes of 0.005*M* iodoacetate, pH 7.0) according to the procedure of Marsh (1952). Muscle samples were frozen in liquid nitrogen and powdered for analysis according to the procedures of Borchert and Briskey (1965).

**Myoglobin, Succinic Dehydrogenase, and Red Fiber Content.** Myoglobin was determined (Poel, 1949) on samples isolated 1440 minutes post-mortem. Succinic dehydrogenase activity was determined by a modification of the method suggested by Bocek (1964) and reported previously by Beecher *et al.* (1965a). One unit of activity equals 1 mg. of INT formazan produced per 15 minutes of incubation per gram of muscle tissue. The red and white fiber content was determined in muscle samples which were fixed (10% calcium-formol), freeze-sectioned (18 microns) and stained with Sudan Black B (Ogata, 1958). The results are reported as the per cent of red fibers of the total fiber content of 10 muscle bundles.

**Muscle Extract Preparation.** Muscle extracts for the assay of glycolytic intermediates and adenosine triphosphate (ATP) were prepared by adding 6% (w./v.) perchloric acid (0° C.) to the muscle powder (approximately 1.7 ml. per gram of powder), centrifuging (8000 × G for 10 minutes at 4° C.) and adjusting the supernatant to pH 7.0 with 30% (w./v.) KOH. Muscle extracts for the assay of citric acid were prepared by adding 5% (w./v.) phosphotungstic acid in 2*N* $H_2SO_4$ (0° C.) to the muscle powder (approximately 1.7 ml. per gram of powder) and centrifuging (2000 × G for 30 minutes at 4° C.). Aliquots of the supernatant were used directly for citric acid determinations. Muscle extracts for the assay of total lactic dehydrogenase (LDH) activity and the starch gel electrophoretic separation of LDH isozymes were prepared by twice extracting with 0.076*M* tris (hydroxymethylamino) methane, pH 8.65 (1 ml. per gram of powder). Supernatants from both extractions were combined after centrifugation (8000 × G for 10 minutes at 4° C.) and filtered through glass wool.

**Metabolite Determinations.** Concentrations of the following metabolites were determined in extracts from samples excised at all post-exsanguination time periods except 1440 minutes: glucose 6-phosphate (G-6-P)

[1] Department of Biochemistry, Kansas State University, Manhattan, Kan.
[2] Departments of Meat and Animal Science and of Biochemistry, University of Wisconsin, Madison, Wis.

and fructose 6-phosphate (F-6-P) (Hohorst, 1963a), fructose 1,6-diphosphate (F-1,6-diP) (Bücher and Hohorst, 1963), pyruvate (Bücher *et al.*, 1963), lactate (Hohorst, 1963b), ATP (Lamprecht and Trautschold, 1963), and citrate (Ettinger *et al.*, 1952, as modified by Hanson, 1966). Glycogen was isolated and hydrolyzed (Pfleiderer, 1963) on initial and 60-minute samples, and the resulting glucose was determined (glucose oxidase, Glucostat, Worthington Biochemical Corp.).

**Lactic Dehydrogenase Activity and Isozyme Content.** The total LDH activity was determined by the method of Amador *et al.* (1963) and starch gel electrophoretic separation of the LDH isozymes was performed by the method of Poulik (1957) on initial samples only. One unit of LDH activity corresponds to an absorbance change of 0.001 per minute at 25° C., 340 m$\mu$ wave length, 1 cm. light path, and 3 ml. total reaction volume (Amador *et al.*, 1963). Muscle extracts were diluted to $2 \times 10^5$ units of LDH activity per ml., and 6000 units of LDH activity were applied to each starch gel sample slot. Lactic dehydrogenase isozymes were identified by incubating the starch gels in 50 ml. of incubation medium for 1 hour at 37° C. The incubation medium contained 25 mg. of $NAD^+$ (Sigma Chemical Co.), 25 mg. of Nitro Blue Tetrazolium (2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-3,3′-3,3′-dimethoxy-4,4′-diphenylene)-ditetrazolium chloride (Sigma Chemical Co.), 2.5 mg. of phenazine methosulfate (Sigma Chemical Co.), and 5 ml. of 60% sodium lactate made to 50 ml. total volume with 0.125*M* phosphate buffer containing 0.011*M* NaCN, pH 7.4 (Paulson, 1965).

Lactic dehydrogenase isozyme I was identified as the fastest migrating anodal band, LDH V as the slowest migrating anodal band (Brody, 1964). The LDH isozyme content was calculated as the per cent of each isozyme band density to the total density of all bands (recording electrophoresis densitometer, Photovolt Corp., New York).

Statistical analyses were conducted according to Steel and Torrie (1960). Probability levels less than 0.05 were considered significant. All metabolite concentrations are reported as micromoles per gram muscle tissue.

## RESULTS AND DISCUSSION

**Myoglobin Concentrations and Association with Red and White Fiber Content and Succinic Dehydrogenase Activity.** Myoglobin concentrations (Table I) were higher ($P < 0.05$) in the red (*rectus femoris* and *serratus ventralis*) than in the white (*gluteus medius* and *longissimus dorsi*) muscles. A close relationship was observed between the myoglobin concentration, succinic dehydrogenase activity and red fiber content, indicating that in the porcine animal, in vivo, the muscles which contain high myoglobin concentrations (*rectus femoris* and *serratus ventralis*) are red muscles (high in red fiber content) and apparently have a high capability for aerobic metabolism (high succinic dehydrogenase activity), whereas muscles which have low myoglobin concentrations (*gluteus medius* and *longissimus dorsi*) are white muscles (low in red fiber content) and would appear to be oriented toward anaerobic metabolism (low in succinic dehydrogenase activity). Similar results have been obtained on small laboratory animals (Dawson and Romanul, 1964; Ogata, 1958; Romanul, 1964).

**Glycogen.** Initial (immediately after exsanguination) glycogen concentrations (Table II) were similar in the red and white muscles studied. These observations agree with the findings of Bocek *et al.* (1966a), but disagree with the findings of Beatty *et al.* (1963) and Bocek *et al.* (1966b) for rat striated muscle. Bocek *et al.* (1966b), however, have indicated that certain white muscles of the rat may be more sensitive to anoxia than red muscles, and during the preparation procedures, dissection and trimming, glycogen in white muscles might decrease more rapidly than in red muscles. The high lactate levels in all muscles at 0-hour sampling support the idea that they have already responded appreciably to exsanguination anoxia.

Only one third as much glycogen was metabolized by the *rectus femoris* (red) during the first hour post-mortem (Table II) compared to the *serratus ventralis* (red) and both white muscles. Domonkos (1961) and Beatty *et al.* (1963) have also observed a lower degree of glycogen metabolism in red muscle than in white muscle. However, the present studies are unique because they show clearly this phenomenon *in situ* under the apparent anaerobic conditions of the post-mortem state. The *serratus ventralis* (red), although having a high myoglobin concentration, metabolized a quantity of glycogen during the 0- to 60-minute period post-mortem which was similar to that metabolized by the white muscles (*gluteus medius* and *longissimus dorsi*).

**Energy Metabolite Concentrations Post-Mortem.** GLUCOSE 6-PHOSPHATE AND FRUCTOSE 6-PHOSPHATE. Initial concentrations of G-6-P and F-6-P (Figure 1) were similar in the *rectus femoris* (red), *serratus ventralis* (red) and *gluteus medius* (white), but slightly lower ($p > 0.05$) than in the *longissimus dorsi* (white). Rapid decreases in concentrations of G-6-P and F-6-P occurred during the firs

**Table I. Myoglobin Concentration, Succinic Dehydrogenase Activity, and Red Fiber Content of Red and White Muscles**

| Characteristic | Muscle: *Serratus ventralis* (red) | *Rectus femoris* (red) | *Gluteus medius* (white) | *Longissimus dorsi* (white) |
|---|---|---|---|---|
| Myoglobin[a] | 3.09 ± 0.24[b] | 1.72 ± 0.15 | 0.98 ± 0.08 | 0.82 ± 0.05 |
| Succinic dehydrogenase activity[c] | 2.56 ± 0.35[d] | 2.00 ± 0.34 | 1.36 ± 0.18 | 1.17 ± 0.17 |
| Red fiber content[e] | 43.1 ± 2.6[d] | 41.1 ± 3.9 | 29.9 ± 1.8 | 25.2 ± 2.2 |

[a] Milligrams per gram muscle tissue.
[b] Mean value of six observations plus or minus standard error of the mean.
[c] Units of activity per gram muscle tissue, from Beecher *et al.* (1965b).
[d] Mean value of eight observations plus or minus standard error of the mean.
[e] Per cent red fibers of total fiber content, from Beecher *et al.* (1965b).

**Table II. Glycogen Content of Red and White Muscles**

| Glycogen | Muscle *Serratus ventralis* (red) | *Rectus femoris* (red) | *Gluteus medius* (white) | *Longissimus dorsi* (white) |
|---|---|---|---|---|
| Initial[a] (0 minutes) | 43.6 ± 4.4[b] | 37.8 ± 3.7 | 47.2 ± 6.9 | 41.2 ± 12.6 |
| 60 minutes[a] | 28.0 ± 3.9 | 32.1 ± 4.0 | 30.5 ± 7.1 | 15.2 ± 3.7 |
| Metabolized[a] (0–60 minutes) | 15.6 ± 1.2 | 5.7 ± 1.9 | 16.7 ± 1.2 | 26.0 ± 10.5 |

[a] Micromoles glucose equivalents per gram muscle tissue.
[b] Mean value of six observations plus or minus standard error of the mean.

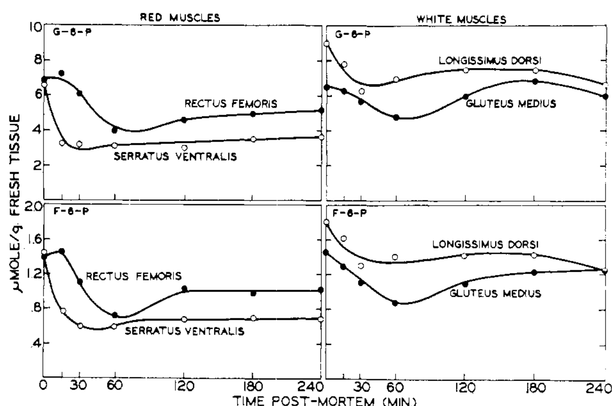


**Figure 1. Post-mortem concentrations of G-6-P and F-6-P in two red and two white porcine muscles**

30 minutes post-mortem in the *serratus ventralis* (red) and *longissimus dorsi* (white), whereas the declines of G-6-P and F-6-P in the *rectus femoris* (red) and *gluteus medius* (white) extended over 60 minutes post-mortem. The initial levels of G-6-P, F-6-P, and F-1,6-diP were considerably higher in the red and white muscles studied than those levels observed in rat heart muscle (Newsholme and Randle, 1964), rat diaphragm muscle (Newsholme and Randle, 1961), cat striated muscle (Pedersen and Sacks, 1965) and insect flight muscle (Sactor and Wormser-Shavit, 1966). Frog striated muscle, incubated with epinephrine, has increased concentrations of G-6-P and F-6-P (Hegnauer and Cori, 1934; Ozand and Narahara, 1964). These findings may mean that the stress and excitement to which pigs are subjected prior to exsanguination (Beecher *et al.*, 1965a) may stimulate glycolysis in striated muscle and account for the high values of G-6-P, F-6-P, and F-1,6-diP in the initial sample.

PYRUVATE AND LACTATE. Initial concentrations of pyruvate (Figure 2) were several-fold higher in the *longissimus dorsi* (white) than in the *rectus femoris* (red) and *gluteus medius* (white). The *serratus ventralis* (red), however, had initial pyruvate concentrations considerably lower than any of the other muscles studied (Figure 2). Pyruvate concentrations declined rapidly during the first 60 minutes post-mortem to low levels (<0.1 μmole per gram tissue) in all four muscles.

Initial lactate concentrations (Figure 3) were slightly lower ($p > 0.05$) in the *serratus ventralis* (red) than in the *rectus femoris* (red), *gluteus medius* (white), and *longissimus dorsi* (white). The post-mortem accumulation of lactate

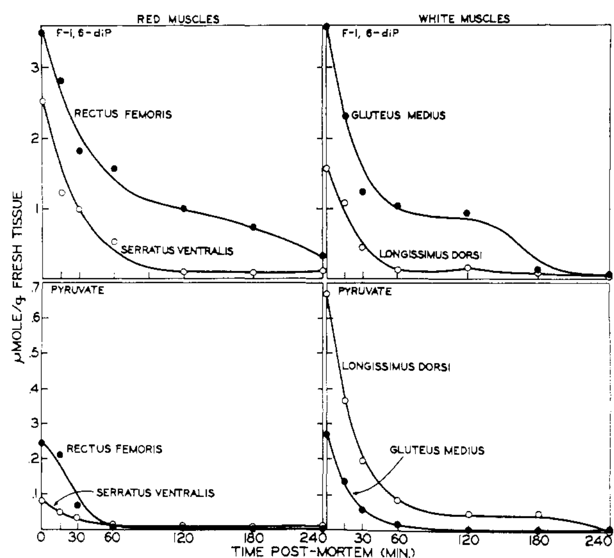


**Figure 2. Post-mortem concentrations of F-1,6-diP and pyruvate in two red and two white porcine muscles**

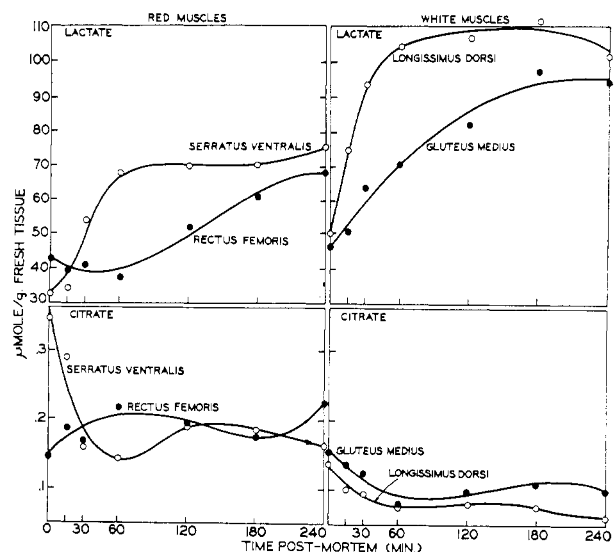


**Figure 3. Post-mortem concentrations of lactate and citrate in two red and two white porcine muscles**

in the *longissimus dorsi* (white) and *serratus ventralis* (red) was rapid during initial sampling periods (to 60 minutes). In contrast, lactate accumulation in the *gluteus medius* (white) was gradual for 180 minutes post-mortem, whereas lactate concentrations in the *rectus femoris* (red) changed little immediately (60 minutes) post-mortem, but subsequently increased during the remaining post-mortem period (Figure 3).

When porcine animals are exsanguinated, their muscles tend to have lower pH values than most other mammalian muscle (Kastenschmidt, 1966); therefore, the initial lactate values are not considered to be unusually high. Nevertheless, considerable glycolysis has obviously occurred (Table II), even in the *rectus femoris* (red). By the time of the initial sampling, the muscles have already been subjected to the anoxia associated with exsanguination. This anoxia and the stimulation which might also be incurred during this period probably account for the high levels of lactate in these muscles (Lister *et al.*, 1968).

CITRATE. The initial citrate content (Figure 3) was severalfold greater and the rate of decline immediately (60 minutes) post-mortem was considerably faster in the *serratus ventralis* (red) than in the *rectus femoris* (red), *gluteus medius* (white) and *longissimus dorsi* (white). However, after the rapid decline had occurred, during the first 60 minutes post-mortem, the citrate concentrations in the *serratus ventralis* (red) were subsequently maintained at or near the high levels observed in the *rectus femoris* (red).

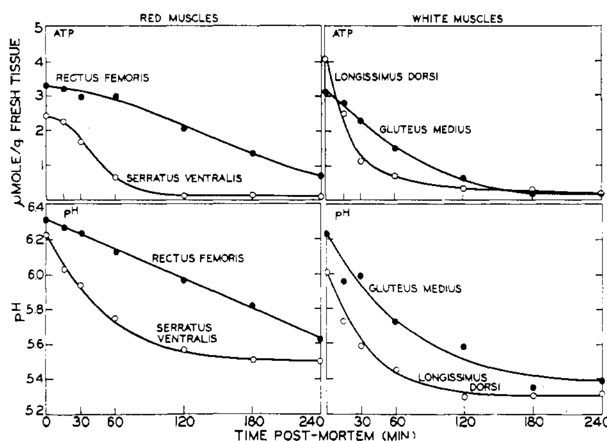


**Figure 4. Post-mortem concentrations of adenosine triphosphate (ATP) and post-mortem pH values in two red and two white porcine muscles**

No evidence was obtained that high citrate concentrations in the *serratus ventralis* inhibited glycolysis; however, compartmentalization of citrate in the *serratus ventralis* might have reduced cytosol concentrations of citrate so that it was insufficient to inhibit phosphofructokinase (Williamson and Jones, 1964). Garfinkel (1965), applying computer simulation to data from rabbit muscle, has shown that the velocity of phosphofructokinase increases greatly as muscle cells change from aerobic to anaerobic metabolism. Citrate was also most effective in inhibiting phosphofructokinase midway between an aerobic and an anaerobic state (Garfinkel, 1965). The rapid accumulation of lactate during the first 60 minutes post-mortem in the *gluteus medius* (white), *longissimus dorsi* (white) and *serratus ventralis* (red), concurrent with the decrease in citrate concentrations, suggested that there was an almost immediate transition to anaerobic glycolysis in these muscles post-mortem.

ATP AND PH. Initial ATP concentrations (Figure 4) were lower ($p < 0.05$) in the *serratus ventralis* (red) but higher ($p < 0.05$) in the *longissimus dorsi* (white) than in the *rectus femoris* (red) and *gluteus medius* (white). These data suggest that the activity of the *serratus ventralis* (red) is tonic in nature and capable of supporting aerobic metabolism, whereas the *longissimus dorsi* (white) has tetanic activity requiring high resting levels of ATP and an orientation toward anaerobic metabolism. Initial pH values were lower ($p < 0.05$) in the *longissimus dorsi* (white) than in the other muscles studied (Figure 4).

Both the ATP concentrations and pH levels (Figure 4) decreased rapidly post-mortem in the *gluteus medius* (white), *longissimus dorsi* (white) and *serratus ventralis* (red), whereas the decrease in ATP content in the *rectus femoris* (red) tended to lag prior to decreasing at a rate slower than in the other muscles studied. The constant pH decline in the *rectus femoris* (red) during early post-mortem periods (60 minutes), despite high ATP and low lactate concentrations, may be due to the increase of free inorganic phosphate (Pi) from the cleavage of the terminal phosphate of ATP. These increases in Pi in conjunction with high F-6-P concentrations could activate phosphofructokinase and easily overcome any inhibitory action of

**Table III. Lactic Dehydrogenase (LDH) Activity and LDH Isozyme Content of Red and White Muscles[a]**

| Lactic Dehydrogenase | Muscle: *Serratus ventralis* (red) | *Rectus femoris* (red) | *Gluteus medius* (white) | *Longissimus dorsi* (white) |
|---|---|---|---|---|
| Total activity[b] | 6.26 ± 0.61[c] | 10.95 ± 1.01 | 12.74 ± 0.67 | 14.95 ± 0.70 |
| Isozyme I[d] | 10.1 ± 0.7 | 4.4 ± 0.2 | 6.6 ± 1.0 | 4.1 ± 0.5 |
| Isozyme II[d] | 12.3 ± 1.5 | 5.7 ± 1.0 | 9.2 ± 1.4 | 7.4 ± 1.4 |
| Isozyme III[d] | 29.5 ± 2.2 | 18.1 ± 1.1 | 22.9 ± 1.9 | 24.1 ± 2.1 |
| Isozyme IV[d] | 14.3 ± 0.9 | 16.3 ± 1.4 | 16.8 ± 0.9 | 18.9 ± 1.6 |
| Isozyme V[d] | 33.8 ± 3.6 | 55.6 ± 2.2 | 44.4 ± 4.4 | 45.4 ± 3.4 |

[a] Muscle samples isolated immediately after exsanguination.
[b] Units × $10^{-5}$ per gram muscle tissue (Amador *et al.*, 1963).
[c] Mean value of six observations plus or minus standard error of the mean.
[d] Per cent density of the total density of all isozyme bands (recording electrophoresis densitometer).

either ATP or citrate (Vinuela *et al.*, 1963). In the *serratus ventralis* (red), initial ATP concentrations were low and probably insufficient to inhibit phosphofructokinase (Parmeggiani and Bowman, 1963; Passonneau and Lowry, 1963), while ADP and AMP probably accumulated during exsanguination to activate phosphofructokinase (Gevers and Krebs, 1966; Passonneau and Lowry, 1964; Underwood and Newsholme, 1965).

LACTIC DEHYDROGENASE (LDH) ACTIVITY AND LDH ISOZYME CONTENT. Total lactic dehydrogenase (LDH) activity (Table III) was higher ($P < 0.01$) in the white muscles (*gluteus medius* and *longissimus dorsi*) than in the red muscles (*serratus ventralis* and *rectus femoris*). These observations support previous findings of Blanchaer *et al.* (1963) and Dawson and Romanul (1964) and serve to emphasize the inverse relationship of lactic dehydrogenase activity with myoglobin content, succinic dehydrogenase activity and red fiber content.

In the *serratus ventralis* (red), LDH isozyme I, II and III content (Table III) was slightly higher and isozyme V content lower than in the other muscles studied. Several investigators (Blanchaer and Van Wijhe, 1962; Dawson and Romanul, 1964; Van Wijhe *et al.*, 1964) have noted high concentrations of LDH isozyme I (heart type) in red muscles and high concentrations of LDH isozyme V (muscle type) in white muscles. Kinetic properties of LDH isozymes indicate that isozyme I (heart type) is active at low pyruvate concentrations, whereas LDH isozyme V (muscle type) is active at high concentrations of pyruvate (Dawson and Kaplan, 1965; Dawson *et al.*, 1964; Plagemann *et al.*, 1960). The *rectus femoris*, although a red muscle, had a still higher LDH isozyme V content than the *gluteus medius* (white) and *longissimus dorsi* (white). These data are in disagreement with previous work (Dawson and Romanul, 1964; Van Wijhe *et al.*, 1964) and suggest that even though the *rectus femoris* is a red muscle, it still maintains a high level of lactic dehydrogenase activity and may be capable of a high degree of anaerobic metabolism during periods of anoxia.

LITERATURE CITED


Amador, E., Dorfman, L. E., Wacker, W. E. C., *Clin. Chem.* **9,** 391 (1963).
Beatty, C. H., Peterson, R. D., Basinger, G. M., Bocek, R. M., *Am. J. Physiol.* **210,** 404 (1966).
Beatty, C. H., Peterson, R. D., Bocek, R. M., *Am. J. Physiol.* **204,** 939 (1963).
Beecher, G. R., Briskey, E. J., Hoekstra, W. G., *J. Food Sci.* **30,** 477 (1965a).
Beecher, G. R., Cassens, R. G., Hoekstra, W. G., Briskey, E. J., *J. Food Sci.* **30,** 969 (1965b).
Blanchaer, M. C., Van Wijhe, M., *Am. J. Physiol.* **202,** 827 (1962).
Blanchaer, M. C., Van Wijhe, M., Mozersky, D., *J. Histochem. Cytochem.* **11,** 500 (1963).
Bocek, R. M., Oregon Regional Primate Research Center, Beaverton, Ore., private communication, 1964.
Bocek, R. M., Basinger, G. M., Beatty, C. H., *Am. J. Physiol.* **210,** 1108 (1966a).
Bocek, R. M., Peterson, R. D., Beatty, C. H., *Am. J. Physiol* **210,** 1101 (1966b).
Borchert, L. L., Briskey, E. J., *J. Food Sci.*, **30,** 138 (1965).
Brody, I. A., *Nature* **201,** 685 (1964).
Bücher, T., Hohorst, H. J., in "Methods of Enzymatic Analysis," H. U. Bergmeyer, Ed., p. 246, Academic Press, New York, 1963.
Bücher, T., Czak, R., Lamprecht, W., Latzke, E., in "Methods of Enzymatic Analysis," H. U. Bergmeyer, Ed., p. 253, Academic Press, New York, 1963.
Dawson, D. M., Kaplan, N. O., *J. Biol. Chem.* **240,** 3215 (1965).
Dawson, D. M., Goodfriend, T. L., Kaplan, N. O., *Science* **143,** 929 (1964).
Dawson, D. M., Romanul, F. C., *Arch. Neurol.* **11,** 369 (1964).
Domonkos, J., *Arch. Biochem. Biophys.* **95,** 138 (1961).
Ettinger, R. H., Goldbaum, L. R., Smith, L. H., Jr., *J. Biol. Chem.* **199,** 531 (1952).
Fritz, P. J., *Science* **150,** 364 (1965).
Garfinkel, D., in "Control of Energy Metabolism," B. Chance, R. W. Estabrook, and J. R. Williamson, Eds., p. 49, Academic Press, New York, 1965.
Garland, P. B., Randle, P. J., Newsholme, E. A., *Nature* **200,** 169 (1963).
Gevers, W., Krebs, H. A., *Biochem. J.* **98,** 720 (1966).
Hanson, C. P., Ph.D. thesis, University of Wisconsin, Madison, Wis., 1966.
Hegnauer, A. H., Cori, G. T., *J. Biol. Chem.* **105,** 691 (1934).
Hohorst, H. J., in "Methods of Enzymatic Analysis," H. U. Bergmeyer, Ed., p. 134, Academic Press, New York, 1963a.
Hohorst, H. J., in "Methods of Enzymatic Analysis," H. U. Bergmeyer, Ed., p. 266, Academic Press, New York, 1963b.
Kastenschmidt, L. L., Ph.D. thesis, University of Wisconsin, Madison, Wis., 1966.
Lamprecht, W., Trautschold, I., in "Methods of Enzymatic Analysis," H. U. Bergmeyer, Ed., p. 543, Academic Press, New York, 1963.
Lawrie, R. A., *Nature* **170,** 122 (1952).
Lister, D., Sair, R. A., Will, J. A., Cassens, R. G., Hoekstra, W. G., Briskey, E. J., 1968 submitted for publication.
Marsh, B. B., *Biochim. Biophys. Acta* **9,** 127 (1952).
Needham, D. M., *Physiol. Rev.* **6,** 1 (1926).
Newsholme, E. A., Randle, P. J., *Biochem. J.* **80,** 655 (1961).
Newsholme, E. A., Randle, P. J., *Biochem. J.* **93,** 641 (1964).
Ogata, T., *Acta Med. Okayama* **12,** 216 (1958).
Ogata, T., *J. Biochem.* **47,** 726 (1960).
Ozand, P., Narahara, H. T., *J. Biol. Chem.* **239,** 3146 (1964).
Parmeggiani, A., Bowman, R. H., *Biochem. Biophys. Res. Comm.* **12,** 268 (1963).
Passonneau, J. V., Lowry, O. H., *Biochem. Biophys. Res. Comm.* **13,** 272 (1963).
Passonneau, J. V., Lowry, O. H., in "Advances in Enzyme Regulation," G. Weber, Ed., Vol. **2,** p. 265, Pergamon Press, New York, 1964.
Paulson, G. D., M.S. thesis, University of Wisconsin, Madison, Wis., 1965.
Pedersen, P. L., Sacks, J., *Arch. Biochem. Biophys.* **112,** 548 (1965).
Pfleiderer, G., in "Methods of Enzymatic Analysis," H. U. Bergmeyer, Ed., p. 59, Academic Press, New York, 1963.
Plagemann, P. G. W., Gregory, K. F., Wroblewski, F., *J. Biol. Chem.* **235,** 2282 (1960).
Poel, W. E., *Am. J. Physiol.* **156,** 44 (1949).
Poulik, M. D., *Nature* **180,** 1477 (1957).
Romanul, F. C., *Arch. Neurol.* **11,** 355 (1964).
Sactor, B., Wormser-Shavit, E., *J. Biol. Chem.* **241,** 624 (1966).
Slater, E. C., in "The Structure and Function of Muscle," G. H. Bourne, Ed., Vol. **2,** p. 105, Academic Press, New York, 1960.
Steel, R. G. D., Torrie, J. H., "Principles and Procedures of Statistics," p. 101, McGraw-Hill, New York, 1960.
Underwood, A. H., Newsholme, E. A., *Biochem. J.* **95,** 868 (1965).
Van Wijhe, M., Blanchaer, M. C., George-Stubbs, S. St., *J. Histochem. Cytochem.* **12,** 608 (1964).
Vessell, E. W., Pool, P. E., *Proc. Natl. Acad. Sci.* **55,** 756 (1966).
Vinuela, E., Salas, M. L., Sols, A., *Biochem. Biophys. Res. Comm.* **12,** 140 (1963).
Williamson, J. R., Jones, E. A., *Nature* **203,** 1171 (1964).
Williamson, J. R., *J. Biol. Chem.* **240,** 2308 (1965).



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