THE DISTRIBUTION OF HISTOCHEMICALLY DEMONSTRABLE SUCCINIC DEHYDROGENASE AND OF MITOCHONDRIA IN TONGUE AND SKELETAL MUSCLES*

By M. WACHSTEIN, M.D., and E. MEISEL, Ph.D.

(From the Department of Pathology, St. Catherine's Hospital, Brooklyn)

Plate 129

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The distribution of histochemically demonstrable succinic dehydrogenase activity in various mammalian species has been described by a number of investigators (15, 17, 21, 25, 26). Enzymatic activity can be demonstrated in various organs often limited to certain anatomical structures. In the kidney, for instance, proximal convoluted tubules show a marked staining reaction, while glomeruli show none. In the heart all muscle fibers show, almost uniformly, considerable dehydrogenase activity. On the other hand, distinct variations in the staining intensity of individual fibers have been reported in skeletal muscles. In this paper, attention is drawn to the fact that the distribution of histochemically demonstrable succinic dehydrogenase in various muscles is similar to that of stainable mitochondria. Several muscles were examined and it was found that the tongue exhibited uniform enzymatic activity in all muscle fibers, a phenomenon otherwise seen only in the heart.

Material and Methods

Muscles were removed from rats and rabbits immediately after sacrifice. In addition, human material obtained at autopsy performed within 6 to 8 hours after death was also utilized. Muscles of all three species were taken from the lower extremities (rectus femoris), back (longissimus dorsi), abdominal wall (rectus abdominis), diaphragm, masseter, and tongue. Additional sections from the heart were regularly included. Unfixed frozen sections, 10 to 15 μ in thickness, were stained for succinic dehydrogenase activity using the technique of Seligman and Rutenburg (25) with some modifications (28). The incubation lasted from 10 to 60 minutes. Enzymatic activity was indicated by the deposition of reduced neotetrazolium (diformazan) in granular or occasionally crystalline form. Tissue blocks from heart, masseter, diaphragm, and tongue (rat and rabbit) were also fixed in a potassium dichromate–formalin solution and the paraffin sections were stained with iron hematoxylin according to Regaud's method (16) for the demonstration of mitochondria.

RESULTS

a. Succinic Dehydrogenase Activity:—In general, material from recently sacrificed animals gave more consistent staining results than human tissues

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removed at autopsy. However, most of the preparations from autopsy material were of good quality. The applicability of the succinic dehydrogenase technique to human autopsy material (heart and kidney) has been previously reported (27, 28 *a*).

In the various muscles investigated there occurred an unequal deposition of Diformazan. There was found, however, a constant pattern of variability in the various muscle groups, which was similar in the three species examined. The dye was deposited in the form of round or oval granules measuring approximately 1 to 2 μ. Occasionally it was deposited in recognizable crystalline structures. These dye deposits were arranged within the cytoplasm in a linear longitudinal fashion obviously lying between the muscle fibrils (Fig. 4). Occasionally, muscle fibers showed a more intense deposition of these granules in a narrow peripheral zone. In many fibers, no relation to cross-striation was noticed. Occasional ones, however, contained dye deposits arranged in a transverse fashion. These granules formed bands which were mostly located along the I discs, as evidenced by observation under the polarization microscope (Fig. 5). Dense accumulation of granules along these discs imparted a purplish beaded appearance. Nuclei showed no dye deposits. In all preparations, muscle fibers were the only structures in which reduction of neotetrazolium took place. A faint reaction was however noticed in the cytoplasm of the basal layer of the squamous cell epithelium covering the tongue.

In the muscles taken from the extremities, the back and the abdominal wall, a common distribution pattern was found with many larger muscle fibers showing only few granules while the fibers of small diameter often contained many more purplish diformazan deposits (Figs. 1 and 2). The cytoplasm of larger fibers remained either unstained or showed a slight pink hue. In sections taken from the diaphragm, more muscle fibers showed an intense staining reaction than in the muscle taken from the extremities. The majority of muscle fibers belonged to an intermediate type which contained a moderate amount of formazan granules. In sections taken from the masseter the staining reaction was considerably more marked. The majority of fibers showed dense accumulation of granules while only occasional ones were of the intermediate type. Finally, in the tongue all muscle fibers showed an almost uniform degree of high enzymatic activity. Within a few minutes of incubation, all the interlacing muscle bundles showed a strong reduction of neotetrazolium (Fig. 3).

b. Mitochondrial Stains:—In the heart muscle, mitochondria were found in all muscle fibers in large numbers. In preparations from the tongue, a similar appearance was observed. Mitochondria were arranged in dense longitudinal rows within all muscle fibers. In contrast, preparations from the leg muscle and the diaphragm showed mitochondria only in some muscles fibers, mostly in those of small diameter, while adjacent fibers showed only very scanty mitochondria or none at all.

COMMENT

In 1839, Schwann (24) first observed discrete sarcoplasmic granules in muscles. This observation was confirmed and extended by Kölliker (13). It soon became apparent that the amount of sarcoplasm, as well as of sarcoplasmic granules, varied considerably in different animals and in various muscles of the same animal. In general, the most constantly active muscle possessed the greatest amount of sarcoplasm and granules (12). However, the occurrence of histologic differences in content of granules and in fiber diameter have no direct relationship to the speed and nature of the contraction process (6).

A thorough examination of the staining characteristics of the sarcoplasmic granules was made by Bullard (3). He confirmed Kölliker's observation that the interstitial[1] granules of the striated muscles may be divided into true interstitial granules and into fat droplets. Both these components are factors bringing about the dark or cloudy appearance of muscle fibers. The application of phase microscopy as well as of the electron microscope has markedly advanced our knowledge concerning the structure of these granules (9, 11, 30). In electron micrographs of sectioned striated muscle (1, 2) and heart (31), mitochondria have a membrane and internal structure identical with that described in the mitochrondria of other organs by Palade (18).

Isolated mitochondria contain a high concentration of enzymes which are responsible for the oxidative breakdown of various substrates (5, 10, 14, 29). Within the muscle they appear to be the sole site of the succinic oxidase system and of several flavoproteins including $DPNH_2$ oxidase and cytochrome *c* reductase (22). *In vitro*, they are able to oxidise all members of the tricarboxylic acid cycle (23). A rough quantitative relationship between the number of mitochondria and oxidative activity has been demonstrated by several investigators. (5, 9, 19).

The histochemical technique for succinic dehydrogenase affords a simple method for the demonstration of one of the oxidative enzymes in muscle tissue. As has been pointed out by Goddard and Seligman for freshly teased preparation of thyroid (7) and liver (8), and by Malaty and Bourne (15) for frozen sections of skeletal and heart muscle, the reduced tetrazolium is deposited in the vicinity of mitochondria. Some of the stained granules appear to be genuine cell structures while others are, at least in part, due to dye deposited outside the organelles. The dye deposits are arranged in closely packed chains lying between muscle fibrils. Occasionally, an arrangement of granules in a transverse fashion, predominantly along the I bands, is recognized. This is in good agreement with findings in electron micrographs of sectioned muscles which show a predominating position of mitochondria opposite the I bands (1). A diffuse staining of cross striations, as described by Calcutt (4) in teased preparations

[1] "Interstitial" refers to the position of the granules in between the myocardial fibrils within the cytoplasm of striated muscle cells.

from mouse muscle, was seen occasionally only in human postmortem material. This probably represents non-specific absorption of reduced dye.

The distribution of formazan granules in various muscles is strikingly similar to that of the mitochondria in appropriately stained sections and is also in good correlation with biochemical findings on isolated mitochondria. As early as 1909, Regaud and Favre described large amounts of stainable mitochondria in the muscle fibers of the rabbit tongue (20). We observed the same phenomenon in both heart and tongue and a corresponding abundance of histochemically demonstrable succinic dehydrogenase activity. A similar correlation was also noted in the skeletal muscles. In the latter, however, stainable mitochondria appeared to be less numerous than the granules visualized with the succinic dehydrogenase technique. A similar observation has been made in preparations from the thyroid (7). Whether this phenomenon is due to unspecific absorption of reduced tetrazolium to cell structures other than mitochondria is not clarified at present.

Bennett (1) has recently pointed out that the mitochondria are prominent structures in electron micrographs of sectioned muscles but that they are not readily demonstrable with the light microscope. It would appear that the stain for succinic dehydrogenase affords a simple method for their visualization. A surprising observation was the abundance of dehydrogenase activity in the masticatory muscles and particularly in the tongue. The reason for this somewhat unexpected finding is at present not apparent and awaits further investigation.

SUMMARY

Various skeletal muscles show considerable variations in histochemically demonstrable succinic dehydrogenase activity. In muscles from the lower extremities, the back, and the abdominal wall, only some fibers reveal evidence of enzymatic staining. In the diaphragm and masseter more fibers react positively. In the tongue, however, all fibers show marked activity comparable to that found regularly in the heart. There is a close similarity in the distribution of histochemically demonstrable succinic dehydrogenase and stainable mitochondria in tissue sections.

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EXPLANATION OF PLATE 129

All figures are from frozen sections cut at 10 to 15 μ and stained for succinic dehydrogenase without counterstain.

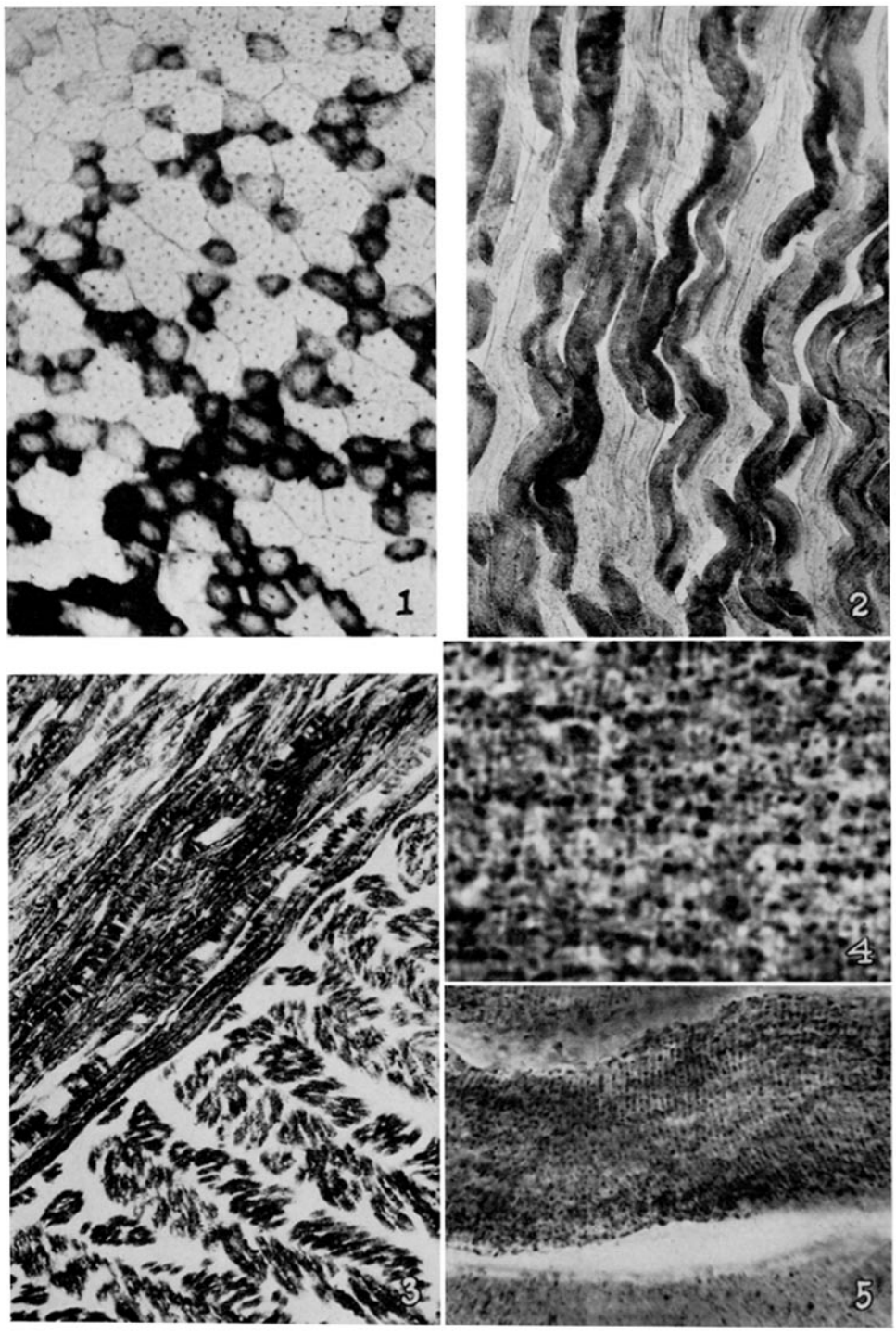
FIG. 1. Rectus femoris muscle from a rabbit shows activity mainly in smaller muscle fibers. × 100.

FIG. 2. Rectus femoris muscle from a patient who died from retroperitoneal sarcoma, removed 1 hour after death. × 100.

FIG. 3. Tongue from rabbit. Notice uniform activity in interlacing muscle bundles. × 100.

FIG. 4. Higher magnification of the section depicted in Fig. 3. Notice the arrangement of the granules in a longitudinal linear fashion. × 1600.

FIG. 5. Higher magnification from the section of human muscle depicted in Fig. 2. Notice the transverse arrangement of the granules which lie predominantly along the I discs. × 750.



(Wachstein and Meisel: Succinic dehydrogenase and mitochondria)