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Abstract

From the histochemical studies of succinic dehydrogenase on the striated muscle of the fish, frog, bird and mammal, the following results were obtained. (1) The red muscle fiber shows a higher succinic dehydrogenase activity, while the white muscle fiber a lower activity. The third type of muscle fiber "medium fiber", which is intermediate in the succinic dehydrogenase activity between the red and white muscle fiber, is observed practically in all of the striated muscle of mammals. (2) There is a good parallelism between succinic dehydrogenase activity and stainability to Sudan black B among the three types of muscle fibers. (3) From the nature of the constituent fibers, muscles can be divided into three groups, i. e., gastrocnemius type, soleus type and diaphragma type. (4) Those belonging to the gastrocnemius type are composed of three types of fibers, i. e., those of large size, low in activity of succinic dehydrogenase reaction and low in sudanophilicity; those of small size, high in enzymatic activity and in sudanophilicity; and those of medium size, moderate in enzymaticactivity and in sudanophilicity. (5) Those belonging to the soleus type, are composed of fibers almost equal in size which can be divided into two by the enzymatic activity and sudanophilicity, excepting the few with low enzymatic activity. (6) Those belonging to diaphragma type, are composed of three kinds of fibers showing different enzymatic activity as in the case of gastrocnemius type, but there is no correlation between the size and the enzymatic activity and sudanophilicity differing from the latter. (7) The difference in succinic dehydrogenase reaction as demonstrated among three types of fibers is due to the difference in number or in activity of mitochondria. (8) The pigeon breast muscle is composed mostly of red muscle fibers, and a few white muscle fibers, while the sparrow breast muscle is composed only of red muscle fibers. (9) The bloody colored muscle of the fish corresponds to the red muscle of the mammals. The white muscle of the fish is composed of three types of fibers. (10) The frog muscle is cmposed of three types of fibers.

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A HISTOCHEMICAL STUDY OF THE RED AND WHITE MUSCLE FIBERS

PART I ACTIVITY OF THE SUCCINOXYDASE SYSTEM IN MUSCLE FIBERS

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Since SELIGMAN and RUTENBERG[12] published the histochemical technique for succinic dehydrogenase using blue tetrazolium as hydrogen acceptor, histochemical studies of the enzyme system in mammalian striated muscles have been reported by several workers[2,7,8,10]. All of them stated that the mammalian striated muscle fibers reacted with a varying intensity, some of the fibers being dark colored and others pale.

Since the classic observation by RANVIER[9], it has been well established that the striated muscles of the rabbit consist of two different kinds of muscle, namely, red and white muscles. And it was further shown that the red muscle was a slow-contracting muscle, while the white muscle was a rapid-contracting muscle. GRÜTZNER[4] reported that the muscles of the higher mammals, including man, are composed of an intimate mixture of clear and opaque fibers, and showed that the former is of rapid contraction type and the latter of slow contraction type. DENNY-BROWN[3] has described that the red muscle fibers can be stained intensly with Sudan III, while the white muscle fibers faintly. In this report, the author will describe that there exists generally three types of muscle fibers, which show different succinic dehydrogenase activity, in a great variety of animals, namely, mammals, frogs, and fishes.

MATERIALS AND METHODS

The striated muscles of healthy adult frogs, fishes, sparrows, pigeons, mice, rats, guinea pigs, rabbits, cats, and monkeys of both sexes were used. The muscles were removed immediately after killing the animals anesthetized with ether. In the case of fishes and frogs, the muscles were removed after pithing.

For the histochemical demonstration of succinic dehydrogenase the

method discovered by SELIGMAN and RUTENBERG[11] and modified by WACHSTEIN et MEISEL[12] was applied. For lipids staining, the frozen sections of wet tissues, were cut at 30 μ, and immersed momentarily in 70% alcohol and then stained at room temperature with Sudan black B saturated in 70% alcohol for 20 min. Thereafter, the excess dye was removed by rinsing them quickly in 50% alcohol. Then the sections were washed in water and covered with glycerin.

OBSERVATIONS AND RESULTS

From the nature of the constracting fibers, muscles can be divided into three groups, i. e., gastrocnemius type, soleus type and diaphragma type.

Gastrocuemius type : as a typical example of the gastrocnemius type muscle, the findings on *M. gastrocnemius* of the cat will be described first. This muscle is composed of large fibers and small ones as shown in Figs. 1 and 2. The large fibers appearing clear (W) are stained pale showing the low activity of the enzyme and contain a few active granules. These fibers are stained faintly with Sudan black B, too, as shown in Fig. 3, indicating that these fibers may belong to the white muscle fibers according to the concept presented by DENNY-BROWN[3]. As is seen in Figs. 1 and 2, besides these white muscle fibers, there are small ones, almost half in diameter of white muscle fibers. They show a higher succinic dehydrogenase activity and contain a large number of active granules. They have an intense affinity to Sudan balck B, showing they may be the red muscle fibers. Judging from the histochemical patterns of succininc dehydrogenase and Sudan black B staining, however, it seems that there exists a third type of muscle fibers (Figs. 1, 2, and 3 (M)), whose diameter is almost two-thirds of that of the white muscle fibers,and which show a moderate enzymatic activity,intermediate between these of the red and the white muscle fibers, having the moderate number of the active granules. This type of fiber is named tentatively *"medium fiber"* in this paper. Then it can be said that this muscle consists of three types of fibers, i. e., red, white and medium fibers. In *M. gastrocnemius* of the cat, the percentage of the white, medium and red fibers is 33% : 19% : 48% respectively.

Besides *M. gastrocnemius* it has been revealed that in the cervical muscles (*M. biventer cervicis*, *M. complexus*, *M. longus colli* and *M. longus capitis*), back muscles (*M. trapezius*, *M. rhomboideus* and *M. serratus anterior*), abdominal muscles (*M. rectus abdominis*) and

limb muscles (*M. biceps brachi*, *M. biceps femoris*, *M. sartorius*, *M. gracilis* and *M. tibialis anterior*) of the cat, all of them are composed of fibers of the three different types in different percentages from each other. It has been further observed that the distribution of these three types of fibers is very different in each muscle. Generally, the muscle which is located near the body surface is rich in white muscle fibers, while the deep muscle is rich in medium and red muscle fibers. And in the same muscle, for example in *M. gastrocnemius*, the external parts are rich in white muscle fibers, while internal parts are rich in red and medium ones.

Soleus type : the muscles belonging to this type are deep red by naked eye. As shown in Figs. 4, most of the muscle fibers of *M. soleus* give an intesnse activity of succinic dehydrogenase except those few muscle fibers which show a low enzymatic activity. But even in these highly active muscle fibers, the two kinds of fibers can be distinguished from the activity of succinic dehydrogenase reaction (Fig. 4). The one (R_1) will be the typical red fibers, which is highly active in the succinic dehydrogenase being stained deeply by neotetrazolium and very rich in active granules, while the other (R_2) is slightly less active and slightly poorer in granules, though its enzymatic activity is higher than that of large fibers found in *M. gastrocnemius*. The fibers of R_2 may be classified as the medium fibers of *M. gastrocnemius*. In the staining pattern of Sudan black B, the former are stained intensly, while the latter less intensly stained as shwon in Figs. 5 and 6. The diameter of these two muscle fibers is almost the same and practically twice the diameter of the small muscle fiber of *M. gastrocnemius*. *M. semitendineus* of the cat also is classified as one of this type.

Diaphragma type : as shown in Fig. 7., the muscle fibers belonging to this type, showed almost the same pattern as that of gastrocnemius type, being composed of small, medium and large fibers. However, in this muscle type some of large fibers give a strong succinic dehydrogenase reaction and are stained deeply by Sudan black B. And yet there are some small fibers giving very slight reactions as that of large fibers of gastrocnemius. Then, in this type of muscle, there is no correlation between the size and histochemical reactions. Of course there can be seen some medium fibers like those in *M. gastrocnemius* as in Fig. 7.

The muscles of other mammals : the observation on various mammals other than cats (mice, rats, guinea pigs, rabbits and monkeys) has given almost the same results as in the case of cats as shown in Figs. 9, 10 and 11.

Pigeon breast muscle : this muscle is a deep red muscle by naked eye. Histochemical observations show that the fibers giving a high succinic dehydrogenase activity may correspond to the red muscle fibers distributed with a small number of large pale fibers (Fig. 12).

Sparrow breast musle : this muscle is also deep red, but different from that of pigeon, and it consists solely of one type of fibers, i. e., those with high succinic dehydrogenase activity (Fig. 13).

Bloody colored muscle of the fish (carp) : this muscle is made of only one type of fibers, whose succinic dehydrogenase activity is high, especially near the sarcolemma (Fig. 14).

White muscle of the fish (carp) : this muscle is composed of three types of fibers giving a similar picture to those of *M. gastrocnemius* of mammals. The small fibers are rich in active granules, medium fibers moderate and the large fibers poor (Fig. 15).

Sartorius of the frog : this muscle is pinkish by naked eye and composed of three types of fibers. But the frog muscles show lower succininc dehydrogenase activity as compared with the mammalian muscle (Fig. 16).

DISCUSSION

STEFANO LORENZINI first pointed out the striking difference in color among certain muscles of the limbs in the rabbit by naked eye (1678). And in 1880 the difference in the physiologic significance between these two muscles has been reported by RANVIER[9], who revealed that the white muscles are rapid-contracting and the red ones are slow-contracting. DENNY-BROWN (1929)[3] revealed that both of these two muscles are composed of red and white muscle fibers, showing that the red muscles contain more red fibers than white fibers and the white ones have more white fibers clearly distinguishable by Sudan III staining. Recently KONDO of our laboratory, revealed the same result by using Sudan black B instead of Sudan III, showing the Sudan black B to be superior to these Sudan III for the differentiation of these fibers.

As is mentioned above, by naked eye, the muscles of the mammals can be divided into two groups, i. e., the red and white muscle. However, judging from the results led by the histochemical observation, these muscles can be classified into three groups with different ratios of their constituting fibers from each other as described in detail, i. e., the gastrocnemius type, the soleus type and the diaphragma type.

(1) *Gasstrocnemius type :* the muscles of this type belong to the white muscles by naked eye. In the muscles belonging to this type, there

can be distiguished the three types of muscle fibers, i. e., the red, medium and white muscle fibers as described above, and there are close relationship between their enzymatic activity, sudanophilicity and size ; small fibers, namely red muscle fibers, having strongest enzymatic activity show the strongest sudanophilicity, while the large fibers, namely white muscle fibers, the weakest enzymatic reaction and the weakest sudanophilicity. And the medium sized ones are also of intermediate strength between the above two in their histochemical reactions.

(2) *Soleus types :* The muscles belonging to this type are deep red by naked eye. The muscle fibers of this type show an intense activity of succinic dehydrogenase except a few with a low enzymatic activity. And in these highly active muscle fibers, the two kinds of muscle fibers, i.e, red and medium fibers can be distinguished from the activity of succinic dehydrogenase reaction and sudanophilicity. The diameter of these two muscle fibers is almost the same and practially twice the diameter of the small muscle fibers of *M. gastrocnemius*. The few muscle fibers, which show a low enzymatic activity, will be classified as the white muscle fibers of *M. gastrocnemius*. *M. semitendineus* of the cat also revealed to be classified as one of this type.

(3) *Diaphragma type :* the histochemical observations show this muscle to be composed of the three types of muscle fibers, red, medium and white fibers as in the case of the gastrocenmius type. However, in this type of muscle there is no intimate correlation between the size of fiberes and their enzymatic activity or sudanophilicity.

Recently NACHMIAS and PADYKULA (1958)[5] reported that the three types of muscle fibers can be observed in the external ocular muscle of the rat and the diaphragma of the cat and that in other muscles only two types of fibers can be observed in the same muscle. However, in the present study, the author demonstrated that most of the muscles are consisted of three types of fibers.

It is interesting to note that the pigeon breast muscles, which have been often used for respiratory experiments by many investigators, are composed almost of very highly succinic dehydrogenase active fibers. The bloody colored muscles of the fish seem to correspond to the red muscle fiber of mammals, from the succinic dehydrogenase activity.

Succinic dehydrogenase is one of the enzymes related to the citric acid cycle. ODA[6] has demonstrated, using the electron microscope that cytochemical reactions of the succinic dehydrogenase system are limited in mitochodria. Besides these, recent biochemical studies reveal that many enzymes in the muscle are associated with the mitochondria, espe-

cially all of those in the citric acid cycle, and those responsible for the oxidation of fatty acids. Therefore, it is reasonably inferred that three types of muscle fibers, which have different numbers of mitochondria in them or vary in enzymatic activity of mitochondria, differ in their respiratory activity as well as in their physiological function. The medium fiber seems to be of a functional and metabolic activity intermediate between the red and white muscle fibers.

SUMMARY

From the histochemical studies of succinic dehydrogenase on the striated muscle of the fish, frog, bird and mammal, the following results were obtained.

(1) The red muscle fiber shows a higher succinic dehydrogenase activity, while the white muscle fiber a lower activity. The third type of muscle fiber "*medium fiber*", which is intermediate in the succinic dehydrogenase activity between the red and white muscle fiber, is observed practically in all of the striated muscle of mammals.

(2) There is a good parallelism between succinic dehydrogenase activity and stainability to Sudan black B among the three types of muscle fibers.

(3) From the nature of the constituent fibers, muscles can be divided into three groups, i. e., gastrocnemius type, soleus type and diaphragma type.

(4) Those belonging to the gastrocnemius type are composed of three types of fibers, i. e., those of large size, low in activity of succinic dehydrogenase reaction and low in sudanophilicity ; those of small size, high in enzymatic activity and in sudanophilicity ; and those of medium size, moderate in enzymatic activity and in sudanophilicity.

(5) Those belonging to the soleus type, are composed of fibers almost equal in size which can be divided into two by the enzymatic activity and sudanophilicity, excepting the few with low enzymatic activity.

(6) Those belonging to diaphragma type, are composed of three kinds of fibers showing different enzymatic activity as in the case of gastrocnemius type, but there is no correlation between the size and the enzymatic activity and sudanophilicity differing from the latter.

(7) The difference in succinic dehydrogenase reaction as demonstrated among three types of fibers is due to the difference in number or in activity of mitochondria.

(8) The pigeon breast muscle is composed mostly of red muscle fibers, and a few white muscle fibers, while the sparrow breast muscle is com-

posed only of red muscle fibers.

(9) The bloody colored muscle of the fish corresponds to the red muscle of the mammals. The white muscle of the fish is composed of three types of fibers.

(10) The frog muscle is cmposed of three types of fibers.

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EXPLANATION OF FIGURES

Fig. 1. Gastocnemius of the cat, cross section, succinic dehydrogenase. Note the three types of fibers, namely, the small fiber (R) has a high enzyme activity and contains more granules per unit area than the white fiber. And the large fiber (W) shows a lesser activity, and the third type of fiber, namely, *medium fiber* (M) shows a moderate activity. ×100.

Fig, 2. Gastrocnemius of the cat, cross section, succinic dehydrogenase. Note the three types of fibers. × 400.

Fig. 3. Gastrocnemius of the cat, cross section, Sudan black B staining. Note the same localization of fibers with succinic dehydrogense. × 400.

Fig. 4. Soleus of the cat, cross section, succinic dehydrogenase. Note the existence of a high active type of fiber (R_1) and slightly lesser active type of fiber (R_2). × 100.

Fig. 5. Soleus of the cat, cross section, Sudan black B. Note the two types of fibers (R_1 and R_2). × 100.

Fig. 6. Soleus of the cat, cross section, Sudan black B. × 400.

Fig. 7. Diaphragma of the cat, succinic dehydrogenase. Note the three types of fibers. × 100.

Fig. 8. Gastrocnemius of the mouce, succinic dehydrogenase. Note the three types of fibers. × 100.

Fig. 9. Gastrocnemius of the rat, succinic dehydrogenase. Note the three types of fibers. × 400.

Fig. 10. Tibialis anterior of the rabbit, succinic dehydrogenase. Note the three types of fibers. × 400.

Fig. 11. Gracilis of the monkey, succinic dehydrogenase. Note the three types of fibers. × 400.

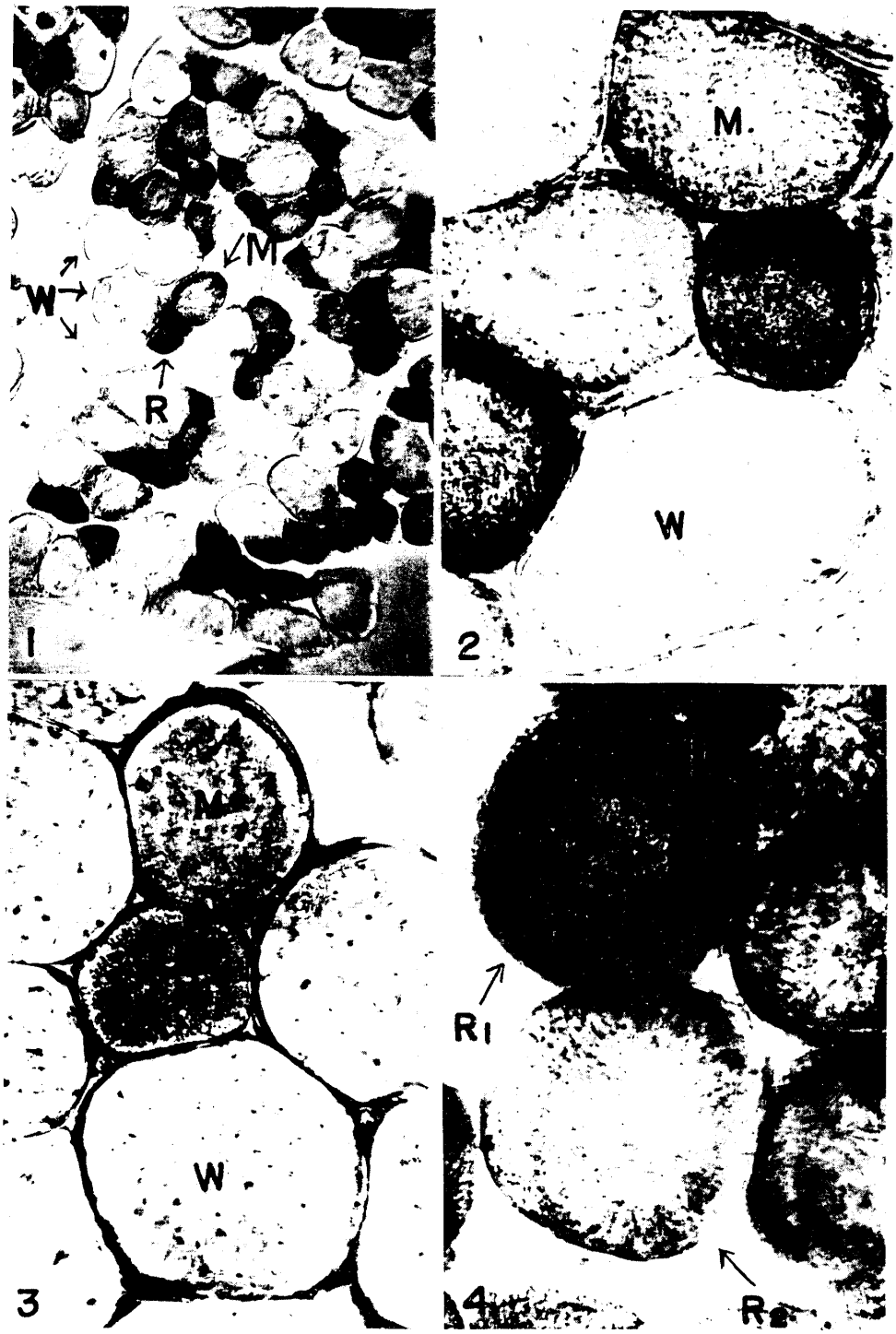
Fig. 12. Breast muscle of the pigeon, succinic dehydrogenase. Note the smaller fibers have the greater deposition of diformazan than the larger fibers. × 100.

Fig. 13. Breast muscle of the sparrow, succinic dehydrogenase. Note only one type of highly active fibers exists. × 100.

Fig. 14. Bloody colored muscle of the fish, cross section, succinic dehydrogenase. Note that only one type of highly active fibers exists. The enzyme activity is especially high in the subsarcolemmal position. × 100.

Fig. 15. White muscle of the fish, cross section, succinic dehydrogenase. Note the three types of fibers. × 100.

Fig. 16. Sartorius of the frog, succinic dehydrogenase. Note the three types of fibers. × 100.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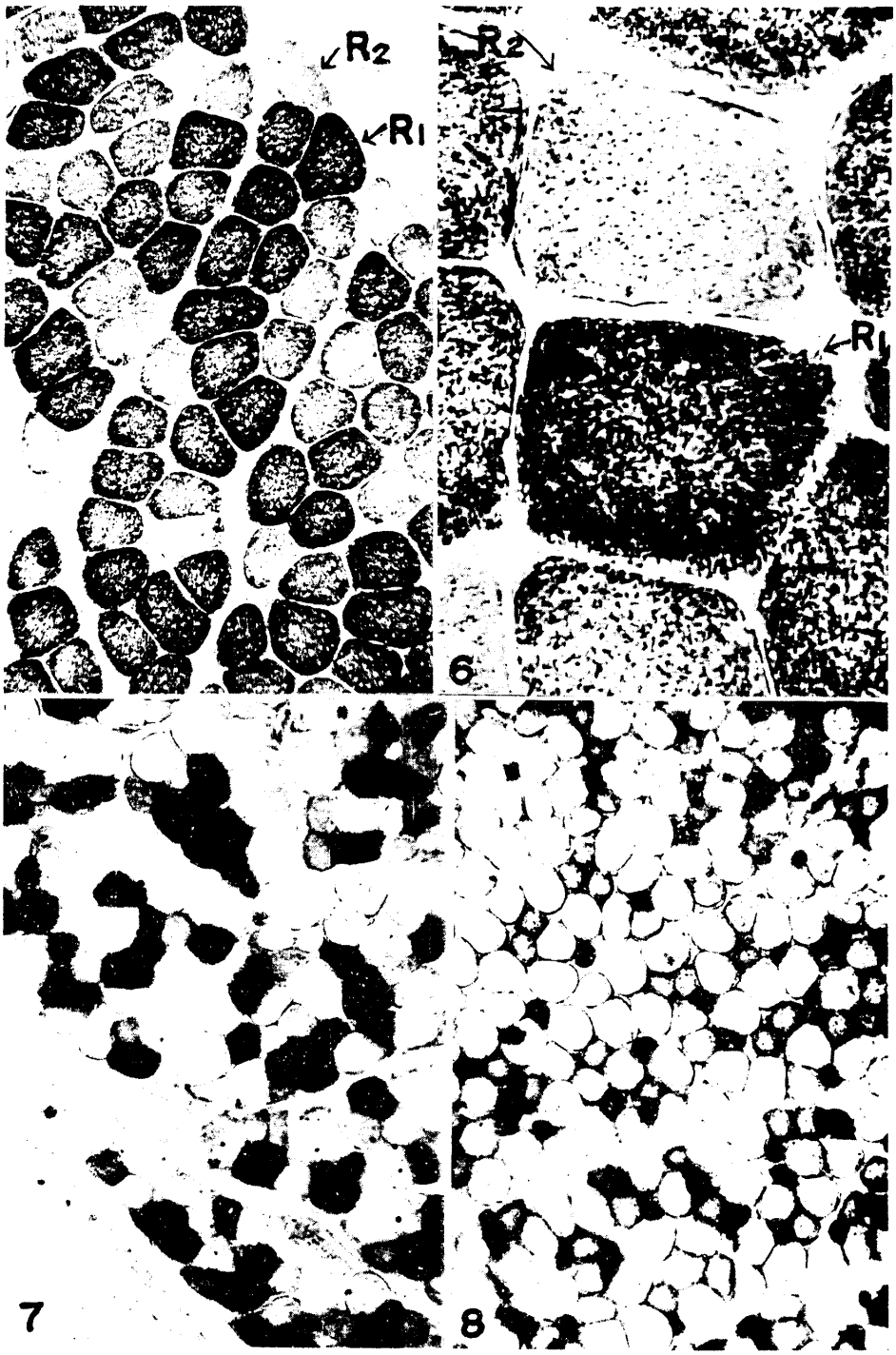
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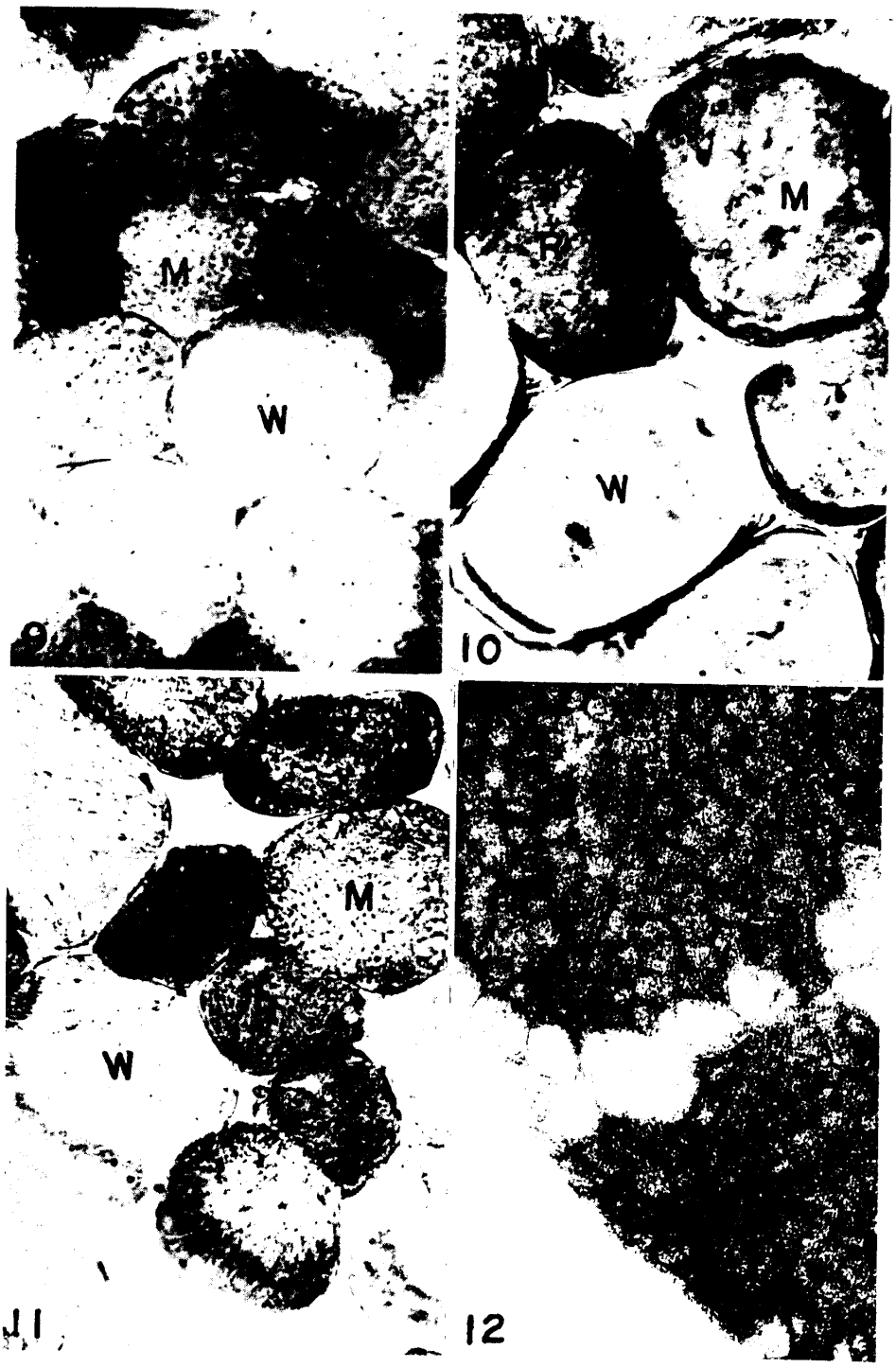
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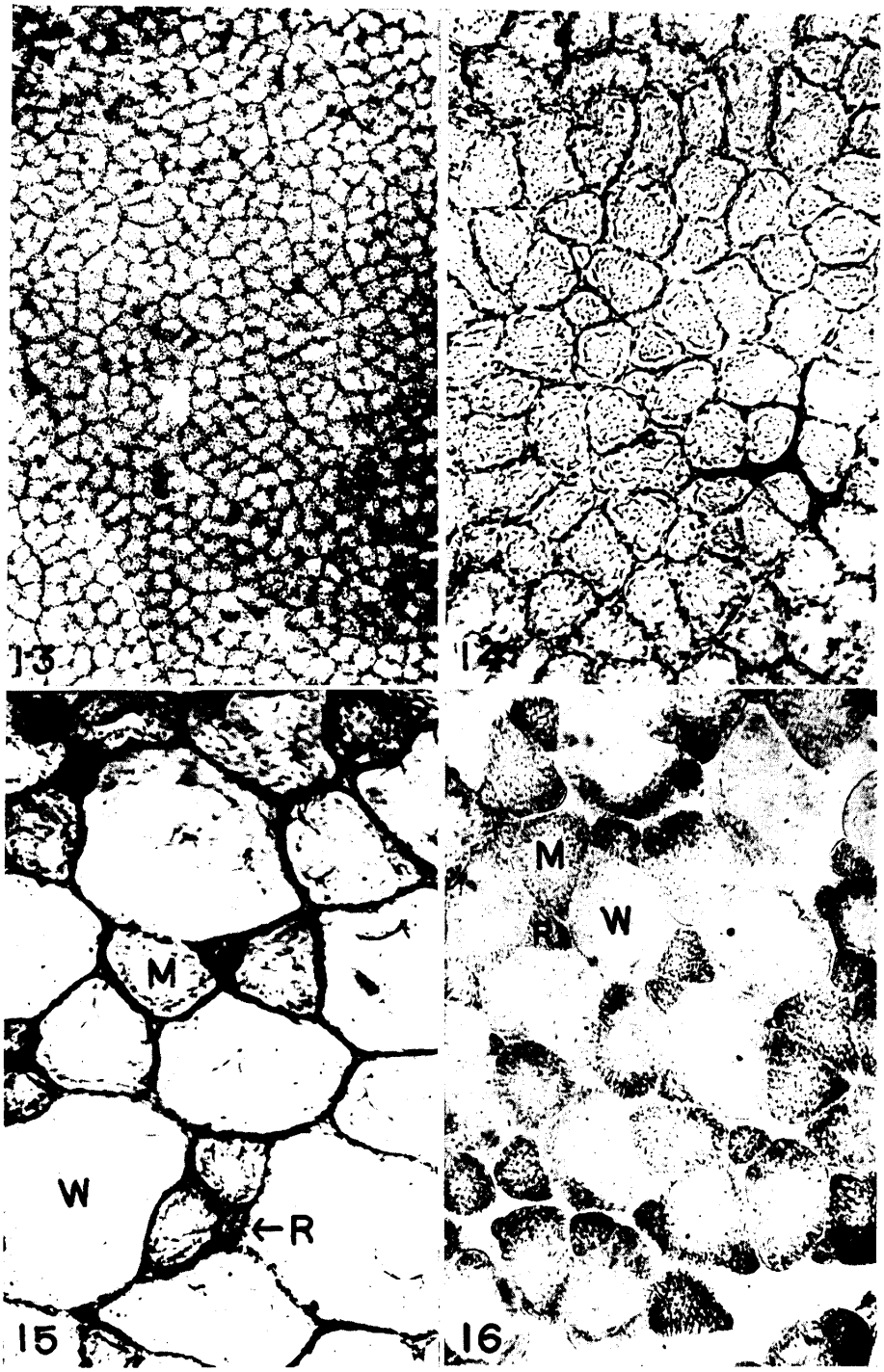
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