to obtain regulatory proteins. When tested, well washed actomyosin obtained by this procedure also had no Ca-sensitizing protein factor (not shown in Figure). It was thus concluded that both procedures presented in Figs. 1 and 2 were desirable enough to eliminate troponin–tropomyosin complex from myofibril.

Effect of postmortem storage of muscle on the sedimentation behavior of desensitized actomyosin

The sedimentation patterns of the desensitized actomyosins obtained by the procedure of Fig. 2 are shown in Fig. 3. Both actomyosins from 0–MF and 8–MF gave almost the same patterns consisting of three peaks of 21.2, 17.5 and 5.6 S for the former, and 20.8, 17.8 and 5.6 S for the latter, respectively. According to the value of $s_{20,w}$ of rabbit myosin reported by many workers,[12] the slowly-sedimenting component of 5.6 S was identified to be myosin released from actomyosin in 0.6 M KCl and other two components, to be actin-myosin complexes. As compared with the result on natural actomyosins from fresh and aged muscles reported by Okitani *et al.*,[13] the amount of the released myosin of desensitized actomyosin was remarkably greater than those of the two fast-sedimenting components. Since this difference may be caused by the elimination of regulatory proteins from actomyosin, the elimination of regulatory proteins seems to weaken the interaction between myosin and actin.

Effect of postmortem storage of muscle on the property of α-actinin

Some properties of the crude α-actinin extracted from myofibrils through the procedure of Fig. 1 were examined to investigate the effect of postmortem storage of muscle on α-actinin. As shown in Fig. 4, the ATPase enhancing ability of α-actinin from 8-MF did not differ from that of α-actinin from 0-MF. The sedimentation patterns of α-actinins from both 0-MF and 8-MF were almost the same, the $s_{20,w}$ of them being 6.0 S, as shown in Fig. 5. These two results in agreement with those of Arakawa *et al.*[4] may indicate that little change has occurred in the property of α-actinin during the postmortem storage of muscle.

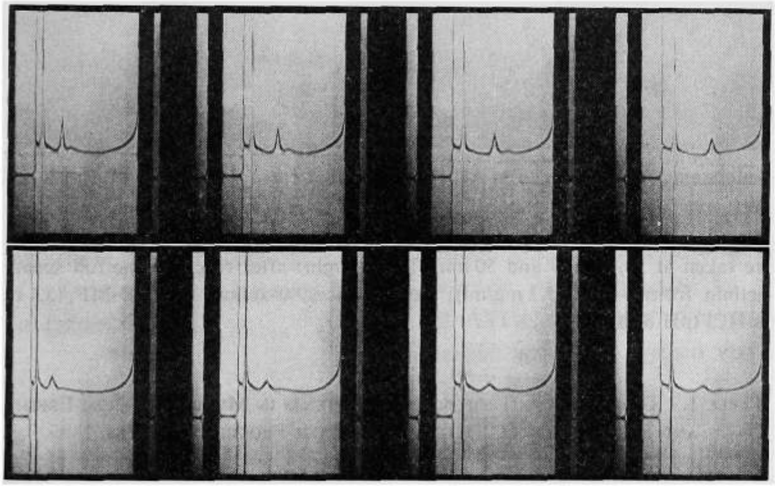

FIG. 3. Sedimentation Patterns of Desensitized Actomyosins Obtained from Myofibrils of Fresh and Stored Muscles.

Desensitized actomyosin prepared by the procedure of Fig. 2 was ultracentrifuged at 55,430 rpm and 10°C. Photographs were taken at 15, 20, 25 and 30 min (left to right) after reaching the full speed. Upper traces, actomyosin from 0–MF (4.3 mg/ml); Lower traces, actomyosin from 8–MF (6.2 mg/ml) in 20 mM Tris-HCl (pH 8.0).

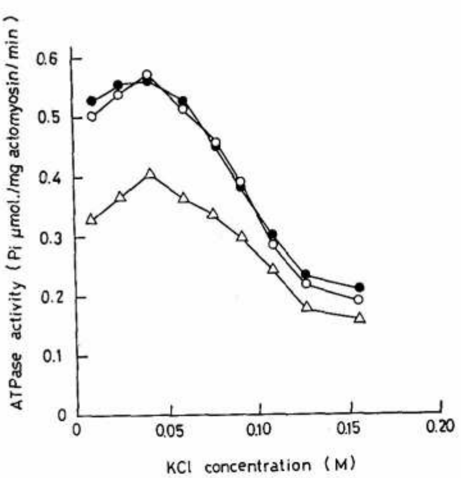

FIG. 4. Effect of α-Actinin on ATPase Activity of Desensitized Actomyosin.

ATPase assay: 0.125 mg/ml desensitized actomyosin obtained from 0-MF by the procedure of Fig. 1, 1 mM ATP, 1 mM $MgCl_2$, 25 mM Tris-HCl (pH 8.0) and KCl at the concentration cited on abscissa in the absence (triangles) or the presence (circles) of 0.05 mg/ml α-actinin. ○, α-actinin from 0-MF; ●, α-actinin from 8-MF.

Effect of postmortem storage of muscle on the amount and the property of troponin-tropomyosin complex

It may be reasonable to consider that, if myofibrils are treated through the procedures of Figs. 1 and 2 until Ca-sensitivity of myofibrils is completely removed, the amount of troponin-tropomyosin complex extracted at that time would reflect the content of the complex in myofibrils. Thus, the contents of troponin-tropomyosin complex obtained by these procedures were measured and shown in Tables I and II. The results indicated that the content of troponin-tropomyosin complex decreased to 70 or 80% of the initial one during 8 days storage. Each content of troponin and tropomyosin in the complex can be determined by the isoelectric precipitation because at pH 4.6 troponin is soluble and tropomyosin precipitates.[9] As shown in Tables I and II, the application of this method revealed clearly that troponin decreased more rapidly than tropomyosin during aging of muscle. About

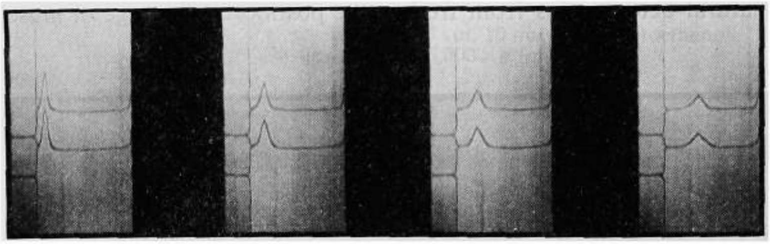

FIG. 5. Sedimentation Patterns of α-Actinins Obtained from Myofibrils of Fresh and Stored Muscles.

α-Actinin prepared by the procedure of Fig. 1 was ultracentrifuged at 60,000 rpm and 10°C. Photographs were taken at 10, 20, 30 and 50 min (left to right) after reaching the full speed. Upper traces, α-actinin from 0-MF (3.2 mg/ml); Lower traces, α-actinin from 8-MF (3.1 mg/ml) in 20 mM Tris-HCl (pH 8.0).

TABLE I. TROPONIN AND TROPOMYOSIN CONTENTS IN MYOFIBRILS FROM FRESH AND STORED MUSCLES DETERMINED BY THE PROCEDURE OF FIG. 1

Myofibril	Troponin–tropomyosin complex		Troponin		Tropomyosin	
	mg/g myofibril	%	mg/g myofibril	%	mg/g myofibril	%
0–MF	72.4	100	22.2	100	50.2	100
1–MF	72.2	99.7	22.5	101	49.7	99.0
8–MF	57.2	79.0	13.1	59.0	44.2	88.0

TABLE II. TROPONIN AND TROPOMYOSIN CONTENTS IN MYOFIBRILS FROM FRESH AND STORED MUSCLES DETERMINED BY THE PROCEDURE OF FIG. 2

Myofibril	Troponin–tropomyosin complex		Troponin		Tropomyosin	
	mg/g myofibril	%	mg/g myofibril	%	mg/g myofibril	%
0–MF	72.5	100	28.7	100	43.8	100
1–MF	70.8	97.7	24.5	85.4	46.3	106
8–MF	53.1	73.2	16.5	57.5	36.6	83.6

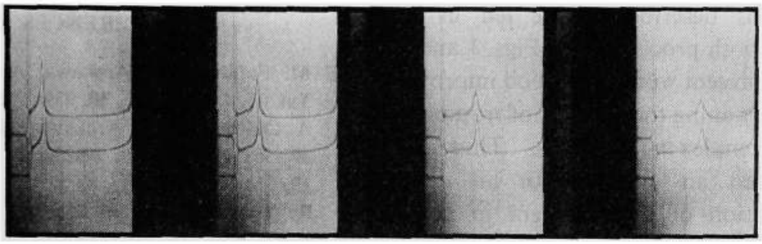

FIG. 6. Sedimentation Patterns of Troponin-Tropomyosin Complex Obtained from Myofibrils of Fresh and Stored Muscles.

Troponin-tropomyosin complex prepared by the procedure of Fig. 1 was ultracentrifuged at 55,430 rpm and 10°C. Photographs were taken at 30, 50, 75 and 105 min (left to right) after reaching the full speed. Upper traces, troponin-tropomyosin complex from 8–MF (3.0 mg/ml); Lower traces, troponin-tropomyosin complex from 0–MF (3.6 mg/ml) in 0.15 M KCl–10 mM Tris-maleate (pH 7.0).

40% of the initial content of troponin were lost whereas only 10 to 20% of that of tropomyosin were lost in 8-day storage of muscle.

Figure 6 shows the sedimentation diagrams of troponin-tropomyosin complex from myofibrils. The $s_{20,w}$ values of the complex from 0-MF were 4.6 S for the main peak and 2.6 S for the slowly-sedimenting one, while those of 8-MF were 4.8 and 2.6 S, respectively. No significant difference in the sedimentation patterns between 0-MF and 8-MF could be observed, although there was a notable difference in the share of troponin content between them, as presented in Tables I and II.

DISCUSSION

The present study showed that the postmortem storage of muscle decreased both troponin and tropomyosin contents in myofibril and that troponin decreased more rapidly than tropomyosin. This result contradicts the conclusion of Arakawa *et al.*[4] who have indicated that postmortem storage does not affect the amount of troponin-tropomyosin complex extracted from rabbit myofibril. Such discrepancy might be accounted for by the difference of the effectiveness of extraction procedures. In the present work, whether the extraction was completely performed or not was always checked by measuring the Ca-sensitivity of washed myofibril. In order to detect the contamination of myofibrillar proteins other than troponin-tropomyosin complex in the fraction precipitated between 40 and 75% ammonium sulfate saturation, properties of the fraction were examined by the measurement of ATPase activity. The result showed that neither actin nor myosin was contaminated in the fraction.

Maruyama *et al.*[14] have reported that the amount of troponin plus tropomyosin in muscle is about 8% of the total myofibrillar proteins. The corresponding value obtained by us, 7.2%, is in good agreement with that of Maruyama *et al.*, considering some loss during the preparation. On the other hand, the value obtained by Arakawa *et al.*[4] is far