After prolonged treatment with nicotine, the heart beat frequency did not increase with Morindin. When the order of treatment was reversed the effect of nicotine was unaffected (Fig. 2). Hence continuous

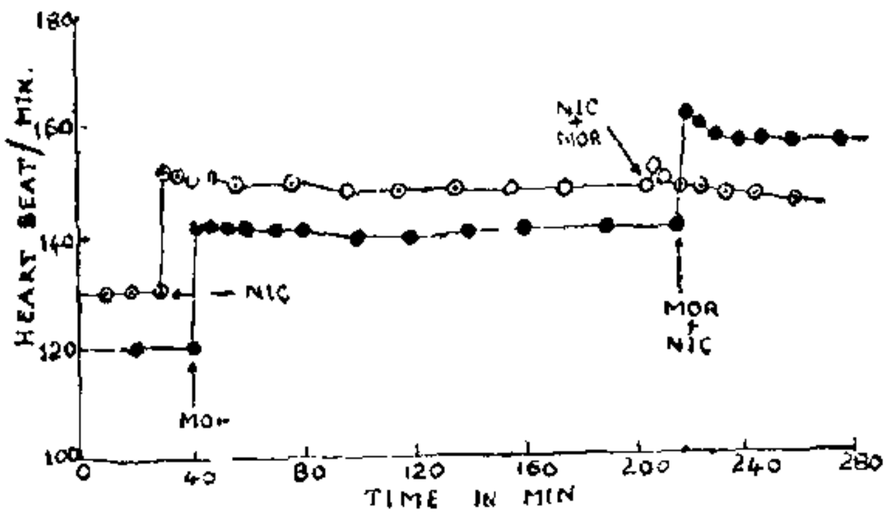

FIG. 2. Effect of nicotine (NIC, 5×10^{-6}) and morindin (MOR, 5×10^{-6}) on the isolated cockroach heart.

administration of nicotine lowers the stimulating action of the cardiac ganglia and completely paralyses in about 3–4 hours (Naidu[5]). Prolonged treatment by Morindin does not paralyse the cardiac ganglia.

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1. Taylor, A., Mekenna, G. F. and Burlage, H. M., *Texas Reports on Biology and Medicine*, 1956, 14, 538.
2. Bhatnagar, S. S. *et al.*, *The Wealth of India*, A Dictionary of Raw Materials and Industrial Products, C.S.I.R., New Delhi, 1962, 6, 423.
3. Rao, P. S. and Reddy, G. C. V., *Indian Journal of Chemistry* (in press).
4. Krijgsman, B. J., Dresden, D. and Berger, N. E., *Bull. Ent. Res.*, 1950, 41, 141.
5. Naidu, M. B., *Ibid*, 1955, 46, 205.

EFFECTS OF ULTRASONICATION, STORAGE AND FREEZE-THAWING ON THE MYOFIBRILLAR-ATPase ACTIVITY OF SKELETAL MUSCLES

THE ATPase activity of the myosin has been correlated with the speed of muscle shortening[1], and this has been studied extensively in the muscles of different mammals, lower vertebrates and invertebrates. The importance of ATPase activity has also been shown with respect to the muscle structure and its regulatory mechanisms[2]. Very few data are available on myofibrillar-ATPase activity of skeletal muscles under different conditions. The present study has been, therefore, intended to obtain some useful information in this direction. Both Ca^{++} and Mg^{++}–ATPase assays have been considered, since Ca^{++} has a role to play in contractibility of muscles whereas Mg^{++} has great physiological significance in various metabolic and regulatory processes involved in the muscle study.

In the present study besides the control, experiments were carried out under four experimental conditions (Table I): (i) fresh myofibrils ultrasonicated[3] at 0° C for 2 minutes with 15 seconds intervals by sonic irradiation at 20 kc with a Bronwill Biosonik Probe delivering 120 W, (ii) fresh myofibrils were stored at 8° C and the enzyme activity assayed after 70 hours and 120 hours intervals, (iii) myofibrils frozen at −20°C for 70 hours and 120 hours, thawed to room temperature and kept in cold (2–5° C) till the assays were completed, and (iv) muscle pieces *in toto* were frozen at −20° C for 120 hours and myofibrils were prepared after thawing the muscle pieces to room temperature and keeping in cold (2–5° C) till the assays were completed.

These studies were carried out on the *Pectoralis major* muscle of common weaver bird (*Ploceus philippinus*). In all, ten animals were sacrificed, the muscle of each animal was assayed for all the above four experimental conditions and also one set for control (normal); ATPase activity was measured within 12 hours. The myofibrils were obtained basically according to the method of Perry and Grey[4]. ATPase assays were carried out at pH 7·5 and 37° C. In the Ca^{++}-ATPase assay incubation medium, 40 mM Tris-HCl, 40 mM KCl, 10 mM $CaCl_2$, 0·2 ml of myofibrils and 3 mM of ATP were used, whereas in Mg^{++}-ATPase assay the concentration of $CaCl_2$ was 0·2 mM and in addition to this 3 mM of $MgSO_4$ solution was used, the rest of the reactants were the same in the both the assays. The final volume of the reactants in both assays (Ca^{++} and Mg^{++}-activated) was 1·5 ml. The reaction was started with the addition of ATP and was stopped by the addition of 1·5 ml of 10% trichloroacetic acid. The amount of Pi liberated and protein content present were measured by the methods of Reckstein and Herron[5] and Gornall *et al.*[6] respectively. All the values of specific-ATPase activity were expressed as μ moles of Pi liberated/mg protein/min.

It is evident from Table I that following ultrasonication, the increase in activity of Ca^{++} and Mg^{++}-ATPase assays is about 68% and 62% respectively. The increase in the activity of myofibrils after ultrasonication can be explained on the basis that sonic radiation is used to break the cell membranes and to release the cell contents; thus fragmentation may take place due to which the enzyme activity increases.

TABLE I

Effects of ultrasonication, storage and freeze-thawing on the myofibrillar-ATPase activity of skeletal muscles ATPase-activity expressed as μ moles of Pi liberated/mg protein/min.

Status of myofibril	Specific-ATPase activity			
	Ca^{++}-ATPase	P*	Mg^{++}-ATPase	P*
Control	0·302 ± 0·003		0·380 ± 0·023	
Ultrasonication	0·508 ± 0·002	< 0·001	0·615 ± 0·004	< 0·001
Storage (8° C) :				
for 70 hours	0·185 ± 0·006	< 0·001	0·227 ± 0·006	< 0·001
for 120 hours	0·166 ± 0·005	< 0·001	0·213 ± 0·004	< 0·001
Storage (− 20° C) :				
for 70 hours	0·168 ± 0·007	< 0·001	0·226 ± 0·014	< 0·001
for 120 hours	0·156 ± 0·005	< 0·001	0·205 ± 0·011	< 0·001
From frozen muscle (− 20° C) and stored for 120 hours	0·258 ± 0·005	< 0·001	0·298 ± 0·006	< 0·001

Values expressed as : means ± S.E. (standard error).

* Probability of significant difference in experimental values with respect to the controls.

Table I further shows that the values of Ca^{++} and Mg^{++}-ATPase assays during storage (8° C) and freezing (−20° C) of myofibrils decrease by 39 to 49%. However a minimum decrease in activity upto 15–20% was observed in myofibrils prepared from frozen muscle after storing for 120 hours (Table I). By and large an overall effect of various treatments was almost similar in both the Ca^{++} and Mg^{++}-activated ATPases. The decrease in the enzyme activities during storage (at 8° C and −20° C) can be explained on the basis of denaturation and also increased association from intermolecular hydrogen bonding and Van-der Waal forces. Formation of polymeric units of enzymes at low temperature could lead to decrease in specific activity[8].

On the basis of the results, it is clearly indicated (Table I) that the ATPase assay of skeletal muscle in fresh myofibrils, within 12 hours, appears ideal under normal circumstances when all facilities are available to carry out the assay. However, as revealed by the present studies, when the immediate facilities for either the preparation of myofibrils or the ATPase assay are not readily available, in cases where the work with rare animals is involved in any remote part of the world, the ATPase assay can be carried out in myofibrils prepared from frozen muscle, after storing for 120 hours at −20° C without much loss of enzyme activity.

The fact that when the muscle is frozen at −20° C for a period upto 120 hours, yet it shows only a 15–20% decrease in ATPase activity, further confirms the validity of the procedure of histochemically demonstrable Ca^{++} activated ATPase in muscle sections after freezing the muscle and storing at −20° C for a few days in a cryostat[9]. These studies thus reveal the pattern of differential effects of various treatments on myofibrillar-ATPase in pure isolated preparation of myofibrils.

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1. Barany, M., *J., Gen. Physiol.*, 1967, **50**, 197.
2. Lehman, W. and Szent-Gyorgyi, A. G., *Ibid.*, 1975, **66**, 1.
3. Blanchaer, M. C. and Griffiith, T. J., *Can. J. Biochem.*, 1966, **44**, 1527.
4. Perry, S. V. and Grey, T. C., *Biochem. J.*, 1956, **64**, 184.
5. Rockstein, M. and Herron, P. W., *Anal. Chem.*, 1951, **23**, 1500.
6. Gornall, A. G., Bardawill, C. J. and David, M. M., *J. Biol. Chem.*, 1949, **177**, 751.
7. Lehninger, A. L., *Biochemistry*, Worth Publishers, Inc., New York, 1975.
8. Meryman, H. T., *Cryobiology*, Academic Press, Inc. (London) Ltd., 1966.
9. Talesara, C. L. and Narang, V., Unpublished data.