Summary

Catalase preparations of different origin indicate different behaviour during the splitting of the H_2O_2. The monomolecular reaction constant in spite of the same amount of enzyme can be changed by variation of the beginning concentration of H_2O_2, or remain constant. An interpretation of the first case through inactivation of the enzyme leads to no satisfactory explanation.

ATPase Activity of Myofibrils and Granules Isolated from Muscle

Action of Progesterone and Desoxycorticosterone

It was explained in some recent researches made in our Institute[1] that the contraction of myofibrils isolated from guinea pig skeletal and heart muscle is inhibited by progesterone and desoxycorticosterone added in small amounts. As a continuation of the investigations on this question, the reaction of ATPase activity of both myofibrils and granules from muscle and heart towards the same two hormones was now tested. The results are described in this paper.

The fibrils were isolated from myocardic and skeletal muscle of guinea-pig according to SCHIKE and HASS[2], a very pure preparation being obtained with this method. Since tryptic digestion is involved in this method, a new procedure was also tried in order to avoid this step, since trypsin might produce some modifications of enzyme activity. The tissue was cut to slices with a freezing microtome and the slices kept in 0·25 M sucrose. The suspension was homogenated in a blendor for 30 s, then centrifuged for 10 min at 600 g. The sediment was again suspended in 0·25 M sucrose and submitted to a new centrifugation for 3 min at 400 g in order to eliminate the larger impurities. The supernatant fluid was then centrifuged for 10 min at 2000 g and the sediment particles washed with pH 6·9 borate buffer, until a perfectly clear supernatant fluid was obtained. The sediment consisted of pure myofibrils without cytoplasmatic elements contaminatio.

Many methods have been described in the last years permitting the separation of cytoplasmic granules (mitochondria?) from skeletal and heart muscle homogenate (PAUL et coll.[1], HARMAND and FEIGELSON[2], GREEN and coll.[3], PLAUT and PLAUT[4]). Since all these methods did not give optimal results because of the great contamination with myofibrils and cellular debris material, a new method was developed, including the preparation of these sections with a freezing microtome prior to homogenization in blendor. The guinea-pig cardiac and skeletal muscle was cut in 15μ slices with a freezing microtome and the slices kept in 50 ml of 0·25 M sucrose. The slices suspension was then homogenated in blendor for 45 s. The homogenate was centrifuged for 10 min at 600 g and the sediment was washed with 0·25 M sucrose and centrifuged again at 600 g for 10 min. The combined supernatant fluids were centrifuged at 12 000 g for 15 min. A Servall type S-S-1 centrifuge was used for this investigations and all procedures were performed in a cold room at + 5°C.

A very fine mitochondria preparation was obtained. The granules had characteristic spherical shape and appeared to be smaller than liver mitochondria. No modification was observed in their shape after suspension in water. They were able to be maintained for 2 days at 2°C without any evident modification of shape and enzymatic activity, but complete disintegration occured after this time. The term "mitochondria" is used in this paper for indicating these granules.

The ATPase activity of myofibrils and mitochondria was determined in the following manner[5]. As a rule the test system was composed of 1 ml myofibrils and mitochondria suspension, 1 ml substrate solution (1×10^{-3} M ATP), 0·05 ml of 0·1 M $CaCl_2$, and 0·35 ml of borate buffer, pH 6·9 (containing 0·05 M boric acid and 0·008 M NaOH), amounting to a total of 2·4 ml. The mixture was

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ATPase activity expressed by P/mg N

	N°	Normal	With progesterone	With desoxycorticosterone
Heart mitochondria	1	111·2	64·4	75·5
	2	89·5	65·8	86·2
	3	102·0	73·9	74·0
	4	105·3	79·4	82·5
	5	94·7	68·0	70·8
	Media	*100·54*	*70·18*	*73·80*
Skeletal muscle mitochondria	1	140·5	125·8	92·5
	2	185·5	145·5	147·3
	3	164·4	134·2	114·2
	4	170·0	140·0	135·5
	5	159·4	128·0	130·4
	Media	*162·52*	*134·70*	*123·98*
Skeletal muscle myofibrils prepared without trypsin	1	116·9	76·2	72·2
	2	120·3	98·5	90·8
	Media	*118·60*	*87·35*	*81·50*
Skeletal muscle myofibrils prepared with trypsin	1	125·5	100·5	107·5
	2	150·0	113·0	101·5
	Media	*137·75*	*106·70*	*104·50*
Heart myofibrils prepared without trypsin	1	98·1	78·6	75·3

incubated for 30 min at 37°C, with occasional agitation. At the end of the incubation period, the fibrils and mitochondria were precipitated by addition[1] 2 ml 20% trichloroacetic acid. After centrifuging, the inorganic P content of the clear supernatant fluid was determined with a BECKMAN Spectrophotometer by the method of FISKE and SUBBAROW[2], on 1 ml aliquot. To establish the influence upon enzymatic activity of progesterone and desoxycorticosterone, similar systems were prepared in which 0·35 ml borate buffer was replaced by either 0·35 ml of desoxycorticosterone glucoside 1×10^{-3} M solution or 0·35 ml of 1×10^{-3} M suspension of progesterone, which is not soluble in water. Appropriate blank determinations were carried out on mixtures from which either the mitochondria and fibrils or the substrate had been omitted. The P amounts obtained were substracted. Corrected values were expressed as a ration γ P/mgN.

The total nitrogen content was determined by usual micro Kjeldahl method.

The results of experiments are summarized in the Table.

As shown in the Table, desoxycorticosterone, as well as progesterone, produced a strong inhibitory action on ATPase activity of both cardiac and skeletal muscle fibrils and mitochondria. Such an inhibition was about 30% for heart mitochondria and 20% for muscle mitochondria. Similar inhibition values were obtained with both cardiac and skeletal muscle fibrils. Higher activities were shown by fibrils prepared with the first method, including tryptic digestion, than by those isolated without trypsin. This fact agrees with PERRY's observation on fresh fibrils.

On the grounds of the results of the present experiments, the inhibition on ATPase activity by both steroid hormones may be responsible for the inhibition of the contraction of myofibrils which was previously described.

Since no considerable differences between mitochondria and fibrils ATPase activities were observed, the question arises whether muscular granules are derived from the disrupture of myofibrils.

Further researches on this problem are in progress.

MARIA ASSUNTA MOR

Department of General Pathology and Bacteriology, University of Genoa, March 21, 1953.

Riassunto

L'autrice ha studiato l'attività ATPasica dei mitocondri e miofibrille isolate da muscolo e cuore di cavia ed ha trovato: 1) Il progesterone e il desossicorticosterone esercitano una azione inibente su tale attività. 2) Le miofibrille preparate con tripsina hanno una attività maggiore di quelle preparate con saccarosio. 3) Non esiste notevole differenza tra attività ATPasica dei mitochondri e quella delle miofibrille.

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Action of Papain and of Trypsin on the Morphology and some Enzymatic Activities of Isolated Mitochondria

It was shown in previous experiments that some enzymatic principles present in liver homogenates exert an inactivating influence on succinoxidase of isolated mitochondria. Although it was reported by MEYER[1], and more recently by ZOLLINGER[2], that mitochondria show a great resistance against proteases (pepsin in acid, trypsin in alcaline medium), the action of some purified proteases, namely papain and trypsin, on the morphology and on the enzymatic activities of isolated mitochondria was studied in this work in order to see whether the inactivating influence of homogenates might be connected with their protease activity.

Papain used in this work was a crude preparation from the latex of *Carica papaya*. Trypsin was a crystalline preparation from pancreas, isolated according to KUNITZ[3]. Protease activity of both enzymes was tested by titrating with 0·01 N NaOH the acidic groups liberated from a denaturated hemoglobin 2% solution after an incubation time of 30 min at 38°C with 1 mg of the enzyme material. Both papain and trypsin were found to be very active, and devoid of desoxyribonuclease, ribonuclease, lecithinase and lipase activities, as tested respectively with the methods of LASKOWSKI and SEIDEL[4], KUNITZ[5], MACFARLANE[6], WILLSTÄTTER[7].

Mitochondria were isolated from 1 g liver with the technique of HOGEBOOM *et al.*[8], by centrifugation of 0·25 *M* sucrose 10% homogenates, albino rats from a selected strain being used as experimental animals. After a previous centrifugation at 1500 g for 15 min, which produced sedimentation of tissue débris, unbroken cells, red cells, nuclei and of some mitochondria, the main portion of mitochondria was collected by a second centrifugation at 12,000 g in the SS-I SERVALL Centrifuge, in the cold room at a maximal temperature of 5°C. Washings were made by resuspension with 0·25 *M* sucrose and sedimentation at 12,000 g for 30 min. Morphological observations were made with the ZEISS-WINKEL phase contrast microscope. Turbidimetric readings were made with a BECKMAN Mod. DU spectrophotometer, by measuring the extinction at 750 mμ.

Enzymatic activities were studied at 38°C in a WARBURG conventional apparatus, the experiments lasting 1 h. After the final sedimentation of mitochondria, the pellet on the bottom of the centrifuge tube was suspended into 6 ml of 0·067 *M* phosphate buffer solution (pH 7·4) and the homogeneous suspension was distributed into 2 WARBURG vessels, 3 ml for each of them. 0·2 ml of sodium succinate, or of 0·2 *M* dl-alanine or 1 *M* sodium lactate were added respectively for the study of succinic acid oxidase, d-aminoacid oxidase and lactic acid oxidase, final concentration of substrates being 0·0125 *M* for succinate and dl-alanine and 0·0625 *M* for lactate. 0·20 ml KOH 30% were placed in the central well. Gaseous environment was air.

Cytochrome oxidase activity was studied by the method of STOTZ *et al.*[9]. 0·2 ml of a mitochondrial suspension prepared by diluting the particles isolated from 1 g of tissue to 5 ml with 0·067 *M* phosphate buffer were used as enzyme source. Concentration of added cytochrome *c* was $2{\cdot}4 \times 10^{-4}$ *M* and that of hydroquinone was 7 mg pro vessel. Controls for autoxidation

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