Als Versuchsobjekt diente in allen Versuchen die «Toronto»-Variante CN 2000 von *C. diphtheriae*, die von Dr. F. V. LINGGOOD, Wellcome Research Laboratories, Beckenham, England, freundlichst zu unserer Verfügung gestellt wurde.

L. JÄNNES

Arzneimittelfabrik Orion Oy., Mikrobiologische Abteilung, Helsinki, Finnland, den 6. Mai 1953.

Summary

The possible role of tricarboxylic acid cycle in the metabolism of *C. diphtheriae* was studied. The occurence of succinic, malic, citric, pyruvic, oxaloacetic, and α-ketoglutaric acid in the culture filtrate and, on the other hand, the dehydrogenation of succinic, malic, citric, and acetic acid by freeze-dried bacteria could be demonstrated.

On the Inactivating Properties of Liver Homogenates on Succinoxidase Activity of Isolated Mitochondria

Many techniques have been described in recent years permitting the fractionation of the cell. It was found that many enzymatic activities, and particularly those concerned with the respiratory processes, are localized in the "mitochondrial fraction" (HOGEBOOM *et al.*[1]; SCHNEIDER[2]). It was established in a previous paper of the author[3] that fresh serum from many kinds of animals produces slight acceleration of the mitochondrial succinoxidase activity, which is changed to a strong inhibition after incubation at 37°C for 30 min. This effect was attributed to the lipase content of the tested sera.

It seemed interesting to study if a similar inactivating property is present also in liver homogenates. This paper shows that some inactivating principles, probably of enzymatic nature, are in fact contained in the supernatant fluid after the sedimentation of mitochondria and microsomes from liver homogenates.

Mitochondria were isolated with 0·25 *M* sucrose from 10% liver homogenates. White rats from a selected strain and weighing 90–100 g, were used in these experiments. After a first centrifugation at 1500 g for 15 min, which produced the sedimentation of tissue débris, undamaged cells, nuclei, red cells and some mitochondria, the main portion of mitochondria was sedimented by a second centrifugation at 12,000 g in the SS-I SERVALL Centrifuge, in the cold room at 5°C. The supernatant fluid after this centrifugation, which contained microsomes and the soluble part of the cytoplasm, was submitted to a new centrifugation at 22,000 g for 2 h. The sediment was mainly made up of microsomes. Mitochondria were washed twice with 0·25 *M* sucrose, and then suspended in 0·067 *M* phosphate buffer solution (pH 7·4). Tests for succinoxidase activity were made manometrically in a WARBURG conventional apparatus at 38°C, by measuring the O_2-uptake of a suspension of mitochondria corresponding to 0·25 g of the tissue in the presence of 0·0125 *M* sodium succinate, 0·2 ml 30% KOH being placed in the central well of the vessels. Neither $CaCl_2$ nor cytochrome *c* were added in the main experiments, because it was observed in previous work that no influence is exerted by these substances when the experiments are made with an excess of enzyme material. Succinoxidase activity of homogenates was similarly studied, a suspension corresponding to 0·25 g of tissue beeing placed in each vessel. Each experiment lasted 1 h, but readings were made every 10 min. N content of samples of the preparations was determined at the end of each experiment by microkjeldhal technique and QO_2 (N) values (i.e. mm^3 O_2 consumed in 1 h/mg of N) were calculated. In a first series of experiments the effect of incubation at 38°C on succinoxidase activity of liver homogenates and isolated mitochondria was studied. Mitochondria and homogenates were prepared from 1 g liver, and the final volume of each suspension was 12 ml, 0·067 *M* phosphate buffer (pH 7·4) being used as a suspension medium. Incubation at 38°C was performed in a thermostat, and the succinoxidase activity of 3 ml samples withdrawn from each suspension was determined at periods of 0,1, 2, 3 h. The whole procedure was made in sterile vessels, in order to avoid any bacterial interference. Sterility controls were made at the end of each experiment. Table I shows that, while the enzymatic activity of the homogenates decreases strongly as a consequence of incubation at 38°C, that of the mitochondrial suspension decreases at a very lower rate. After 3 h of incubation, succinoxidase activity of homogenates is decreased 47·5%, that of mitochondria only 16·9%.

Table II

Difference of activity between mitochondria isolated from fresh homogenates and mitochondria isolated from homogenates incubated at 38°C

Time of incubation	Mitochondria from fresh homogenates	Mitochondria from incubated homogenates
Non-incubated	218·0	
1 h	215·0	170·1
2 h	200·6	140·6

In a second series of experiments, succinoxidase activity of mitochondria isolated from homogenates incubated at 38°C for 1 h and for 2 h was compared with that of mitochondria isolated from a fresh homogenate

Table I

Influence of incubation at 38°C on succinoxidase activity of liver homogenates and mitochondria (The data are expressed at $QO_2(N) \pm \sigma$ = standard deviation)

	Number of experiments	Non-incubated controls	Samples incubated for		
			1 h	2 h	3 h
Homogenates	5	51·6 ± 0·9	40·8 ± 1·9	33·1 ± 0·9	27·1 ± 1·4
Inactivation %			20·9	35·8	47·5
Mitochondria	5	216·5 ± 2·3	210·7 ± 0·4	199·3 ± 2·0	179·5 ± 2·1
Inactivation %			2·5	10·5	16·9

[1] G. H. HOGEBOOM, A. CLAUDE, and R. D. HOTCHKISS, J. Biol. Chem. *165*, 615 (1946).
[2] W. C. SCHNEIDER, J. Biol. Chem. *165*, 585 (1946).
[3] M. U. DIANZANI, Exper. 7, 102 (1951).

and incubated at 38°C for 1 and for 2 h. As shown in Table II, succinoxidase activity of mitochondria isolated from incubated homogenates is remarkably lower than that of mitochondria isolated from fresh homogenates.

In other experiments, the effect of the incubation with supernatant fluid on the succinoxidase activity of isolated mitochondria was studied. Mitochondria from 1 g of liver were suspended into 12 ml of 0·067 *M* phosphate buffer, divided into 4 aliquots of 3 ml each and allowed to stand in a thermostat at 38°C. 2 ml of the supernatant fluid coming from the same homogenate were added to 3 of 4 aliquots, while the first one was used as a control at time *O*. Succinoxidase activities of incubated aliquots were tested after 1, 2, 3 h. After the incubation time,

Table III
Influence of incubation with supernatant fluid on succinoxidase activity of isolated mitochondria

	Non-incubated controls	Samples incubated with supernatant fluid for		
		1 h	2 h	3 h
Number of experiments . .	3	3	3	3
$QO_2(N)$	211·9	185·2	174·4	155·9
$\sigma \pm$	3·5	4·4	5·0	5·2
Inactivation %		12·6	17·7	26·4

each sample was submitted to a centrifugation at 22,000 g for 30 min and succinoxidase activity was tested on the sediment. As shown in Table III, incubation with the supernatant fluid produces a definite inactivation of the enzymatic activity. This inactivation did not occur when mitochondria were incubated with boiled, instead of with crude, supernatant fluids. Potentiometric determinations of pH of the suspensions were made after 3 h of incubation, but the very slight decrease which was observed seemed not sufficient to justify such a strong inactivation of enzymatic activity.

In another group of experiments, the influence of the microsome fraction on mitochondria succinoxidase was studied after incubation for 1, 2 and 3 h at 38°C, but no influence was observed.

It appears that one or more principles, probably of enzymatic nature, contained in the supernatant fluid are responsible for the inactivation phenomenon observed.

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Department of General Pathology, University of Genoa, March 8, 1953.

Résumé

L'auteur a étudié l'action des liqueurs surnageantes après la sédimentation des mitochondries et des microsomes de l'homogénate de foie sur l'activité succinoxidasique des mitochondries isolées. Il a trouvé une action inhibitrice qui disparaît après ébullition. Les microsomes n'ont aucune influence.

Substances fluorescentes du type ptérinique dans la peau ou les yeux de la grenouille *(Rana nigromaculata)* et leurs transformations photochimiques

Goda[1], en 1941, a nommé une substance fluorescente isolée de la peau dorsale de *Rana nigromaculata*. Selon lui, le pyrrol-chrome joue un rôle physiologique important.

Nous avons signalé dans d'autres travaux les propriétés des substances fluorescentes du type ptérinique des divers animaux (vers à soie[1], papillon, carpe[2], crapaud[3],grenouille[4],etc.) et le rôle de telles substances dans la mélanogénèse[5].

Nous donnerons ici brièvement nos résultats sur les substances fluorescentes de *Rana nigromaculata*.

1° Présence de plusieurs substances fluorescentes dans la peau dorsale (noire) et ventrale (blanche), dans les yeux, et leurs propriétés. Nous avons proposé le nom collectif de «rana-chrome» pour les substances fluorescentes extraites de tels tissus par le méthanol, l'ammoniaque ou l'acide acétique. Les rana-chromes sont des substances désignées ici (Tableau) par des chiffres (1–6) qui correspondent aux termes d'une échelle basée sur la valeur décroissante de *Rf* (dissolvant; butanol: acide acétique : eau 4 : 1 : 1) du chromatogramme sur papier.

On découpe ces taches fluorescentes du chromatogramme et on en extrait la substance par le méthanol, l'ammoniaque ou l'eau. Ainsi nous avons examiné les propriétés et les spectres d'absorption des pigments, dont quelques-uns ont pu être rapportés à des substances déjà connues (Tableau).

Les rana-chromes sont en général thermostables. Le rana-chrome 1 est un cristal cubique, coloré en jaune clair, dont la solution aqueuse a la fluorescence bleu ciel. Cette substance correspond donc au pyrrol-chrome même et n'est pas tout à fait identique à la fluorescyanine qui pourtant avait été désignée comme étant le pyrrol-chrome par les savants français[6] (voir Tableau).

Propriétés de plusieurs substances fluorescentes isolées de la peau et les yeux de *Rana nigromaculata*

Substances fluorescentes	Couleur de la fluorescence à pH 7	Valeur de *Rf*
Rana-chrome 1, pyrrol-chrome	bleu ciel	0,46
Rana-chrome 2, riboflavine	jaune	0,43
Rana-chrome 3	bleu ciel	0,40
Rana-chrome 4, fluorescyanine ou ichtyoptérine (?)	violet	0,33
Rana-chrome 4B	bleu ciel	0,24
Rana-chrome 5, acide d'Angier	bleu ciel	0,22
Rana-chrome 6, flavine-adénine-dinucléotide . . .	jaune	0,06

Le rana-chrome 4 est un cristal aciculéiforme blanc. La solution aqueuse présente une fluorescence violette, elle est réductible par l'hydrosulfite, mais récupère, par simple agitation dans l'air, sa fluorescence. D'ailleurs, cette substance a donné jusqu'à présent la même valeur

[1] T. Goda, J. Fac. Sci. Imp. Univ. Tokyo, Sec. IV *5*, 305 (1941).

[1] T. Hama, H. Aruga et Y. Maki, Zool. Mag. *58*, 219 (1949).

[2] T. Hama, T. Goto et K. Kushibiki, Science (Japon) *22*, 478 (1952). Il y a deux sortes de Cyprinus purple *A* et *B* dans la peau et les écailles de la carpe, la première correspond à la fluorescyanine.

[3] T. Hama, T. Goto, K. Oi et K. Kushibiki, Science (Japon) *22*, 542 (1952). Nous avons nommé «Bufo-chrome» une substance isolée de la peau du *Bufo vulgaris*, leur solution aqueuse ayant la fluorescence bleue ciel.

[4] T. Hama, Zool. Mag. *61*, 89 (1952).

[5] T. Hama et T. Goto, Zool. Mag. *61*, 88 (1952).

[6] M. Polonovski, P. Gonnard et A. Baril, Enzymologia *14*, 311 (1951).