SON and LENICQUE[1] noted an overall decrease in mitochondria in *P. miliaris*.

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Zusammenfassung

Zytologische Untersuchungen an Embryonen von zwei Seeigelarten haben in bezug auf die Lokalisation der Mitochondrien keinen Unterschied zwischen animaler und vegetativer Hemisphäre ergeben. Vorläufige Versuche, mit Hilfe einer quantitativen Methode die Mitochondrien in Homogenaten zu zählen, ergaben eine starke Zunahme auf dem Stadium der Blastula und eine anschliessende Abnahme der Mitochondrienzahl pro Embryo.

[1] T. GUSTAFSON and P. LENICQUE, Exp. Cell Res. *3*, 251 (1952).

Factors Influencing the Fatty Acid Oxidation of Tumor Mitochondria with Special Reference to Changes in Spontaneous Mouse Hepatomas

WEINHOUSE and CHAIKOFF *et al.* gave a conclusive demonstration that slices of tumors are capable of oxidizing fatty acids[1]. Cell-free preparations of a number of tumors did not show this property, however[2]. It is the object of this short communication to report on some of the factors influencing the oxidation of fatty acids by tumor mitochondria *in vitro*, and especially on changes in this respect occuring in mitochondria from spontaneous hepatomas. So far as we are aware, the present data furnish the first positive evidence of fatty acid oxidation by tumor mitochondria.

The hepatomas studied arose in a high incidence in two year old female F_1(♀ C_{57} Black × ♂ C_3He) mice[3] which were kindly put at our disposal by Dr. O. MÜHLBOCK.

The tumors were always divided in two groups, consisting of hepatomas of less than 1 g, which we shall call early stage tumors, and tumors weighing 2–10 g, which are designated here as fully developed tumors. Mitochondria, prepared from both specimens in isotonic (0·25 m) sucrose[4], were washed twice in a volume of cold isotonic sucrose equal to twice the original tissue weight, and the suspension prior to incubation–the preliminary suspension– was made in a (0·13 m) KCl-(0·013 m) phosphate buffer (pH 7·4). After the mitochondrial suspension was pipetted into Warburg flasks containing the proper additions (Table I), only the mitochondria from the early stage tumors showed an oxidation of octanoate with α-oxy caproate and L-malate as "sparkers" of fatty acid oxidation[5].

In a previous publication[6], it was shown that mitochondria prepared from many mouse tumors exhibit a marked "adenosine triphosphatase (ATPase) activity", as measured by the complete inhibition of fatty acid oxidation of normal liver mitochondria after addition of these tumor mitochondria. Most tumor mitochondria showed this high "ATPase activity" irrespective of the fact whether the preliminary suspension was made in isotonic sucrose or in KCl-phosphate buffer. At the same time it was found that no oxidation of octanoate took place by these mitochondria *per se* after addition of NaF (0·01 m) diphosphopyridine nucleotide (10^{-4} m) and coenzyme A (10 γ).

The mitochondria from the fully developed hepatomas also showed a high "ATPase activity", when the preliminary suspension was made in the KCl-phosphate buffer, but, when isotonic sucrose devres as the suspending medium, only a moderate or slight "ATPase activity" was recorded. This was more or less anticipated in view of the apparent lack of octanoate oxidation by the mitochondria from the preliminary KCl-phosphate suspension. After addition of fluoride–a well-known inhibitor of ATPase–a small oxygen consumption resulting from octanoate oxidation could sometimes be seen. Mitochondria from the preliminary sucrose suspension were somewhat variable in their oxidative behaviour, sometimes showing an oxygen consumption with octanoate as the substrate. Isolation of the mitochondria with isotonic sucrose containing 0·001 ethylenediamine tetraacetate (versene) resulted in an enhanced oxidation.

The "ATPase activity" of the mitochondria from the early stage tumors was considered as small or absent, because even the mitochondria from the preliminary KCl-phosphate suspension were capable of oxidizing octanoate; this was confirmed by measurements of the "ATPase activity" in the way indicated.

The absence of "ATPase activity" does not necessarily mean that tumor mitochondria in general are able to effect fatty acid oxidation. Mitochondria prepared from two different transplanted interstitial cell tumors of the mouse testis never showed such ATP splitting activities as would influence the fatty acid oxidation of normal liver mitochondria appreciably. The mitochondria from one of these tumors, prepared either with or without versene, were, however, unable to oxidize fatty acids under the conditions of the present experiments. The mitochondria from the other testis tumors, prepared with sucrose-versene, showed a vivid oxidation of octanoate. Another example may be mentioned of a transplanted granulosa cell tumor of the mouse ovary. Mitochondria prepared from this tumor with isotonic sucrose and suspended in KCl-phosphate buffer had a moderate or marked "ATPase activity". When the preliminary suspension was made in isotonic sucrose and added to normal liver mitochondria, no influence on the fatty acid oxidation of the liver mitochondria could be detected, but the tumor mitochondria *per se* showed no octanoate oxidation. When the whole procedure of preparing the tumor mitochondria was carried out with 0·001 m versene included in the isotonic sucrose solution, a very large oxygen consumption resulted from octanoate oxidation.

Changes in the mitochondrial nitrogen content of the spontaneous hepatomas were also noted. The nitrogen content of the mitochondria per 100 mg fresh tissue of the early stage hepatomas was always found to be higher than the corresponding values of the fully developed tumors, e.g. 0·39 against 0·28 mg N on the average. This may be the result of the greater blood content of the latter. The division of the spontaneous hepatomas into the two groups mentioned was merely

[1] S. WEINHOUSE, R. H. MILLINGTON, and C. E. WENNER, Cancer Res. *11*, 845 (1951). – D. D. CHAPMAN, G. W. BROWN Jr., I. L. CHAIKOFF, W. O. DAUBEN, and N. O. FAUSCH, Cancer Res. *14*, 372 (1954).

[2] C. G. BAKER and A. MEISTER, J. Natl. Cancer Inst. *10*, 1191 (1950).

[3] O. MÜHLBOCK, unpublished.

[4] W. C. SCHNEIDER and G. H. HOGEBOOM, J. Biol. Chem. *183*, 123 (1950).

[5] N. WATERMAN, C. J. BOS, and T. J. BARENDREGT, Enzymologia *15*, 307 (1952). – P. EMMELOT and C. J. BOS, Enzymologia *17*, 13 (1954).

[6] P. EMMELOT and C. J. BOS, Biochim. Biophys. Acta *16*, 620 (1955).

Table I.–Oxidation of Octanoate by Tumor Mitochondria and by Liver Mitochondria in the presence of Tumor Mitochondria. Additions: octanoate ($1{\cdot}25 \times 10^{-3}$ m), α-oxy caproate (3×10^{-3} m), L-malate (7×10^{-5} m), ATP (7×10^{-4} m), cytochrome c (10^{-5} m), diphosphopyridine nucleotide (DPN, 10^{-3} m), Mg^{++} (5×10^{-3} m), in a total volume of 1·6 ml (0·06 m) KCl-(0·013 m) phosphate buffer (pH 7·4) including 0·6 ml suspension of tumor mitochondria in KCl-phosphate buffer (KCl-P), or 0·25 m sucrose ± versene (0·001 m). NaF was added in one experiment in a final concentration of 0·01 m. In the "ATPase assay" (lower part of table) 0·3 ml suspension of liver mitochondria in KCl-phosphate buffer and 0·3 ml suspension of tumor mitochondria in KCl-phosphate or 0·25 m sucrose ± versene were added, DPN and malate were omitted. Oxygen consumption was calculated as the difference between oxygen uptake in the presence of octanoate + α-oxy caproate (+ DPN) and that of α-oxy caproate (+ DPN). Protein-N was determined by a micro-Kjehldahl procedure. In the experiments in which tumor and liver mitochondria were incubated together, the N-content of the former is listed in the table. The N-content of the latter was always the same as that of the liver mitochondria incubated separately.

Mitochondria from	preliminary suspension	mg N per flask	$\mu l O_2$ consumed per flask after 60 min
hepatomas:			
"early stage" (I)			
Ia	KCl-P	2·18	177
Ib	KCl-P	2·37	140
"fully developed"(II)			
IIa.	KCl-P	2·28	−3
IIb.	KCl-P	1·81	−4
IIb+F^-	KCl-P	1·81	23
granulosa cell tumor (III)			
IIIa	sucrose	1·06	0
IIIa	sucrose+versene	0·76	246
mouse liver (IV)			
IVa		0·80	60
IVa+Ic	KCl-P	1·68	100
IVb		1·38	117
IVb+Id	sucrose	0·78	140
IVb+IIc	KCl-P	0·89	10
IVb+IIc	sucrose	0·73	60
IVc		1·42	121
IVc+IId	sucrose	1·73	106
IVd		1·26	124
IVd+IIIb . . .	KCl-P	1·01	59
IVe		1·26	115
IVe+IIIc . . .	KCl-P	0·78	14
IVf		0·98	117
IVf+IIIa . . .	sucrose	1·06	130
IVf+IIIa . . .	sucrose+versene	0·76	190

made on account of the weight of the tumors. The results reported were confirmed in more than 25 experiments, only a very few exceptions being noted. It can be concluded that the mitochondria from the fully developed hepatomas are more liable to damage *in vitro*, by which latent ATPase activities become activated. By splitting ATP the forming of the acyl-coenzyme A bond, a necessary requisite for fatty acid oxidation, is precluded.

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Zusammenfassung

Mitochondrien aus kleinen und grossen Spontanhepatomen bei $F_1(C_{57}Bl \times C_3He)$ Mäusen weisen verschiedene Adenosintriphosphataseaktivitäten auf, was sich 1. an der Hemmung der Fettsäureoxydation normaler Lebermitochondrien nach Zugabe von Tumormitochondrien und 2. an der Fettsäureoxydation der Hepatommitochondrien selbst zeigen lässt. Die Mitochondrien gewisser Tumoren vermochten Caprylsäure nur zu oxydieren, wenn die Adenosintriphosphatase möglichst wenig aktiviert war durch Zugabe von Verseen zum Aufarbeitungsmedium.

Sur la présence de β-alanine dans les tissus des plantes supérieures

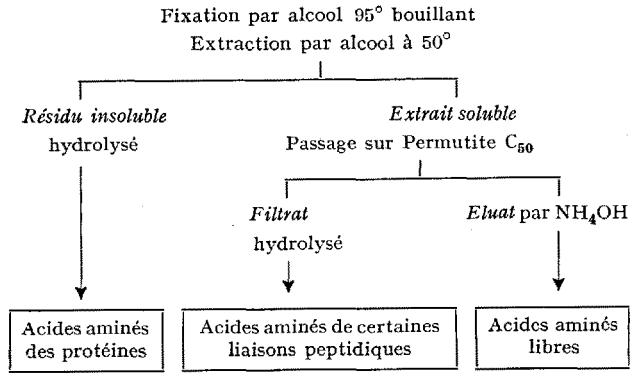
La β-alanine a été identifiée parmi les acides aminés libres des végétaux supérieurs par plusieurs auteurs: STEWARD et THOMPSON[1] dans des tissus variés; HUNT[2] dans les racines et les nodules de légumineuses, AUCLAIR et MALTAIS[3] dans les extraits de pois.

Par contre, au cours de travaux portant sur les protéines des tissus végétaux, il n'a jamais été décelé de β-alanine dans les hydrolysats. Peu de recherches ont porté sur les acides aminés constituant des polypeptides.

Cependant, VIRTANEN et MIETTINEN[4] ont identifié la β-alanine chez le Pois (feuilles), à la fois parmi les acides aminés libres, et comme constituant des polypeptides.

Au cours de travaux d'orientations différentes sur le métabolisme protidique, nous avons rencontré la β-alanine dans certains extraits obtenus à partir de feuilles de blé et de *Bryophyllum daigremontianum* BERGER (M.L.C.) et de divers tissus végétaux cultivés *in vitro*: tissus normaux de racines de scorsonère, tissus de Crown-gall de scorsonère, tissus de Crown-gall de topinambour (C.L.).

Les techniques de séparation employées sont résumées dans le schéma suivant:



Le passage sur la Permutite C_{50} (méthode de BOULANGER et BISERTE[5]) permet de retenir les acides aminés

[1] F. C. STEWARD et J. F. THOMPSON, Ann. Rev. Plant. Physiol. *1*, 233 (1950).

[2] G. E. HUNT, Amer. J. Bot. *38*, 452 (1951).

[3] J. L. AUCLAIR et J. B. MALTAIS, Nature *170*, 1114 (1952).

[4] A. I. VIRTANEN et J. K. MIETTINEN, Biochem. Biophys. Acta *12*, 181 (1953).

[5] P. BOULANGER et G. BISERTE, Bull. Soc. Chim. Biol. *33*, 1930 (1951).