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.

P. EMMELOT and C. J. BOS

Department of Biochemistry, Antoni van Leeuwen-Hoekhuis, the Netherlands Cancer Institute, Amsterdam, April 14, 1955.

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

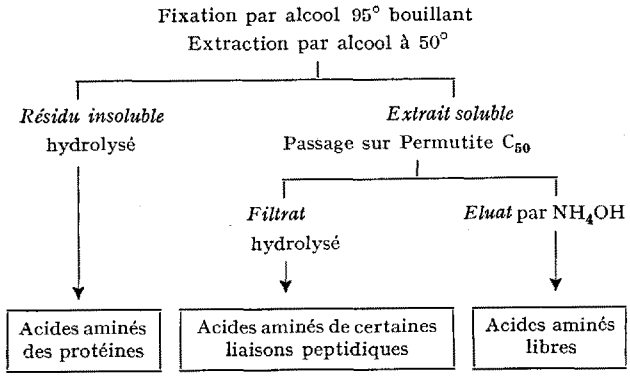
La β-alanine a été identifiée parmi les acides aminés libres des végétaux supérieurs par plusieurs auteurs: STEWARD et THOMPSON[1] dans des tissus variés; HUNT[2] dans les racines et les nodules de légumineuses, AUCLAIR et MALTAIS[3] dans les extraits de pois.

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).

libres et certains polypeptides courts. D'autres substances (polypeptides longs, glucides, etc.) traversent la colonne et constituent le filtrat, qui, après hydrolyse, fournit les acides aminés de corps solubles contenant des liaisons peptidiques. L'élution des acides aminés libres retenus par la résine est faite par NH_4OH N. Les hydrolyses sont faites par HCl 6 N à 100° pendant 24 h. Des distillations répétées sous vide permettent d'éliminer la plus grande partie de HCl. La purification s'effectue ensuite par passage sur permutite C_{50} et élution comme précédemment. La séparation est faite par chromatographie sur papier à 2 dimensions selon le système de DENT[1]. La β-alanine apparaît en bleu clair au réactif à la ninhydrine.

Les résultats relatifs à la présence de β-alanine dans les tissus étudiés sont indiqués dans le tableau suivant:

	Acides aminés libres	*Fraction peptidique*	*Fraction protéique*
Racines de scorsonère . . .	+	+	–
Crown-gall scorsonère . . .	–	+	–
Crown-gall topinambour .	+	+	–
Feuilles de blé	–	+	–
Feuilles de bryophyllum. .	–	+	–

On retrouve ainsi les données antérieurement acquises: présence occasionnelle de β-alanine parmi les acides aminés libres, absence totale parmi les acides aminés d'origine protéique. De plus, on peut remarquer qu'elle est toujours présente dans la fraction peptidique. D'après VIRTANEN[2] la β-alanine libre proviendrait de la décarboxylation de l'acide aspartique. VIRTANEN et MIETTINEN[3] pensent aussi que, dans la fraction peptidique, la β-alanine serait liée à des sucres réducteurs par son groupe NH_2 devenu plus réactif du fait de son éloignement du groupe carboxyle. On peut émettre également l'hypothèse qu'il s'agit de la β-alanine constitutive de l'acide panthoténique entrant lui-même dans la composition du «coenzyme A».

MARIE LOUISE CHAMPIGNY et
C. LIORET

Laboratoire de Photosynthèse, Centre national de la Recherche scientifique, Gif-sur-Yvette, le 28 mai 1955.

Summary

β-alanine has always been found in peptide hydrolysates of various higher plants, but never in protein hydrolysates. An assumption that the β-alanine so found is a constituent of coenzyme A has been suggested.

[1] C. E. DENT, Biochem. J. *43*, 169 (1948).
[2] A. I. VIRTANEN, Nature *142*, 674 (1938).
[3] A. I. VIRTANEN et J. K. MIETTINEN, Biochem. Biophys. Acta *12*, 181 (1953).

Histochemical Demonstration of Reductase Activity in the Nucleus of Chicken Erythrocyte[1]

Recently RUBINSTEIN and DENSTEDT[2] have demonstrated the presence of Krebs cycle enzymes in the nuclei of chicken erythrocytes. The work of the above investigators was criticized by STERN and TIMONEN[1] who were concerned with a possible contamination of the nuclear preparation by postulated cytoplasmic particles or with a possible absorption by the isolated nuclei of cytoplasmic enzymes made soluble through freezing-thawing techniques. The interesting original work and the subsequent criticism led us to examine these nuclei histochemically for the presence of enzymes concerned with reductase activity.

Histochemical work, using basic techniques previously described[2], confirms the actual presence of general reductase and specifically of succinic dehydrogenase activity in the nuclei of chicken erythrocytes.

Four tetrazolium salts were tested: 2, 3, 5-triphenyl tetrazolium chloride (TTC); 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INTC); 2, 2'-(p-diphenylene)-bis-2-(3, 5-diphenyl) tetrazolium chloride, or neotetrazolium (NT); and 3, 3'-dianisole-bis-4, 4'-(3, 5-diphenyl) tetrazolium chloride, or blue tetrazolium (BT)[3].

The first two are monotetrazolium, the latter ditetrazolium compounds. The concentrations used were 1 mg/ml. The different salts varied not as much in the final results as in the time of incubation required to obtain formazan pigment. The INTC was preferred as it gave faster reduction and more minute granulation. Purification of the tetrazolium salts by removal of contaminating metal by previous treatment with a chelating agent, diphenylthiocarbazone, was very effective in decreasing the time of incubation, particularly in the case of the INTC.

Different methods, whose characteristics will be discussed elsewhere, were used in order to achieve satisfactory histochemical results. The reaction was performed both in intact cells as well as in nuclei, which were isolated by freezing and thawing. Freezing and thawing were used for demonstration of succinic dehydrogenase as freezing destroys the so called "endogenous" dehydrogenase.

Potassium cyanide and versene (tetrasodium salt of ethylenediaminotetraacetic acid) at concentrations from 0·01 *M* to 0·1 *M* proved to be very effective on the final histochemical result, probably by affecting the membrane permeability[4] and enhancing the enzymatic activity[5].

The specificity of the mechanism involved in the reduction was confirmed by absence of formazan formation in the case of succinic dehydrogenase on leaving out the succinate or on adding fluoride or malonate in addition to succinate.

Results under each test condition were highly reproducible and the number of formazan crystals per cell was fairly uniform. The crystals were localized at the inner surface of the nuclear membrane; and only very rarely were crystals encountered at the center of the nucleus. No tetrazolium reduction was observed with or without succinate in the cytoplasm of the intact cell, as well as in the supernatant fluid from which nuclei had been isolated.

[1] This work was supported in part by a Research Grant from the National Cancer Institute, U. S. Department of Health, Education and Welfare, and in part by an Institutional Grant to the Detroit Institute of Cancer Research from the American Cancer Society.

[2] D. RUBINSTEIN and O. F. DENSTEDT, J. Biol. Chem. *204*, 623 (1953).

[1] H. STERN and S. TIMONEN, J. Gen. Physiol. *38*, 41 (1954).

[2, 3] B. PEARSON and V. DEFENDI, J. Histochem. Cytochem. *2*, 248 (1954). – V. DEFENDI and B. PEARSON, J. Histochem. Cytochem. *3*, 61 (1955).

[3] Obtained from the Pal Chemicals Limited, London, England.

[4] S. R. MERKEL and W. J. NICKERSON, Proc. Nat. Acad. Sci. *39*, 1008 (1953).

[5] S. M. ALTMANN and E. M. CROOK, Nature *171*, 76 (1953).