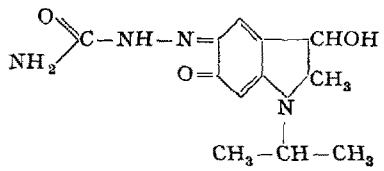
Technique

a) Il possède la formule



Sa préparation et ses propriétés ont été décrites dans cette revue par C. BEAUDET (sous presse).

b) Le temps de saignement moyen a été déterminé en suivant rigoureusement les indications de ROSKAM et PAUWEN[1]; le temps de saignement moyen modifié a été recherché 15 minutes après l'injection du 1032 L.

a) Injection intraveineuse de 5 µg/kg de L 1032 dl

N°	Temps de saignement moyen		Modification	
	avant	après	en min et sec	en %
1	2'10"	1'24"	– 0'46"	– 35,3
2	2'16"	1'08"	– 1'08"	– 50
3	2'20"	0'57"	– 1'23"	– 60
4	2'38"	1'14"	– 1'24"	– 53
Moyenne arithmétique	2'21"	1'11"	– 1'10"	– 49,5

Résultats

1° Le 1032 L, injecté en solution aqueuse, à raison de 100 µg à 2 mg, en une fois, au lapin anesthésié au chloralosane, ne possède aucune activité sur la pression artérielle même après section des vagues. La répétition des doses a engendré dans un cas une hausse lente et durable de la pression artérielle. Le 1032 L est sans action sur la membrane nictitante du chat.

2° L'activité hémostatique, en fonction de la dose, figure dans les tableaux ci-dessous. L'écart statistique est de ± 20 secondes.

b) Injection intraveineuse de 25 µg/kg de L 1032 dl

N°	Temps de saignement moyen		Modification	
	avant	après	en min et sec	en %
1	2'11"	1'26"	– 0'45"	– 34,3
2	2'30"	0'59"	– 1'31"	– 60,7
3	2'19"	0'55"	– 1'24"	– 60
4	2'08"	1'18"	– 0'50"	– 39
Moyenne arithmétique	2'17"	1'09"	– 1'07"	– 48,5

On voit donc que le 1032 L est aussi actif que l'Adrénoxyl préparé à partir des diverses variétés optiques d'adrénaline[1]. L'activité n'est pas proportionnelle à la dose, ce que DEROUAUX avait déjà constaté pour la monoxime de l'adrénochrome[2].

Discussion

L'aleudrine, dont provient le 1032 L, ne possède aucune propriété sympathicomimétique excitatrice; elle est hypotensive d'emblée, dilate les bronches et inhibe le tonus de l'utérus vierge de chatte. Les rapports entre la fluorescence, les actions inhibitrices et la structure chimique des amines sympathico-mimétiques ont été récemment développés par BACQ, FISCHER et LECOMTE[3]. Rappelons simplement ici qu'après administration d'adrénolytiques (933 F), les effets inhibiteurs de l'adrénaline sont comparables à ceux d'une même dose d'aleudrine[3].

Il est toutefois impossible de conclure que l'activité hémostatique de l'Adrénoxyl et du 1032 L sont en rapport avec les propriétés inhibitrices des amines dont elles dérivent. La dioxyéphédrine, qui rentre selon le travail de BACQ, FISCHER et LECOMTE dans le même groupe d'amines que l'adrénaline et l'aleudrine, ne possède aucune activité sur le temps de saignement[4].

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Laboratoires de Pathologie médicale, de Pathologie générale, Université de Liège et Société belge de l'Azote, le 10 octobre 1950.

Zusammenfassung

Das Semicarbazon des Oxydationsproduktes von Aleudrin wurde geprüft. Es wirkt hämostatisch, und zwar in gleichem Ausmaß wie das Semicarbazon des Adrenochroms.

[1] J. ROSKAM et L. PAUWEN, Arch. int. Pharm. Thér. *57*, 450 (1937).

[1] C. BEAUDET, P. TRABERT et F. HENAUX, Arch. int. Physiol. *57*, 343 (1950).

[2] G. DEROUAUX, Arch. int. Pharm. Thér. *69*, 142 (1943).

[3] Z. M. BACQ, P. FISCHER et J. LECOMTE, Arch. int. Physiol. *56*, 380 (1949).

[4] G. DEROUAUX, Arch. int. Pharm. Thér. *65*, 125 (1941).

Pyruvic Acid Intermediate Product in Acetate Oxidation by Yeast

The accumulation of succinate and citrate during oxidation of barium acetate by yeast was first observed by WIELAND and SONDERHOFF[1]. On the basis of these results the German authors suggested that the oxidation of acetate occurs according to the THUNBERG-KNOOP scheme and considered the formation of citrate as a side reaction of the oxidative pathway.

SONDERHOFF and THOMAS[2], using trideuteroacetate, found deuterium in the succinate formed. The amount of deuterium in the succinate was lower than in the acetate, and this result cannot be explained on the basis of the oxidation scheme assumed by WIELAND.

LYNEN[3] demonstrated that acetate is oxidized in yeast by the tricarboxylic acid cycle, but here too previous condensation of acetic acid is not excluded, and he pointed out that the results obtained by SONDERHOFF and THOMAS are in accordance with this hypothesis.

[1] H. WIELAND and R. SONDERHOFF, Liebig's Ann. Chem. *499*, 213 (1932).

[2] R. SONDERHOFF and H. THOMAS, Liebig's Ann. Chem. *530*, 195 (1937).

[3] F. LYNEN, Liebig's Ann.Chem. *552*, 270 (1942); *554*, 40 (1943).

In further experiments LYNEN[1] found out that the oxidation of acetate by yeast does not undergo the citric acid step. Moreover, on the basis of the distribution of deuterium, it is possible that part of the non-isotopic fumarate was oxidized to C_2-compounds and in turn entered into the condensation reaction, which gives rise to "light" succinate.

The quantitative relationships of fumarate consumed and succinate formed are in agreement with this supposition.

WEINHOUSE and MILLINGTON[2] have established that the oxidation of Mg and Ba carboxyl-labelled acetates by baker's yeast led to the accumulation of citrate and respiratory carbon dioxide, whose C^{13} content and distribution seemed to support the idea that the tricarboxylic acid cycle is a major oxidative process in yeast. But the American authors do not exclude the simultaneous occurrence to a small extent of the dehydronegative coupling of two acetyl groups to yield succinate according to the THUNBERG-KNOOP scheme.

This oxidative pathway has been postulated by SLADE and WERKMAN[3] to account for the formation of isotopic succinate from C^{13}-labelled acetate during the glucose fermentation by Aerobacter.

In a more recent work WEINHOUSE *et al.*[4] by means of traced acetate demonstrated that over half of the glucose oxidized by the yeast goes through the acetate stage.

KREBS[5], who has considered the possibility of the occurrence of the tricarboxylic acid cycle and THUNBERG-KNOOP cycle in yeast, came to the conclusion that neither the first system nor the second are involved in the oxidation process of yeast because of the apparent absence of the dehydrogenases for the various components of both systems.

According to LYNEN and NECIULLAH[6] the failure of yeast to support dehydrogenation of citrate, α-ketoglutarate, succinate and malate was determined by the impermeability of the cell membrane. In effect by using yeast frozen in liquid air and thawed the authors were able to demonstrate the rapid dehydrogenation of the di- and tricarboxylic acids.

From the above results it seems to follow that two acetate oxidative systems take place in yeast, and the major one seems to be the Krebs's cycle.

The problem of acetate oxidation in yeast has been studied by us with the aid of the phenylhydrazine oxalate (OP salt) as "trapping agent" for ketonic and aldehydic intermediate products[7]. By means of the OP salt we were able to isolate high amounts of pyruvic acid and not oxalosuccinic or oxaloacetic acid and acetaldehyde.

Since the salt fixes the first intermediates in greater amounts than the last one[7], if the KREBS's cycle were a major oxidative process in yeast, in our experiments the first fixed intermediates would have been the oxaloacetic acid or the oxalosuccinic acid and not the pyruvic acid.

On the contrary, assuming that the acetate oxidation occurs by the THUNBERG-KNOOP scheme, the first fixed intermediate would be the oxalacetic acid and then the pyruvic acid and the acetaldehyde.

The failure of the oxaloacetic acid fixation is probably consistent with the belief of several authors[1] that said acid would appear in a particular form closely related to it. As a matter of fact, in the demolition of the aspartic acid by enzymatic preparations of *Bacterium coli* in presence of semicarbazide, LICHSTEIN and UMBREIT[2] have fixed only the pyruvic acid and not the oxaloacetic acid which forms before and probably might be phosphorilated.

The low course of the acetate oxidation will be responsible for difficulty in the fixation of acetaldehyde, supposed to be the intermediate product following the pyruvic acid, as it has been already observed that the OP salt is able to fix the last terms of a process only in rapid processes[3].

Further experiments at the same conditions showed that also the sodium succinate, fumarate and malate can be oxidized to pyruvic acid.

The results obtained in the oxidation of the acetate and of the three dicarboxylic acids do not yet lead to decisive conclusions on the most active oxidation system in the yeast, although they seem to support the WIELAND scheme. More experiments are necessary to establish the position of pyruvic acid in relation to present knowledge of the respiratory systems supposed to exist in yeast.

Experimental results

Oxidation of acetate: 200 g of baker's yeast were suspended in 3500 ml of water containing Na_2HPO_4 0·6%, $(NH_4)_2SO_4$ 0·1% and K_2HPO_4 0·05%, and the suspension was continuously aerated for about 12 hours at 30° to deplete the cells of endogenous nutrients.

Then the content of the flask was divided in two portions of 2500 ml and 1000 ml respectively; 5 g per mille of sodium acetate and 0·75 per mille of OP salt were added to the first portion and the p_H was adjusted to 5·5 by hydrochloric acid; to the second portion only 0·75 g per mille of OP salt were added. The salt was dissolved in distilled water containing 0·3 g of Na_2HPO_4. After 24 hours incubation in air stream the addition of 0·75 g per mille of OP salt to each flask was repeated.

After 48 hours of an additional incubation always in air stream the contents of the flasks were centrifuged and the clear liquids concentrated 1:3 and analysed for fixed products.

Pyruvic acid: was isolated as phenylhydrazone by continuous ether extraction of the concentrated medium, and purified by recrystallisation from alcohol.

Pyruvic acid (as phenylhydrazone)	m.p. 188°	Calculated N% 15,73	Found 15,82

Amount produced: 0·65 g per mille, i.e. about 37% of the acid which can be obtained on the basis of phenylhydrazine present in the OP salt. In the blank test no pyruvic acid was fixed.

[1] F. LYNEN, Liebig's Ann. Chem. *558*, 47 (1947).
[2] S. WEINHOUSE and R. H. MILLINGTON, J. Amer. Chem. Soc. *69*, 3089 (1947).
[3] H. D. SLADE and C. H. WERKMAN, Arch. Biochem. *2*, 97 (1943).
[4] S. WEINHOUSE, R. H. MILLINGTON, and K. F. LEWIS, J. Amer. Chem. Soc. *70*, 3680 (1948).
[5] H. A. KREBS, Adv. Enzymology *3*, 191 (1943).
[6] F. LYNEN and N. NECIULLAH, Liebig's Ann. Chem. *541*, 203 (1939).
[7] V. BOLCATO, Nature *165*, 814 (1950); Enzymologia *14*, 21 (1950); Farmaco *5*, 20, 132 (1950).

[1] G. KALNITSKY and C. H. WERKMAN, Arch. Biochem. *4*, 25 (1944). – L. O. KRAMPITZ, H. G. WOOD, and C. H. WERKMAN, J. Biol. Chem. *147*, 243 (1943). – L. O. KRAMPITZ, M. F. UTTER, and C. H. WERKMAN, J. Bact. *42*, 139 (1941).
[2] H. C. LICHSTEIN and W. W. UMBREIT, J. Biol. Chem. *170*, 329 (1947).
[3] V. BOLCATO, Nature *165*, 814 (1950); Enzymologia *14*, 21 (1950); Farmaco *5*, 20, 132 (1950).

Oxaloacetic acid: the Straub reaction and the determination as phenylhydrazone gave always negative results.

Acetaldehyde: by distillation of the medium with pyruvic acid in a Vigreux column and precipitation of the distillate with 2·4-dinitrophenylhydrazine, yellow precipitates are obtained which do not prove to be acetaldehyde.

At the end of the experience the acetic acid consumption resulted in 80% of the added acid and the p_H changed from 5·5 to 7–7·2, while the oxalic acid of the OP salt was slightly decreased. Under our conditions no citric acid was formed from the acetate.

Oxidation of the sodium succinate, fumarate and malate: 4000 ml of the suspension of yeast impoverished, as explained above, were divided into four fractions, each of 1000 ml to three of them were added 0·5 g of succinate, fumarate and malate respectively and 0·5 g of OP salt each. To the fourth fraction used as a blank test only 0·5 g of OP salt were added. In all fractions the p_H was 5·5. After 24 hours aeration at 30° a further addition of 0·5 g of the respective salt and 0·5 g of the OP salt was made to each of the three tests and only 0·5 g of the OP salt was added to the blank test. The OP salt was dissolved in 100 ml of distilled water in presence of 0·25 g of Na_2HPO_4. After 24 hours additional aeration the contents of the flasks were centrifuged and the clear liquids analysed for intermediates by the given methods.

Pyruvic acid (as phenylhydrazone) from dicarboxilyc acids (mixed)

m.p.	Calculated N%	Found
188°	15·73	15·87

Amount produced: 0·35 g per mille, i.e. about 30% of the acid which can be obtained on the basis of phenylhydrazine present in the OP salt.

In the blank test no pyruvic acid was fixed.

Both in the oxidation of acetate and dicarboxilic acids no contamination was never observed.

Acknowledgements

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Institute of pharmaceutical Chemistry, University of Pavia, July 30, 1950.

Riassunto

Da respirazioni dell'acetato, succinato, fumarato e malato di sodio con il lievito, in presenza di ossalato di fenilidrazina, è stato possibile captare l'acido piruvico come prodotto intermedio della reazione.

Wirkung des Ouabains auf das Aktomyosin der Herzmuskulatur

Mit Hilfe der viskosimetrischen Methode, die von A. Csapó[1] beschrieben worden ist, wurde die Wirkung des Ouabains auf die Reaktion zwischen ATP und Aktomyosin (AM) von Rinderherzen studiert. Der Einfluß von Ca^{++} auf den Effekt der Herzglykoside wurde sowohl an nativem Extrakt wie an gewaschenem AM untersucht. Eine Modifikation der Extraktionsmethode von A. Szent-Györgyi[1] ermöglichte es, lange Zeit hindurch mit einem identischen Extrakt zu arbeiten. Es wurden einfache Ostwald-Viskosimeter verwendet.

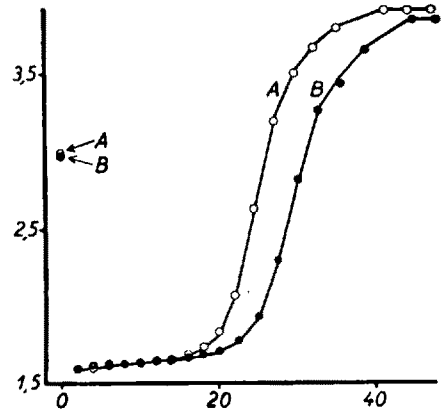

Abb. 1. Wirkung von Ouabain in einer Konzentration von 10^{-5} M auf nativen Aktomyosinextrakt von Rinderherzen.

Ordinate: Relative Viskosität. Abszisse: Zeit in Minuten nach ATP-Zugabe. Kreise: Ohne Ouabain. Punkte: Mit Ouabain.

Untersuchung an nativem Muskelextrakt. Die Ouabainwirkung wurde für Konzentrationen von 10^{-4} M bis 10^{-7} M untersucht, und der Effekt macht sich in diesen Fällen in einem hemmenden Einfluß auf die ATP-ase bemerkbar. Für die Konzentrationen 10^{-4} M und 10^{-5} M beträgt die Hemmung der ATP-ase-Wirkung ca. 20% (Abb. 1). Ouabain in einer Konzentration von 10^{-6} M gibt eine ca. 8%ige Hemmung der Enzymaktivität. In einem Falle, der jedoch nicht reproduziert werden konnte, ergab sich für diese Konzentration eine 45%ige Hemmung. Die Konzentration 10^{-7} M ergab keine feststellbare Wirkung.

Am Wiederaufbau – die spontane Viskositätssteigerung nach Spaltung des ATP – übt das Ouabain eine schwache hemmende Wirkung aus, aber eine Veränderung der Form der Kurve entsteht nicht.

Ca^{++} stimuliert die Myosin-ATP-ase[1] und hemmt das wasserlösliche Enzymsystem[2]. Ein Extrazuschuß von 0,001 M Ca^{++} verursacht in diesem nativen Extrakt sowohl eine Hemmung der Enzymaktivität wie des Wiederaufbaues. Die hinzugefügte Menge Ca^{++} scheint jedoch keinen Einfluß auf die Wirkung des Ouabains zu haben.

Untersuchung an gewaschenem AM. Die Reinigung geschah durch drei in schneller Folge vorgenommene Waschungen. Die ATP-ase-Aktivität ist in dem gewaschenen AM schwächer als in dem nativen Extrakt. Die Form der Kurve ist in den beiden Fällen ebenfalls verschieden. Ouabain verursacht in einer Konzentration von 10^{-5} M und 10^{-6} M eine Stimulation der Myosin-ATP-ase (Abb. 2). Die hemmende Wirkung am Wiederaufbau, die für den nativen Extrakt beobachtet wurde, tritt bedeutend stärker in dem gewaschenen AM hervor. In der Konzentration 10^{-7} M zeigt das Ouabain keine Wirkung. Interessant ist die Veränderung, die nach einer Sonderzugabe von 0,001 M Ca^{++} entsteht. Das Ouabain verursacht jetzt einen Wiederaufbau, welcher

[1] A. Csapó, Acta Physiol. Scand. *19*, 100 (1949).

[1] A. Szent-Györgyi, Acta Physiol. Scand. *9*, Suppl. 25 (1945).

[2] W. W. Kielley und O. Meyerhof, J. Biol. Chem. *174*, 387 (1948).