morphologie et la distribution de l'acide ribonucléique dans le nucléole et le suc nucléaire.

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Summary

The effects of dinitrophenol, usnic acid and A.T.P. on nucleated and non-nucleated halves of *Amoeba proteus* have been studied.

In regenerating nucleated halves of *Acetabularia mediterranea*, dinitrophenol and usnic acid induce deep changes in the morphology and chemical composition of the nucleus. It is concluded that the nucleus depends on energy production in the cytoplasm for the maintenance of both its structure and its composition.

Influence of Isonicotinic Acid Hydrazide (INH) and 1-Isonicotinyl-2-isopropyl Hydrazide (IIH) on Bacterial and Mammalian Enzymes[1]

All basic antibiotics and synthetic basic antituberculous substances so far tested reduce the activity of bacterial diamine oxidase[2] and of a guanidine-splitting enzyme present in *Mycobacterium smegmatis*[1]. Since INH and IIH are bases, we investigated their possible influence upon bacterial diamine oxidase and guanidine deamidinase. In order to obtain some information about the mode of action of the two new antituberculous drugs on the organism of the host, the behaviour of the mammalian diamine oxidase and monoamine oxidase against INH and IIH was tested. Some typical results are presented in the following tables.

Experiments were carried out at 38°. All results, from which the blanks have been subtracted, are calculated in terms of micromoles per one hour (Q). Further experimental details can be obtained from previous publications[2]. (See Tables I and II.)

As expected the two drugs inhibit diamine oxidase (putrescine) and guanidine deamidinase (agmatine) of *M. smegmatis*. Toward diamine oxidase INH is still measurably active at a concentration (4 µg per milliliter) which is within the therapeutical range. When cell-free preparations of guanidine deamidinase are exposed to higher concentrations of the drugs, again a decrease of enzyme activity is found. (See Table III.)

While streptomycin, viomycin, and neomycin are much less effective on the oxidative deamination of diamines by preparations of mammalian origin than by

[1] In part presented at the Federation Meeting, New York, April 18, 1952, and at the Meeting of the Society of Illinois Bacteriologists Chicago, May 23, 1952.

[2] E. A. ZELLER, C. A. OWEN, Jr., and A. G. KARLSON, J. Biol. Chem. *188*, 623 (1951). – C. A. OWEN, A. G. KARLSON, and E. A. ZELLER, J. Bact. *62*, 53 (1951). – E. A. ZELLER, L. S. VAN ORDEN, and W. F. KIRCHHEIMER, Feder. Proc. *10*, 273 (1951), *11*; 316 (1952).

[1] E. A. ZELLER, L. S. VAN ORDEN, and W. F. KIRCHHEIMER, Feder. Proc. *10*, 273 (1951), *11*, 316 (1952).

[2] E. A. ZELLER, C. A. OWEN, Jr., and A. G. KARLSON, J. Biol. Chem. *188*, 623 (1951). – C. A. OWEN, A. G. KARLSON, and E. A. ZELLER, J. Bact. *62*, 53 (1951). – E. A. ZELLER, L. S. VAN ORDEN, and W. F. KIRCHHEIMER, Feder. Proc. *10*, 273 (1951); *11*, 316 (1952). – E. A. ZELLER, *Oxidation of Amines*, in J. B. SUMNER and K. MYRBÄCK, *The Enzymes*, vol. II (Academic Press, New York, 1951), p. 536.

Table I
Mycobacterium smegmatis

25 mg of wet packed bacteria (streptomycin-sensitive) are suspended in phosphate buffer at pH 7·1. Total volume: 2 ml; incubation: 6 h. Determination of ammonia (putrescine) with *Conway*-units and of the degradation of the guanidine group of agmatine with a modification of the procedure of DUBNOFF and BORSOOK[1].

Substrates	Inhibitor	Q	Inhibition
$2 \cdot 10^{-3}$ *M* putrescine	–	0·5 µMoles NH_3	–
M putrescine	$3 \cdot 10^{-5}$ *M* INH	0·4	20%
M putrescine	$3 \cdot 10^{-5}$ *M* IIH	0·2	60%
$2 \cdot 10^{-3}$ *N* agmatine	–	0·5 µMoles guanidine	–
N agmatine	$3 \cdot 10^{-5}$ *M* INH	0·4 groups disappearing	20%
N agmatine	$3 \cdot 10^{-5}$ *M* IIH	0·4	20%

[1] J. W. DUBNOFF and H. BORSOOK, J. Biol. Chem. *138*, 381 (1941).

Table II
Cell-free extract from M. smegmatis

Washed bacteria in aqueous suspension treated with 9 kilocycles-vibration for 1 h. Supernatant, obtained after centrifugation for 30 min with 10,000 g, contains 1·4 mg of nitrogen per ml. 0·1 ml of it is used in a total volume of 2 ml. Incubation with agmatine for 1 h at pH 7·1 (phosphate buffer).

Substrate	Inhibitor	Q Guanidine groups disappeared	Inhibition
$2 \cdot 10^{-3}$ *M* agmatine	–	2·4 µMoles	–
M agmatine	$5 \cdot 10^{-4}$ *M* INH	1·4	42%
M agmatine	$5 \cdot 10^{-4}$ *M* IIH	1·5	38%

Table III
Purified diamine oxidase of hog kidney cortex

Activity of diamine oxidase peparation: liberation of 84 mM of NH_3 per hour and per gram of nitrogen from 0·01 M cadaverine. In a total volume of 2 ml 0·079 mg of diamine oxidase-N was present, together with 2 μg of purified catalase (*Armour*). Incubation in phosphate buffer at pH 7·1 for 1 h.

Substrate	Inhibitor	Q	Inhibition
10^{-2} M cadaverine	—	3·5 μAtoms O_2	—
M cadaverine	$5 \cdot 10^{-4}$ M INH	1·7	51%
M cadaverine	$5 \cdot 10^{-4}$ M IIH	2·9	17%
M cadaverine	—	4·0 μMoles NH_3	—
M cadaverine	$5 \cdot 10^{-4}$ M INH	3·3	18%
M cadaverine	$5 \cdot 10^{-4}$ M IIH	4·0	0%

Table IV
Monoamine oxidase of rat liver mitochondria

Mitochondria prepared by differential centrifugation in 0·88 M sucrose (see references[1] and[2]). Mitochondria from 0·2 g liver (male Sprague-Dawley rats) are suspended in a total volume of 2 ml of phosphate buffer at pH 7·1. Incubation: 2 h.

Substrate	Inhibitor	Q Oxygen uptake	Inhibition
$5 \cdot 10^{-2}$ M tyramine	—	4·2 μAtoms	—
M tyramine	$1 \cdot 10^{-3}$ M INH	4·4	0%
M tyramine	$4 \cdot 10^{-5}$ M IIH	1·0	77%

bacteria[1], the two new drugs have a considerable action on mammalian diamine oxidase. The effective concentration (69 μg of INH per milliliter), however, is higher than the ordinary therapeutical concentration.

The deamination is obviously less reduced than the oxidation. In case of INH, only 0·51 atoms of oxygen are required for the liberation of one atom mole of ammonia. Thus, for the first time, it has been shown that the process of oxidative deamination occurs in two steps: (1) deamination and (2) oxidation. A partial separation of the two steps (enzymes) was also achieved in the course of the purification of diamine oxidase[1]. (See Table IV.)

The marked inhibition of monoamine oxidase is complete with 10^{-4} M INH, and is still 49% with $2 \cdot 10^{-3}$ (5 μg per milliliter). Since monoamine oxidase is considered to be an important agent in the inactivation of adrenaline and noradrenaline, this strong inhibition of the enzyme may be connected with some side reactions produced by this drug, indicating a sympathetic stimulation[2].

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Zusammenfassung

Isonikotinsäure-hydrazid (INH) und 1-Isonikotinyl-2-isopropyl-hydrazin (IIH) hemmen Diaminoxydase und Guanidindeamidinase von *Mycobacterium smegmatis* und verhalten sich in dieser Hinsicht gleich wie basische Antibiotika und basische tuberkulostatische Chemotherapeutika. Die beiden neuen Tuberkuloseheilmittel wirken auch auf eine gereinigte Diaminoxydase aus Schweinenierenrinde. Hierbei wird die Oxydation viel stärker als die Desaminierung beeinflusst, was zu einer teilweisen Aufspaltung des Prozesses der oxydativen Desaminierung führt. Auffallend stark ist die Hemmung der Monoaminoxydase der Mitochondria aus Rattenleber durch IIH, eine Reaktion, die möglicherweise im Zusammenhang mit unerwünschten Nebenreaktionen bei der therapeutischen Anwendung dieses Heilmittels steht, die unter dem Bild einer Sympathikuserregung verlaufen können.

[1] J. R. FOUTS and E. A. ZELLER, unpublished results.
[2] I. J. SELIKOFF, E. H. ROBITZEK, and G. G. ORNSTEIN, Quart. Bull. Sea View Hosp. *13*, 17 (1952).

[1] G. H. HOGEBOOM, W. C. SCHNEIDER, and G. E. PALLADE, J. Biol. Chem. *172*, 619 (1948).
[2] G. C. COTZIAS and V. P. DOLE, Proc. Soc. Exp. Biol. Med. *78*, 157 (1951).

Nitrogen Metabolism of *Penicillium Chrysogenum* – Q 176

In the course of our studies on the mechanism of the biosynthesis of penicillin by *Penicillium chrysogenum*–Q 176, it became desirable to ascertain the day-to-day changes in the nature of the free amino acids present in the medium and the mycelium during the growth of the organism. The methods previously described were not considered very convenient in view of the very large number of estimations involved but an opportunity was provided by the recent development of the simple circular paper chromatographic technique in this department by GIRI and his co-workers[1] for rapid detection and estimation of amino acids.

[1] K. V. GIRI, Current Science *20*, 295 (1951). – K. V. GIRI and N. A. N. RAO, Nature *169*, 923 (1952); J. Ind. Inst. Sci. *34* [2], 95 (1952).