paralysie centrale et périphérique...). Ce choc, que l'on retrouve dans l'intoxication par CCl_4 par exemple où il correspond à une accumulation de guanidine[1], peut être combattu par l'administration de cocaïne ou de novocaïne[2] et peut-être de glucose qui est un anticonvulsif dans les chocs guanidiques d'après ELLIS[3].

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Institut Pasteur, Paris, le 24 mai 1948.

Summary

Streptomycin forms an insoluble complex with organic substances of which the isoelectric point is situated in the acid range such as cephalin, oleic, palmitic, and tannic acid.

[1] J. W. TOMB et M. M. HELMY, J. Trop. Med. Hyg. *36*, 265 (1933).
[2] E. FRANK et R. STERN, Arch. exp. Path. Pharm. *90*, 168 (1921).
[3] M. M. ELLIS, Biochem. J. *22*, 941 (1928).

Cyanase, a new Enzyme Catalysing the Hydrolysis of Cyanate

It was recently found that some pharmacological properties of cyanate, e. g. the diuretic action in rats[1], was lost on incubation of cyanate with serum and organ extracts[2]. This was partly due to binding of cyanate by certain proteins[3], but the possibility remained that an enzymic destruction might have taken place as well. A further indication for the probable existence of a mechanism destroying cyanate in liver was obtained in recent experiments by DIRNHUBER and SCHÜTZ[4], when small but significant amounts of cyanic acid were liberated and distilled from brain and other tissues, but no trace was recovered from either fresh liver tissue or after it was incubated under physiological conditions. An investigation was therefore undertaken to see whether an enzyme could be found in tissues which was capable of destroying cyanate.

An enzyme was found in liver, kidney and erythrocytes, capable of catalysing the hydrolysis of cyanic acid into ammonia and carbon dioxide, thus:

$$HCNO + H_2O = NH_3 + CO_2$$

Aqueous extracts were made of organs from freshly killed rats or guinea pigs by grinding the organs with moistened sand and extracting the material with water. 0·4 *M*-phosphate buffer solution (p_H 6·3) was added to the centrifuged extract (1:5, v./v.); 0·2–0·3 ml of this mixture was placed into one side arm of a Warburg vessel. 0·2 ml of a freshly made 0·1 *M* aqueous solution of sodium cyanate was placed in the other side arm. Pure sodium cyanate was made from urea according to BADER, DUPRÉ, and SCHÜTZ[5]. 2·8–3·0 ml 0·4 *M*-phosphate buffer (p_H 6·3) were placed in the main compartment. It can be seen in Fig. 1 that both liver and kidney extracts were active in accelerating the hydrolysis of cyanate at p_H 6·3. Serum, or similarly made extracts of brain or muscle showed no activity at this p_H. No indication was yet found for the extracts having any catalytic action in the opposite direction.

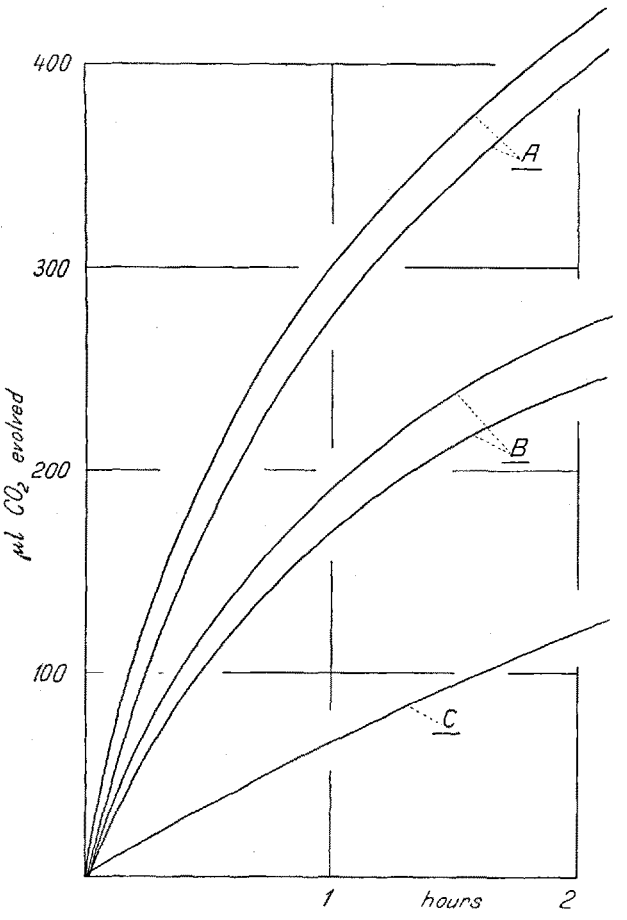


Fig. 1. – Hydrolysis of 1.3 mg sodium cyanate at p_H 6·3 (phosphate buffer, 0·4 *M*), in the presence of liver extract (*A*), and kidney extract (*B*). C = spontaneous hydrolysis.

The p_H optimum of the enzyme obtained from liver was found to lie in the region of 6·2–6·3. At p_H 5·5 the enzyme was inactive and at 6·8 its activity was reduced to less than 40% of that shown at 6·3. The enzyme obtained from liver and kidney was clearly thermolabile. Liver extract incubated for 15 min. at 65° was inactive.

The properties of this enzyme are being further investigated. We propose for this new enzyme the name of "cyanase".

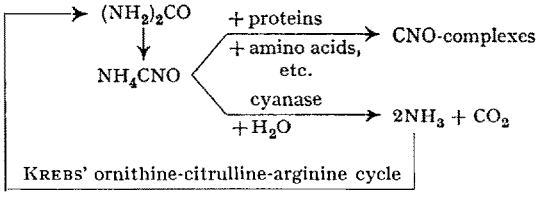
An alternative way to show the activity of the enzyme, was by determining the decrease of cyanate present in the solution, on incubation of the latter with the enzyme. Using the recently developed manometric technique for the determination of cyanate[1], 3 *M*-acetate buffer (p_H 5) is added from one side bulb of a Warburg vessel to the enzyme substrate mixture contained in the main compartment. By this addition any carbonate and bicarbonate present is decomposed, with the consequent evolution of CO_2. When working at room temperature equilibrium is attained again within 10 to 12 min. During this time only negligible amounts of cyanate are destroyed. When, after equilibration, 10% H_2SO_4 is added from the other side bulb, the further evolution of CO_2 then occurring is due to the hydrolysis of cyanate at this low p_H. From the evolved CO_2 the amounts of cyanate can be calculated, since carbonate and bicarbonate were decomposed before.

[1] F. SCHÜTZ, J. Physiol. *105*, 17, P (1946). – K. M. BIRCH and F. SCHÜTZ, Brit. J. Pharmacol. *1*, 186 (1946).
[2] M. MILLINGTON and F. SCHÜTZ, in preparation.
[3] S. B. HOLTHAM and F. SCHÜTZ, Biochim. Biophys. Acta (1948), in the press.
[4] P. DIRNHUBER and F. SCHÜTZ, Biochem. J. *41*, liv (1947); Biochim. Biophys. Acta, in the press, (1948*a*); Biochim. Biophys. Acta, in the press, (1948*b*).
[5] R. BADER, D. J. DUPRÉ, and F. SCHÜTZ, in the press.

[1] P. DIRNHUBER and F. SCHÜTZ, Biochem. J. *42*, 628 (1948).

The above described results have been confirmed by means of this procedure. Moreover, this procedure made it possible to investigate whether the enzyme was still active at neutral or alkaline p_H. The enzyme was still found active at p_H 7·4, though its activity was less than 30% of that shown at p_H 6·3. The physiological role of this enzyme probably consists in catalysing the hydrolysis of cyanate. Particular interest seems to lie in the fact that the substrate of cyanase is the simplest organic nitrogenous compound whose hydrolysis is catalysed by a thermolabile enzyme. Cyanase may, therefore, be helpful in the study of the mechanism underlying processes of hydrolysis.

There may be limitations to its activity *in vivo*. Tissue slices at p_H 7·4 still catalysed the hydrolysis of cyanate, but they were much less active than extracts at the optimal p_H (6·3). Since strong indications have recently been found for the formation of small amounts of cyanate from urea to occur in certain mammalian tissues by isomeric transformation (reverse Wöhler reaction)[1,2], it would seem conceivable that a small proportion of the urea present in the body would start the following cycle.



Cyanate, isomerized from urea, could either react with proteins[1], with several tetrapyrrolic compounds[3], or in a number of other possible ways. Surplus of cyanate may then be hydrolysed by cyanase, thus providing small amounts of ammonia and CO_2. Some of the ammonia thus formed would again be incorporated into urea by the ornithine-citrulline-arginine cycle of KREBS.

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Résumé

Une nouvelle enzyme a été découverte dans le foie, le rein et les érythrocytes. Cette enzyme catalyse l'hydrolyse du cyanate en NH_3 et CO_2. Le substrat de cette enzyme est le plus simple des composés azotés organiques connus aujourd'hui qui soit hydrolysé par une enzyme thermolabile.

[1] B. HOLTHAM and F. SCHÜTZ, Biochim. Biophys. Acta (1948), in the press.

[2] P. DIRNHUBER and F. SCHÜTZ, Biochem. J. *41*, liv (1947); Biochim. Biophys. Acta, in the press (1948*a*); Biochim. Biophys. Acta, in the press, (1948*b*).

[3] R. BADER, P. DIRNHUBER, and F. SCHÜTZ, in preparation.

Über den Einfluß des B_1-Vitamins auf den Zuckerumsatz beim Alloxandiabetes

Die Frage, ob beim Diabetes mellitus eine spezifische Störung des Zuckerhaushaltes oder ob darüber hinaus eine allgemeine stoffwechselphysiologische Schädigung vorliegt, wurde mehrfach untersucht. Man bediente sich dabei neben der Verfolgung des Kohlehydratumsatzes der Bestimmung des Grundumsatzes oder der Ermittlung des Gaswechsels nach Zuckerzufuhr bzw. nach Muskelarbeit. Die Ergebnisse sind nicht einheitlich, erlauben aber trotzdem die Schlußfolgerung, daß die leichteren und mittelschweren Formen des menschlichen Diabetes keine wesentliche Abweichung des Ruheumsatzes aufweisen, obwohl die Zahlenwerte nicht selten eine Neigung gegen die obere Grenze der Norm haben. Bei schweren Diabetesfällen können dagegen recht hohe Grundumsatzwerte angetroffen werden. Die dabei ebenfalls beobachteten Grundumsatzerniedrigungen lassen sich meist auf die damals übliche starke Unterernährung der Diabetiker zurückführen.

Tabelle I

Verhalten des R. Q. und des Grundumsatzes von weißen Ratten vor und nach Behandlung mit Alloxan. Vor Beginn des Versuches fasteten die Tiere 18—24 Stunden

			Grundumsatz pro 24 Std. und 1 m² Oberfl. in Kalorien
Ratte Nr. 39			
Vor	Alloxan-behandlung	0,74	1075
8 Tage nach		0,72	—
12 „ „		0,69	1182
22 „ „		0,72	1013
Ratte Nr. 41			
Vor	Alloxan-behandlung	0,77	1021
6 Tage nach		0,75	933
12 „ „		0,68	1066
21 „ „		0,69	1144

Der respiratorische Quotient (R. Q.) wurde meist erniedrigt gefunden. Er lag in schweren Diabetesfällen zwischen etwa 0,69 und 0,73 gegenüber ca. 0,8—0,85 beim Gesunden. Muskelarbeit, welche normalerweise den R. Q. schnell auf den Wert von etwa 1,0 oder darüber bringt, kann den R. Q. des Diabetikers unverändert lassen oder sogar etwas herabsetzen, und zwar trotz des begleitenden Mehrverbrauches an Zucker.

Beim experimentellen Diabetes nach Pankreasentfernung wurden starke Erhöhungen des Gaswechsels gefunden, der R. Q. war sowohl im nüchternen Zustand wie nach Zuckerzufuhr erniedrigt. Dies sind die wichtigeren Ergebnisse der älteren Forschung[1].

Die nun bestehende Möglichkeit der Erzeugung des Diabetes mellitus ohne Pankreasentfernung, sondern durch chemische Schädigung der insulinproduzierenden Betazellen mittels Alloxan veranlaßte uns, die Fragen des Gaswechsels beim experimentell gestörten Zuckerstoffwechsel einer erneuten Prüfung zu unterziehen. Wir benutzten dazu weiße Ratten, welche durch zwei bzw. mehrmalige subkutane Injektionen von je 15 mg Alloxan pro 100 g Körpergewicht schwer diabetisch wurden. Nach entsprechender Angewöhnung der Tiere an die Versuchsbedingungen wurde deren Gaswechsel in Ruhe und nach Zuckerzufuhr bestimmt.

a) Mit wenigen Ausnahmen blieb der Grundumsatz trotz der Zuckerverluste und der begleitenden allgemeinen Schädigungen auf normaler Höhe. Der R. Q. lag meist unterhalb des Durchschnittswertes von ca. 0,75—0,76, in mehreren Fällen wurden sogar Werte von 0,68 und 0,69 angetroffen.

[1] S. THANNHAUSER, Lehrbuch des Stoffwechsels (München 1929). – L. KREHL, Pathologische Physiologie (Leipzig 1930). – C. OPPENHEIMER, Hb. Biochemie *8*, 384 (1925).