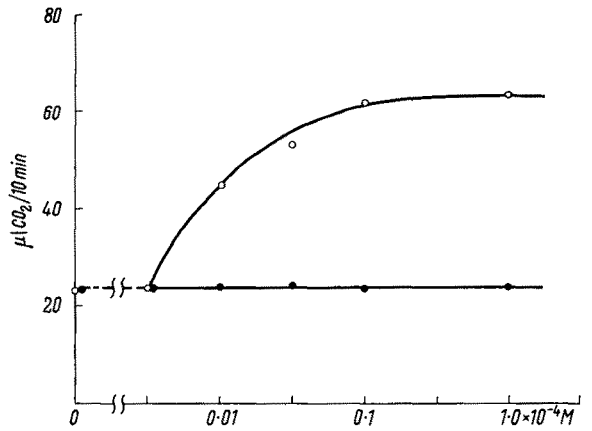
The Interaction Between Pyridoxal-5-Phosphate and Arginine Apodecarboxylase

In previous papers[1], the interaction between pyridoxal-5-phosphate (Py-5-P) and potassium cyanide was described. The resulting addition product, namely a cyanohydrine, which has been characterized by us both spectrophotometrically and ionophoretically, appeared unable to activate the glutamic-oxalacetic apotransaminase prepared and almost completely resolved according to the procedure of O'KANE and GUNSALUS[2]. On the other hand, no competitive action with Py-5-P was observed.

These results suggested that the 4-aldehyde group of Py-5-P is more likely involved in the interaction between Py-5-P and the specific protein portion (apotransaminase) rather than in the formation of a Schiff base between Py-5-P and the aminoacid substrate, as assumed by SCHLENK and FISHER[3] in their well known scheme.



Activity curves of apodecarboxylase preparation after addition of Py-5-P (o—o) and of its cyanohydrine •---•. The first curve is not influenced by previous or simultaneous addition of the cyanohydrine.

In order to verify whether such a rôle of the 4-aldehyde group of Py-5-P is essential in the activation process of apotransaminase only or of other apoenzymes (whose coenzyme is Py-5-P) as well, we have investigated the interaction between Py-5-P and arginine apodecarboxylase.

The enzyme preparation used in our experiments has been obtained from *Esch. coli*, Mac Leod ATCC 10536, according to TAYLOR and GALE[4]. The resolution obtained was about 65%. The decarboxylation reaction was followed in Warburg manometers containing 0·5 ml enzyme preparation, 1·5 ml M/5 acetate buffer pH 5·25 and 0·5 ml M/15 L(+)arginine. Experiments were carried out at 30°C and the evolution of CO_2 was studied for 10 min after addition of substrate (from the side bulb) following equilibration. The specific activity of the arginine apodecarboxylase preparation (in presence of $0·5 \times 10^{-4}$ M Py-5-P) was 102, in terms of μl CO_2 liberated in 10 min/mg protein.

While KCN inhibits arginine apodecarboxylase activity as reported by TAYLOR and GALE[4], the cyanohydrine of Py-5-P does not modify the residual activity of the partially resolved enzyme. Thus, the enzyme preparation previously incubated for 30 min with Py-5-P cyanohydrine, even at a concentration 10^{-4} M, shows the same activity as the enzyme preparation not preincubated.

The activation curve of the arginine apodecarboxylase by Py-5-P is not influenced by the previous incubation of the enzyme with Py-5-P cyanohydrine, independently of the concentration of this latter. Further, the Py-5-P cyanohydrine does not interfere with Py-5-P in the activation process even when added to the apoenzyme simultaneously with Py-5-P (Figure).

As it appears from our results, the cyanohydrine of Py-5-P neither activates apodecarboxylase nor exerts a competitive action in regards to Py-5-P itself, so that the 4-aldehyde group of Py-5-P seems to be involved in the linkage to the apoenzyme.

It can be assumed that such a rôle is probably played by the 4-aldehyde-group in the case of any apoenzyme, whose coenzyme is Py-5-P.

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Institute of Human Physiology, University of Naples (Italy), December 18, 1957.*

Riassunto

Vengono descritti i risultati di indagini sull'interazione tra piridossal-5-fosfato ed apodecarbossilasi argininica.

La cianidrina del piridossal-5-fosfato non attiva l'apoenzima né riduce l'effetto attivante del coenzima naturale. Si presume che il gruppo aldeidico del piridossal-5-fosfato sia interessato nell'attacco all'apoenzima.

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Breakdown of Adenine Nucleotides in Beef Muscle *post mortem* and its Relation to the Content of Ammonia

During the breakdown of adenosine nucleotides in muscle tissue *post mortem*, ammonia and inosinic acid (IMP) is released from adenylic acid (AMP). During autolysis of beef muscle at various temperatures, a different production of ammonia was found[1]. It was less during autolysis at + 37°C than at + 5°C, taking into consideration the increased temperature. To what degree the content of ammonia in muscle *post mortem* is released from them, is studied in this article.

Musculus psoas maior from 4 well nourished young bulls was used. One part of each was hung in a thermostate at + 37°C (100% relative humidity) and irradiated with ultraviolet rays. The greater part of each muscle was placed in a box at + 5°C (70% relative humidity)[2]. The first sample from the muscles was processed 3 h after slaughtering for the chromatography of free nucleotides. The other specimens were taken at different intervals of time. For chromatography, the tissue was extracted three times with 10% trichloracetic acid, which was then ex-

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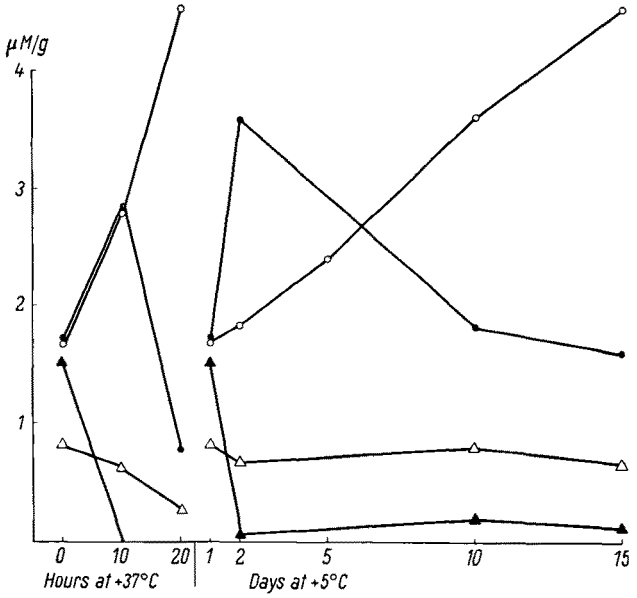
[3] F. SCHLENK and A. FISHER, Arch. Biochem. Biophys. *12*, 69 (1947).

[4] E. S. TAYLOR and E. F. GALE, Biochem. J. *39*, 53 (1945)

[1] Z. DVOŘÁK (not publicated).

[2] Z. DVOŘÁK, Čs. biologie *5*, 236 (1956).

tracted from the solution with ether[3]. The extract was adjusted to pH 8·5 and chromatographied through Dowex 2 (chloride form, 200–400 mesh, 1 × 12 cm) after washing with water and then by elution with 0·01 M NH_4Cl (the total volume of this fraction was about 1200 ml); then by elution with hydrochloric acid in the following concentrations: 0·001 M HCl; 0·003 M HCl; 0·005 M HCl; 0·01 M HCl; 0·02 M NaCl in 0·01 M HCl; 0·05 M NaCl in 0·01 M HCl; 0·2 M NaCl in 0·01 M HCl. 10 eluates 50 ml each were taken from every fraction manually and optical density at 250 mμ and 260 mμ was determinated by use of Beckman spectrophotometer.



The amount of ATP (▲), ADP (△), IMP (●) and hypoxanthine (○) in beef muscle during autolysis at two different temperatures.

In each pattern 10 greater peaks were detected, from which IMP, ADP and ATP were identified. AMP could not be reliably identified, although a little peak in the fraction eluted with 0·003 M HCl supported the possibility of its presence. The quantity was determinated using molecular extinction coefficients: 14200 at 260 mμ for phosphates of adenosine and 13200 at 250 mμ for IMP[4]. The fraction, which was eluted with 0·01 M NH_4Cl was all counted as hypoxanthine, using molecular extinction coefficient 8100 at 260 mμ[5].

Breakdown of nucleotides is distinct from the Figure, where mean values from 4 muscles are indicated. It is evident that, before the first analysis of specimens, the greater part of ATP was already dephosphorylated. That agrees with the high content of IMP and hypoxanthine. The amount of ADP during autolysis at + 5°C remained at the same level. It is probably nucleotide bound in myofibrils, which is not attacked by enzymes at lower temperature[6].

From the amount of hypoxanthine and IMP, the equivalent content of ammonia may be deduced. On the day of slaughter, the amount of both is equivalent to 6 mg% of ammonia in muscle. On the second and tenth day, it is equivalent to 9 and 10 mg% of ammonia respectively. From this fact, it is seen that ammonia is released from nucleotides only up to the second day. The desamination is probably in close connection with the destruction of ATP (the same agrees also for autolysis at + 37°C). As ATP is a criterium for beginning of *rigor mortis* in muscle[7], it may be said that the production of ammonia from nucleotides finishes at the beginning of *rigor mortis*.

The author wishes to express his thanks to Dr. Z. BRADA from the Oncological Institute in Brno for the possibility of measuring with a Beckman spectrophotometer.

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Institute for Research of Meat and Fish, Brno (Czechoslovakia), December 4, 1957.

Zusammenfassung

Der postmortale Zerfall von Adenosintriphosphat im Rindermuskel wurde mit der Ammoniakbildung verglichen. Die Ammoniakbildung aus Nukleotiden ist mit dem Eintreten der Totenstarre beendet.

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Über Pflanzenwachstumsregulatoren*

Beiträge zur Kenntnis der phytotoxischen Wirkung von Triazinen

Die phytotoxische Wirkung von 2-Chlor-4,6-bis-(diäthylamino)-s-triazin wurde erstmals von GAST, KNÜSLI und GYSIN[1] beschrieben. Später wurden weit wirksamere Verbindungen dieser Körperklasse aufgefunden, unter denen besonders Simazin [2-Chlor-4,6-bis-(äthylamino-) s-triazin] hervorsticht[2].

In dem durch Triazine hervorgerufenen Vergiftungsbild tritt normalerweise eine typische Chlorose auf. Es war deshalb naheliegend, die Frage zu prüfen, ob im Verlauf der Vergiftung Störungen in der CO_2-Assimilation, bzw. Stärkebildung auftreten. Für diese orientierenden Untersuchungen wurde *Coleus Blumei* Benth. als Versuchspflanze gewählt, ein Objekt, welches eine intensive Stärkeproduktion aufweist. Etwa 20 cm hohe Pflanzen wurden mit je 50 mg Simazin und Chlorazin behandelt. Als Vergleichsmittel wurde CMU in derselben Dosierung verwendet. Chlorazin wurde in Wasser emulgiert, Simazin und CMU in suspendierter Form appliziert. Pro Topf wurden 50 ml der Versuchslösungen, welche die oben angegebenen Mengen der Wirksubstanzen enthielten, verwendet. Die Flüssigkeitsmenge genügte, um die Topferde vollständig zu durchnässen. Die Pflanzen wurden vorerst während 6 Tagen im Gewächshaus gehalten, dann für zwei Tage ins Dunkle verbracht und hernach erneut belichtet. Nach sechs bzw. acht (nach der Dunkelperiode) und zehn Tagen (nach Beginn der zweiten Belichtung) wurden Blätter entnommen, mit heissem Alkohol vom Chlorophyll befreit und mit Jod-Jodkaliumlösung auf ihren Stärkegehalt geprüft. 6 Tage nach der Behandlung zeigten die mit CMU behandelten Pflanzen bereits als erste Ver-

* 3. Mitteilung (siehe auch die zwei folgenden Arbeiten EXER und ROTH).

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