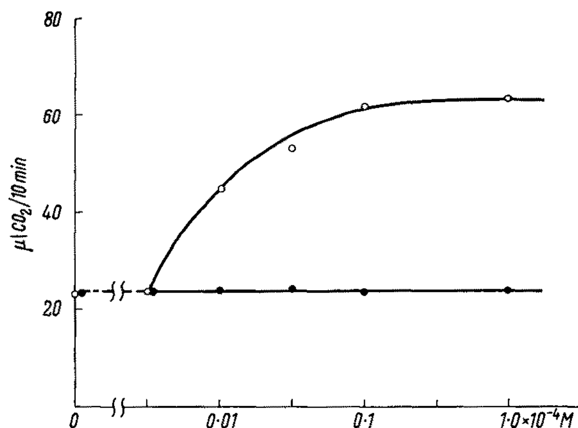
The Interaction Between Pyridoxal-5-Phosphate and Arginine Apodecarboxylase

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 (○—○) 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.

V. BONAVITA and V. SCARDI

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.

* Postal address: S. Andrea delle Dame, 8, Naples.

[1] V. SCARDI and V. BONAVITA, Boll. Soc. Biol. Sper. *33*, 770 (1957); Acta vitaminol. *11*, 117 (1957); Exper. *14*, 7 (1958).
[2] D. E. O'KANE and I. C. GUNSALUS, J. biol. Chem. *170*, 425 (1947).
[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)

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-

[1] Z. DVOŘÁK (not publicated).
[2] Z. DVOŘÁK, Čs. biologie *5*, 236 (1956).