mit Sukzinat («Sukzinat-abhängige Transhydrogenase») oder NADH («NADH-abhängige Transhydrogenase») als Energiequelle untersucht. Reaktionsmilieu siehe Legende zur Tabelle. Ethacrynsäure (Merck, Sharp und Dohme)[7] wurde in verdünntem Bikarbonat gelöst und dem Messansatz ohne vorherige Inkubation direkt zugesetzt. Die Proteinbestimmung erfolgte nach Kjeldahl.

Die Tabelle zeigt, dass Ethacrynsäure die untersuchten energiegekoppelten Transhydrogenase-Reaktionen schon bei einer Konzentration von $5 \times 10^{-5} M$ deutlich hemmt. Somit weisen auch unsere Ergebnisse darauf hin, dass Ethacrynsäure ihre Wirkung bei der Transformierung der Respirationsenergie, d.h. bei einer der Energieübertragungsreaktionen der oxydativen Phosphorylierung entfaltet. Da, wie ERNSTER[4] gezeigt hat, die energieabhängige Transhydrogenase-Reaktion keines phosphorylierten energiereichen Zwischenproduktes bedarf, und andererseits Ethacrynsäure die endergone Transhydrogenase-Reaktion sowohl dann hemmt, wenn ATP, als auch dann, wenn die Respiration als Energiequelle dient, nehmen wir an, dass Ethacrynsäure mit einem nichtphosphorylierten Zwischenprodukt der mitochondrialen Energieübertragung einen inaktiven Komplex bildet.

Wir halten es durchaus für möglich, dass für die pharmakologische Wirkung der Ethacrynsäure neben der Hemmung der Membran-ATPase[1,2], auch die Beeinflussung der Energieübertragung von Bedeutung sein könnte.

*Summary.* Ethacrynic acid, a diuretic drug, inhibits the endergonic reduction of $NADP^+$ by NADH in sonic particles from liver, the energy being supplied either by adding ATP or by respiration.

R. KRAMAR und E. KAISER

*Institut für Medizinische Chemie der Universität, Wien (Österreich), 15. März 1968.*

[1] J. B. HOOK und H. E. WILLIAMSON, Proc. Soc. exp. Biol. Med. *120*, 358 (1965).
[2] D. E. DUGGAN und R. M. NOLL, Archs Biochem. Biophys. *109*, 388 (1965).
[3] Y. GAUDEMER und B. FOUCHER, Biochim. biophys. Acta *131*, 255 (1967).
[4] L. DANIELSON und L. ERNSTER, Biochem. biophys. Res. Commun. *10*, 91 (1963); Biochem. Z. *338*, 188 (1963).
[5] E. C. WEINBACH, Analyt. Biochem. *2*, 335 (1961).
[6] W. W. KIELLEY und J. R. BRONK, J. biol. Chem. *230*, 521 (1958).
[7] Für die Überlassung von Ethacrynsäure sind wir der Fa. Contex (Wien) zu grossem Dank verpflichtet.

## Autoradiographic Studies on Collagen in Plant Nuclei[1]

From biochemical analysis on Walker tumour and rat liver cells, STEELE and BUSCH[2] demonstrated that the residual nuclear protein is rich in collagen. They confirmed the presence of collagen by electron microscopic studies. On the basis of the fact that structure and function of chromosomes are the same in plants and animals, collagen should be present in plant nuclei, if it happens to be an important and integral part of the chromosome. In a previous report, the present workers detected the presence of collagen in plant nuclei[3]. It was reported that the plant nuclei showed typical collagen staining with picro-ponceau with hematoxylin, Mallory's P.T.A.H. and Verhoeff's elastic stain, only when the nucleic acids were removed by hydrolysis with 5% TCA at 90 °C for 15 min followed by digestion with 3 mg/ml solution of pepsin in $N/50$ HCl at pH 2.3 for 6 h at 37 °C and digestion with 1 mg/ml solution of trypsin in phosphate buffer at pH 6.0 for 6 h at 37 °C in succession. That this positive staining in onion nuclei is truly due to collagen, was indicated by the fact that collagenase treatment (1 mg/ml solution in distilled water for 18 h at 37 °C) effaced the typical collagen staining.

Although labelled hydroxyproline would have been the ideal precursor substance for specific labelling of collagen for autoradiographic studies, unfortunately it is not incorporated in collagen[4]. However, radioactive proline can be used for detection of collagen[5,6]. In the present experiment, onion root tip nuclei were incorporated with 30 $\mu$c/ml (sp. act. 1.15 c/m$M$) for 8 h. The root tips were fixed in Carnoy's fixative, dehydrated and embedded in paraffin and sectioned at 5 $\mu$. The tissue sections were mounted alternately on 2 different slides so that 2 sections of the same nucleus could be located on 2 different slides. 1 of the slides was treated with TCA to remove nucleic acids, followed by digestion with pepsin and trypsin in succession. The other one was digested with a solution of collagenase in addition. The concentration, pH and duration of treatment used for each enzyme were the same as that used in stainability studies reported earlier. All the slides were then rinsed in water and then taken up to 50% alcohol. Autoradiographic film was applied to the serial sections. The film was exposed for 15 days and then developed. After air drying the sections were coloured through the emulsion with picro-ponceau to provide a colour image. The autoradiographic grains were counted on 10 $\mu^2$ of chromatin material with the help of a reticule. Such counts were made on 2 adjacent sections of 40 nuclei, which were treated as stated above. The results are presented in the Table.

It was observed that collagen treated nuclei showed 4.5 silver grains/10 $\mu^2$ in contrast to 6.2 in control. These results indicate that in the plant nuclei there is a protein which can be labelled by $H^3$-proline and be removed by collagenase.

Thus, an application of 2 valid and established methods: namely specific staining reaction for collagen and auto-

[1] This investigation was partially supported by a Ford Foundation Grant to the Department of Agricultural Engineering of this Institute. Thanks are due to Prof. V. N. PRASAD, Director, and Prof. A. C. PANDYA, Head of the Department of Agricultural Engineering of this Institute.
[2] W. J. STEELE and H. BUSCH, Expl Cell Res. *33*, 68 (1964).
[3] D. N. DE and S. N. GHOSH, Expl Cell Res. *47*, 637 (1967).
[4] K. JUVA, D. J. PROCKOP, G. W. COOPER and J. W. LASH, Science *152*, 92 (1966).
[5] J. CARNEIRO and C. P. LEBLOND, J. Histochem. Cytochem. *14*, 334 (1966).
[6] R. Ross, in *The Use of Radioautography in Investigating Protein Synthesis* (Ed. C. P. LEBLOND and K. R. WARREN; Academic Press, New York 1965), p. 273.

radiographic studies with $H^3$-proline in root tip cells, proves that there is indeed a detectable amount of collagen present in plant nuclei. It is very likely that collagen represents an inner core material of the chromosome and not an accessary or matrix substance, since partial removal of proteins by either pepsin or trypsin failed to produce any specific staining reaction. This contention has been strengthened by further experiments, to be published later, that like chloramphenicol and 5-methyl tryptophan, collagenase can induce chromosomal aberrations only when effective in pre-DNA-synthetic stage of both mitotic and meiotic cells.

| Treatment | Average No. of grains/10 $\mu^2$ of chromatin |
|---|---|
| Control (TCA + pepsin + trypsin) | 6.2 |
| Treated (TCA + pepsin + trypsin + collagenase) | 4.5 |

*Zusammenfassung.* Mit Hilfe von radioaktiv markiertem Prolin wird autoradiographisch das Vorkommen von Collagen im Matrixeiweiss pflanzlicher Zellkerne festgestellt.

D. N. DE and S. N. GHOSH

*Applied Botany Section, Indian Institute of Technology, Kharagpur (India), 26 April 1968.*

## Action of Adenosine Diphosphate on Human Platelets

Platelets contain both 5-hydroxytryptamine (5-HT) and adenosine triphosphate (ATP); BAKER, BLASCHKO and BORN[1] found most of both these substances in the same layer when homogenized platelets were centrifuged through a sucrose gradient. This, apart from the observations that the amount of 5-HT in normal platelets and the amount they are able to take up varies with the amount of ATP they contain[2], is the only evidence we have that ATP is concerned in the binding of 5-HT in platelets.

It has been known for some years[3] that adenosine diphosphate (ADP) added to platelet-rich plasma causes the platelets to aggregate. According to MACMILLAN[4] this occurs in 2 phases: the first phase is due to the action on the platelet membrane of the added ADP, the second to the action of endogenous ADP derived from the breakdown of ATP in the platelets.

If 5-HT is bound to ATP, the breakdown of part of this ATP might be expected to lead to the release of the associated 5-HT.

Human citrated-platelet-rich plasma containing ADP ($10^{-4}M$) was incubated in polycarbonate tubes for 20 min at 37 °C. During the first 5 min the plasma was stirred mechanically at 3 rev/sec. Aggregation of the platelets was marked in about 30 sec. Another portion of the same platelet-rich plasma to which no ADP had been added was treated similarly. After incubation the tubes were cooled in ice and centrifuged for 5 min at 25,000 *g*. ATP and 5-HT in the platelets was estimated by methods previously described[5]. Platelets treated with ADP lost about $^1/_3$ of their ATP but none of their 5-HT.

The effect of ADP on platelets which had been loaded with 5-HT was then investigated. Part of each sample of platelet-rich plasma was first incubated for 75 min in an atmosphere of 5% $CO_2$ in oxygen[6] with a solution of 5-HT in saline to give a final concentration of 2.4 $\mu$g/ml. Under these conditions the platelets become saturated with 5-HT. Another portion of the same platelet-rich plasma was incubated with an equal volume of saline. The effect of ADP on the loaded and unloaded platelets was then tested as described above. The results are recorded in the Table. Rather less ATP was lost from the loaded than from the unloaded platelets, but the difference is not significant at the 5% level: only the loaded platelets lost 5-HT.

These results show that treatment with ADP does, in fact, lead to a loss of part of the ATP in platelets but that normally no 5-HT is lost. They would be explained if 5-HT were bound to ATP in platelets. Normally more than enough ATP is present for this purpose; only when the platelets contain so much 5-HT that after treatment with ADP there is insufficient ATP remaining to bind it, is 5-HT lost.

Some of the results described here have been confirmed during the preparation of this paper by MILLS, ROBB and ROBERTS[7].

*Zusammenfassung.* Es wird gezeigt, dass der ATP-Gehalt der Thrombocyten in blutplättchenreichem Plasma mit ADP inkubiert abfällt, während deren 5-HT-Gehalt unverändert bleibt. Thrombocyten, die nach 5-HT-Sättigung inkubiert werden, verlieren sowohl ATP wie auch 5-HT.

R. S. STACEY

*Department of Pharmacology and Therapeutics, St. Thomas's Hospital Medical School, London, S.E.1. (England), 17 May 1968.*

Loss of ATP and 5-HT ($\mu$moles/ml packed platelets $\pm$ S.E.M.) from platelets after treatment with ADP ($10^{-4}M$)

| | Before loading with 5-HT | After loading with 5-HT |
|---|---|---|
| ATP | 1.06 $\pm$ 0.24 (33%) | 0.69 $\pm$ 0.07 (23%) |
| 5-HT | 0.01 (< 2%) | 0.38 $\pm$ 0.06 (19%) |

In brackets, % lost. Mean of 5 experiments.

1 R. V. BAKER, H. BLASCHKO and G. V. R. BORN, J. Physiol. *149*, 55P (1959).
2 G. V. R. BORN, G. I. C. I. INGRAM and R. S. STACEY, Br. J. Pharmac. Chemother. *13*, 62 (1958).
3 A. GAARDER, J. JONSON, S. LALAND, A. HELLEM and P. A. OWEN, Nature *192*, 531 (1961).
4 D. C. MACMILLAN, Nature *211*, 140 (1966).
5 P. D. MCCLURE, G. I. C. INGRAM, R. S. STACEY, U. H. GLASS and M. O. MATCHETT, Br. J. Haemat. *12*, 478 (1966).
6 R. S. STACEY, Br. J. Pharmac. Chemother. *16*, 284 (1961).
7 D. C. B. MILLS, I. A. ROBB and G. C. K. ROBERTS, J. Physiol. *195*, 715 (1968).