Reactivity of some Transition Metals on Nuclear Protein Biosynthesis in Rat Liver

The knowledge of nuclear protein biosynthesis is progressively improving[1–9]. Amino acids are incorporated into nuclear proteins where they are found in a genuine chemical bonding. Therefore pure adsorptive effects may be excluded. While it appears that in fact a biosynthesis of proteins or at least of peptides occurs in the nuclei, optimal conditions for in vitro studies still need to be elucidated.

We have designed an in vitro system employing enzymatically active whole rat liver nuclei[10] where ^{14}C-amino acids are readily incorporated. The assay seems to be independent of added nucleoside triphosphates such as ATP and GTP phosphoenolpyruvate and pyruvate kinase. Mg^{++} concentrations higher than 1 m*M* do not improve the rate of amino acid incorporation. Low concentrations of puromycin, chloramphenicol and actinomycin have no effect, either. This suggests the absence of ribosomal contaminations of the nuclear membrane as well as bacterial growth. However, nuclear protein biosynthesis may be inhibited if transition metals are used which are strongly coordinated to SH or S-S groups. The average radius of the metal ions is considerably smaller than that of the antibiotic molecules. This enables the metal ions to pass the nuclear membrane much better, and the blocking of a great number of biochemical reactions, including nuclear protein biosynthesis, is to be expected. Since some transition metals are able to influence the rate of ^{14}C-amino acid incorporation into the nuclei, a comparative study has been performed employing Mn^{++}, Fe^{3+} and Cr^{3+}, which are preferably coordinated to oxygen, and Hg^{++}, which usually shows a great tendency to react with sulphur.

Materials and methods. Reconstituted ^{14}C-protein hydrolysate in 0.01*N* HCl (Lot No. 6703) was obtained through Schwarz, Orangeburg N.Y. HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid), a grade Lot No. 920012, was purchased from Calbiochem, Los Angeles. Sucrose, special enzyme grade Lot No. U1149 from Mann, N.Y. Hydroxide of Hyamine 10-X, Lot No. 1264 from Packard. All other chemicals were of reagent grade quality.

Female albino rats (Wistar) with an average weight of 150 g were used throughout this study. Liver nuclei were isolated at 0–4 °C in sucrose solutions of different densities[11,12]. The nuclear RNA/DNA ratio was 0.25 ± 0.01 as estimated by the procedures of references [13] and [14]. Protein was assayed according to the Lowry method[15].

Incubations were carried out at 37 °C in a volume of 1 ml. If not otherwise stated, the incubation mixture usually contained: nuclei (2–3 mg of nuclear protein/ml), sucrose (225 m*M*), 0.1 μC/ml of ^{14}C-amino acids, phosphate or Hepes buffer (20 m*M*) pH 7.0, $MgCl_2$ (10 m*M*), NaCl (50 m*M*) and KCl (10 m*M*). The reaction was stopped by adding 1 ml $HClO_4$ (10%) to the assay mixture. The precipitate was washed with the same $HClO_4$ solution. After centrifugation the pellet was suspended for 15 min in 3 ml $HClO_4$ (5%) at 90 °C. The non-hydrolyzed proteins were washed with ethanol (80%) dissolved in 0.5 ml of Hyamine, and subjected to radioactive counting as described elsewhere[10].

Results and discussion. The time-dependent ^{14}C-amino acid incorporation of isolated enzymatically active whole liver nuclei is summarized in the Table. Samples which have been kept at ice-bath temperature throughout the experiment did not show a radioactivity higher than 50 cpm/mg protein.

The reactivity of the transition metals Hg^{++}, Mn^{++}, Fe^{3+} and Cr^{3+} on nuclear protein biosynthesis has been compared after a 30 min incubation. The results are plotted in the Figure.

Hg^{++}, which is known to form extremely stable complexes with sulphur and nitrogen derivatives, reduces the ^{14}C-amino acid incorporation down to about 30% of the control. On the other hand, a 30% incorporation refers to the ice-bath value; or, in other words, the synthesis of nuclear proteins can be regarded as totally inhibited. In no case can this be attributed to pure adsorption since Hg^{++} readily forms complexes with the available amino acids and rather an increase of ^{14}C-uptake would be expected. Unfortunately, the mode of inhibition is unknown; the metal ion may have reacted with the nuclear membrane, the proteins or with the polynucleotides[16]. The addition of Fe^{3+} and Mn^{2+} indicates a slight increase in the uptake of ^{14}C-amino acids by the nuclei. A remarkable stimulation can be observed in presence of Cr^{3+}-ions. First it was thought that this stimulation is an artefact due to the adsorption of amino acids to $Cr(OH)_3 \cdot nH_2O$. However, chromium hydroxide will be unstable during the 10% and 5% $HClO_4$ treatment of the precipitated nuclei. In another experiment Al^{3+}-ions were employed to

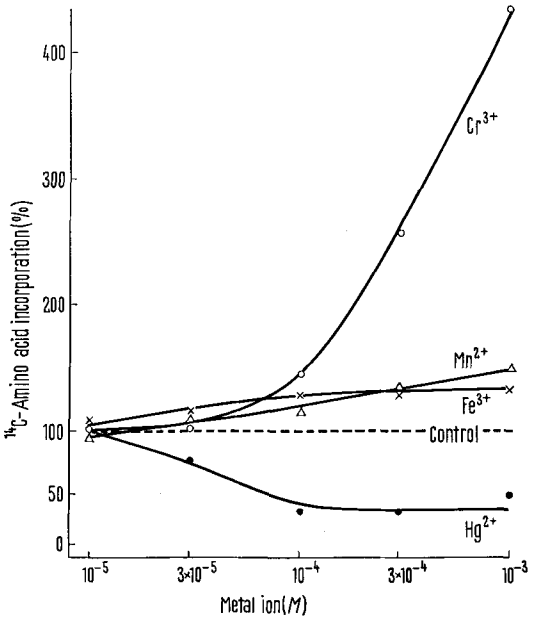
Incorporation of ^{14}C-amino acids in rat liver nuclei

Time (min)	^{14}C-incorporation (cpm/mg protein)
0	50
5	117
10	125
15	157
20	153
30	167
40	200
90	197
120	210
180	217

1 ml of the incubation mixture contained: phosphate buffer, 20 μmoles, pH 7.0; $MgCl_2$, 10 μmoles; NaCl, 50 μmoles; KCl, 10 μmoles; sucrose, 225 μmoles; 0.1 μC ^{14}C-amino acids; nuclei (3.3 mg/ml of nuclear protein).

[1] T. CASPERSSON, *Cell Growth and Cell Function* (Academic Press, New York 1950).
[2] M. M. DALY, V. G. ALLFREY and A. E. MIRSKY, J. gen. Physiol. *36*, 173 (1952).
[3] V. G. ALLFREY, A. E. MIRSKY and S. OSAWA, J. gen. Physiol. *40*, 451 (1957).
[4] R. LOGAN, M. FICQ and M. ERRERA, Biochim. biophys. Acta *31*, 402 (1959).
[5] H. ONO and H. TERAYAMA, Biochim. biophys. Acta *166*, 175 (1968).
[6] F. SANGER, Biochem. J. *39*, 507 (1945).
[7] S. AKABORI, K. OHNO, T. IKENAKA, A. NAGATA and I. HARUNA, Bull. chem. Soc. Japan *25*, 214 (1952).
[8] G. P. GEORGIEV, in *Enzyme Cytology* (Ed. D. B. ROODYN; Academic Press, New York 1967), p. 27.
[9] E. F. ZIMMERMANN, J. HACKNEY, P. NELSON and I. M. ARIAS, Biochemistry *8*, 2636 (1969).
[10] U. WESER and J. KOOLMAN, Z. physiol. Chem., *350*, 1273 (1969).
[11] C. C. WIDNELL and J. R. TATA, Biochem. J. *92*, 313 (1964).
[12] R. R. MCGREGOR and H. R. MAHLER, Arch. Biochem. Biophys. *120*, 136 (1967).
[13] K. BURTON, Biochem. J. *62*, 315 (1956).
[14] R. B. HURLBERT, H. SCHMITZ, A. F. BRUMM and R. VAN POTTER, J. biol. Chem. *209*, 23 (1954).
[15] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[16] U. WESER, Structure Bonding *5*, 41 (1968).

check possibly pure adsorptive reactions of $Al(OH)_3 \cdot nH_2O$, which very much resembles $Cr(OH)_3 \cdot nH_2O$ in its characteristics. No stimulation could be detected. The ^{14}C-amino acid incorporation rate was rather diminished following the addition of Al^{3+}-ions. Further, the counting efficiency did not change when a chromium-Hyamine complex was added to a known amount of ^{14}C.



^{14}C-amino acid incorporation in rat liver nuclei in presence of different transition metals. The incubation mixture was composed of: 24 m*M* Hepes buffer, pH 7.0 (to avoid chelating with metal ions); 10 m*M* $MgCl_2$; 0.1 μC ^{14}C-amino acids; 225 m*M* sucrose; nuclei (3.0 mg protein/ml) and different concentrations of Cr^{3+}, Fe^{3+}, Mn^{2+} and Hg^{2+} added as the chloride compounds.

Of course, this biochemical reactivity of chromium ions is rather unlikely under physiological conditions. On the other hand, it has been postulated that Cr^{3+} should be regarded as a biochemically active and essential metal ion[17]. Finally, the different chemical reactivity of Hg^{++} and other transition metal ions should be emphasized again. Hg^{++}, which is strongly coordinated to sulphur, causes a strong inhibition of the nuclear protein biosynthesis, while those transition metals which are preferably bound to oxygen, stimulate the ^{14}C-amino acid incorporation. Whether or not this correlation will be of relevance has to be elucidated.

Zusammenfassung. An enzymatisch aktiven Leberzellkernen aus Ratten wurde die Reaktivität von Hg^{2+}, Mn^{2+}, Fe^{3+} und Cr^{3+} auf die nukleare Proteinbiosynthese geprüft. Hg^{2+}-Ionen hemmen den ^{14}C-Aminosäure-Einbau vollständig, während Mn^{2+} und Fe^{3+} eine schwache Stimulierung verursachen. Die ^{14}C-Einbaurate ist nach Cr^{3+}-Zusätzen stark erhöht. Es konnte gezeigt werden, dass die Cr^{3+}-Stimulierung nicht durch Adsorption zu erklären ist.

U. Weser und J. Koolman

Physiologisch Chemisches Institut der Universität, D-7400 Tübingen (Germany), 6 October 1969.

[17] W. Mertz, Physiol. Rev. *49*, 163 (1969).

Reparation at Increased Oxygen Supply[1]

The increase of the oxygen concentration in the breathing gas of the rat to about 50% v/v enhances the tensile strength of the healing skin wound by 40%[2]. An augmented synthesis of collagen in vitro has been reported also as the effect of the increased oxygen in the gas phase[3]. This effect could not depend entirely on the requirement of oxygen for the hydroxylation of proline in protocollagen, because the K_m is only about 2.6% v/v[4]. The present experiments show that the increased oxygen supply affects also components other than collagen in the regenerating tissue.

For the experiments with ^{3}H-proline (Figure, a and b), 4 viscose cellulose-sponges (1 × 1 × 2 cm) were implanted s.c. in the backs of male Wistar rats (weight 200 ± 30 g). The animals were closed into boxes where they breathed either air or 40% v/v oxygen. After 10 days the rats were decapitated, samples pooled from 4 sliced granulomas and incubated in 20 ml of Krebs-Ringer phosphate medium with 22.4 m*M* glucose. The gas phase was either air or 40% v/v oxygen. After a preincubation period of 30 min 80 μCi of ^{3}H-proline (The Radiochemical Centre, Amersham, England) were added and the incubation continued for 4 h. The tissue was washed twice with ice-cold water after separating it from the medium by centrifugation at 0 °C and 30,000 *g* for 10 min. The material was homogenized in water, hydrolyzed and the total activity and radioactivity of hydroxyproline were determined[5,6] using Packard Tri-Carb 3320 liquid scintillation spectrometer (Packard Instrument Company Inc., Downers Grove, Illinois, USA). When ^{3}H-proline was used as a label, only 3–10% of the incorporated ^{3}H was recovered in ^{3}H-hydroxyproline. Because proline and hydroxyproline occur in collagen in approximately equal amounts, the radioactivity of collagen was accepted to be twice that of hydroxyproline. The radioactivity of non-collagenous protein was obtained by subtracting the ^{3}H-activity of collagen from the total ^{3}H-activity.

The experiments with ^{3}H-cytidine (Figure, c) were performed analogously except that 30 μCi of the precursor was added. The incubation was stopped by the addition of cold perchloric acid solution to the final concentration of 0.5 *N*. The isolation of RNA was based on hydrolysis in 0.3 *N* potassium hydroxide at + 37 °C for 20 h and subsequent neutralization at 0 °C with perchloric acid which

[1] Supported by institutional grants from the Sigrid Jusélius Foundation and U.S. Department of Agriculture, Foreign Research and Technical Programs Division.

[2] J. Niinikoski, R. Penttinen and E. Kulonen, Acta physiol. scand. suppl. 277, 146 (1966). – E. Kulonen, J. Niinikoski and R. Penttinen, Acta physiol. scand. *70*, 112 (1967). – J. Niinikoski, Acta physiol. scand. suppl. *334*, 72 pp. (1969).

[3] M. Chvapil, J. Hurych and E. Ehrlichová, Z. physiol. Chem. *349*, 211 (1968).

[4] J. J. Hutton, A. L. Tappel and S. Udenfriend, Arch. Biochem. *118*, 231 (1967).

[5] K. Juva, Acta physiol. scand. suppl. *308*, 73 (1968).

[6] D. J. Prockop and P. S. Ebert, Analyt. Biochem. *6*, 263 (1963).