ished. Our findings would suggest that Δ^1-cortisol may impair the intranuclear transport of macromolecule-bound cAMP-H^3 and thereby lessen, but not suppress, the transcriptive effects of the cyclic nucleotide on rat liver chromatin[15,18,19]. On the other hand, it appears reasonable to admit that corticosteriods may act also by inhibiting in part (at the level of transcription?) the synthesis of nuclear macromolecular acceptors of cAMP-H^3, and by simultaneously enhancing the synthesis of a number of cytoplasmic phosphokinases which are activated by the binding of cAMP to their regulatory subunits[20]. Such a different regulation by corticosteroids of the synthesis of nuclear and cytoplasmic cAMP acceptors is supported: 1. by the finding that in hepatocytes the cytoplasmic increment of specific cAMP binding macromolecular sites is percentually greater than their intranuclear simultaneous decrease; 2. by the behaviour of fibroblast-like cells, in which only the nuclear uptake is decreased. A report stating that glucocorticoids do not alter the uptake of cAMP-H^3 by H4-11E hepatoma cells in tissue culture[6] does not contrast with our findings, since hepatoma cells are metabolically different from normal liver cells[6]. The relief of a post-transcriptional role of cAMP has been stressed in several reports[9]. CAMP has been shown to enhance the phosphorylation of ribosome proteins[21], and to influence polypeptide synthesis[22], assembly[23] and release[23,24] at the level of this organelle. Moreover, cAMP has been postulated to modulate, at the level of mRNA translation, adrenal[25], adenohypophyseal[26] and thyroid[27] protein synthetic processes. A microsomal protein, strongly binding cAMP, has been indicated as an essential factor in the induction by cAMP of the release of enzymes from rat liver polysomes[23]. In this frame, our results suggest that glucocorticoid hormones increase in the hepatocyte the cytoplasmic utilization (and thereby metabolic effects[1-5,7]) of cAMP.

Riassunto. I meccanismi che operano alla base dell'effetto «permissivo» dei corticosteroidi sulle azioni metaboliche dell'adenosin-monofosfato-3′,5′-ciclico (cAMP) sono stati indagati in colture primarie di fegato di Ratto. I risultati ottenuti indicano che i glucocorticoidi, mentre diminuiscono l'utilizzazione del cAMP nel nucleo degli epatociti, la aumentano a livello del citoplasma. Viene avanzata l'ipotesi che gli steroidi inibiscano la sintesi delle chinasi proteiche nucleari cAMP-dipendenti e/o il loro trasferimento nel nucleo e, contemporaneamente, stimolino la sintesi delle chinasi citoplasmatiche. Per converso, si è appurato, in parallelo, che il cAMP ha effetti alquanto limitati sul destino del cortisolo (e metaboliti) legato a proteine nella cellula epatica.

U. ARMATO, ENRICA DRAGHI and PAOLA G. ANDREIS

Tissue Culture Laboratory of the Department of Human Anatomy, University of Padova Medical School, Via A. Gabelli 37, Padova (Italy), 18 July 1973.

[18] T. A. LANGAN, J. biol. Chem. *244*, 5763 (1969).
[19] L. A. DOKAS and L. J. KLEINSMITH, Science *172*, 1237 (1971).
[20] A. KUMON, H. YAMAMURA and Y. NISHIZUKA, Biochem. biophys. Res. Commun. *41*, 1290 (1970).
[21] J. E. LOEB and C. BLAT, FEBS Lett. *10*, 105 (1970).
[22] P. DE NAYER and M. DE VISSCHER, Archs int. Physiol. Biochim. *79*, 1021 (1971).
[23] C.-C. CHUAH and I. T. OLIVER, Biochemistry *10*, 2990 (1971).
[24] E. A. KHAIRALLAH and H. C. PITOT, Biochem. biophys. Res. Commun. *29*, 269 (1967).
[25] L. D. GARREN, G. N. GILL and G. M. WALTON, Ann. N.Y. Acad. Sci. *185*, 210 (1971).
[26] F. LABRIE, G. BÉRAUD, M. GAUTHIER and A. LEMAY, J. biol. Chem. *246*, 1902 (1971).
[27] S. LISSITZKY, S. MANTE', J. C. ATTALI and G. CARTOUZOU, Biochem. biophys. Res. Commun. *35*, 437 (1969).

On the Activity of Cathepsin C in Human Embryonic Kidney Cell Cultures Infected with *Herpesvirus hominis* (*Herpes simplex*)

One of the possible results of the viral infection of susceptible cells in tissue culture are cytopathic effects (CPE), morphological and physiological changes which result from a changed metabolism and an interruption of the stimulation for the cellular replication.

The importance of lysosomal enzymes for the majority of biological processes was obvious soon after the discovery of these subcellular structures[1,2]. The damage of the lysosomes and the release of their enzymes can in certain diseased conditions and after death cause the lysis of the cell.

Already ALLISON and MALLUCCI[3,4] established that release of lysosomal enzymes into the cytoplasm makes an important contribution to cytopathic effects. FINE et al.[5] reported that cells infected with *Herpes simplex* virus undergo different lysosomal and cytopathic changes, which could be correlated with increased accumulation of acid phosphatase and infectious virus in the extracellular fluid. LA PLACA et al.[6] found that acid phosphatase and β-glucuronidase are released from the cells after infection with some toga- and enteroviruses. Lactic dehydrogenase and β-glucuronidase were detected in the tissue culture fluid and in the cellular lysates from cell cultures infected with rabies virus[7]. The activation of lysosomal enzymes was detected from the day 4 of infection on without any indication of CPE. REEVES and CHANG[8] investigated acid phosphatase in the cell infected with vaccinia, fowl plague virus, poliovirus type 2 and adenovirus type 4. The increase of enzyme activity accompanied the first detectable CPE.

It seemed therefore of some interest to test the hypothesis that cathepsins as lysosomal components are an important factor contributing to the destruction of cells infected with viruses. The effect of these hydrolytic enzymes was already investigated in organisms after ionizing irradiation[9,10].

[1] C. DE DUVE, *Lysosomes*, Ciba Foundation Symposium *1* (J. and A. Churchill, London 1963).
[2] C. DE DUVE, Protoplasma *63*, 95 (1967).
[3] A. C. ALLISON and L. MALLUCCI, *J.* exp. Med. *121*, 463 (1965a).
[4] L. MALLUCCI and A. C. ALLISON, J. exp. Med. *121*, 477 (1965b).
[5] D. L. FINE, R. S. LAKE and E. H. LUDWIG, J. Virol. *5*, 226 (1970).
[6] M. LA PLACA, B. G. BARBANTI and M. PORTOLANI, G. Microbiol. *17*, 79 (1969).
[7] A. M. DIAZ, F. J. DIAZ, P. YAGER and G. M. BAER, Infection Immun. *4*, 546 (1971).
[8] M. W. REEVES and G. C. CHANG, Microbios, Cambridge *4*, 167 (1971).
[9] R. N. FEINSTEIN and J. C. BALLIN, Proc. Soc. exp. Biol. Med. *83*, 6 (1953).
[10] D. LEBEZ, S. KLEMENC-ŠEBEK and L. STRAUCH, Proc. IInd Int. Conf. on the Peaceful Uses of Atomic Energy, Geneva *22*, 533 (1958).

Materials and methods. Our experiments were performed in human embryonic kidney cell cultures infected with an adapted strain Z of *Herpesvirus hominis*. The cells were grown in milk bottles with 0.5% lactalbumin in Hanks' balanced salt solution with 10% bovine serum and 100 IU/ml of penicillin and streptomycin (growth medium). At the time of the experiment the bottles contained approximately 2.5 million cells in a monolayer. Previous to the infection the cells were washed with saline (0.85% NaCl). The cells giving 50% CPE 48 h after infection were infected with 1 ml viral suspension and 9 ml of the maintenance medium (0.5% lactalbumin in Hanks' balanced salt solution without phenol red and 1% calf serum and usual doses of antibiotics) and incubated at 37 °C. On the day after infection the virus titer in the tissue culture medium was 2 $\log_{10}$/ml. For each assay of enzymes 2 bottles have been used.

The samples of the cells and the tissue culture fluids were collected at the time of the infection (time 0), 4, 8, 24 and 44 h after the infection. Each experiment was repeated 5 times.

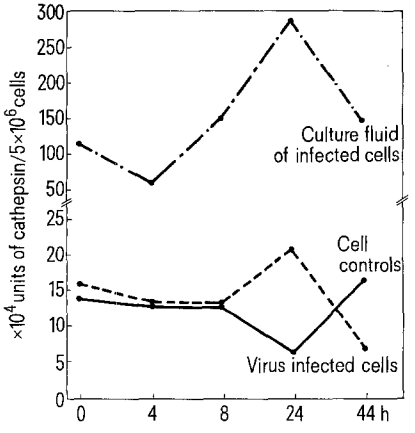

Fig. 1. The activity of cathepsin C in human embryonic kidney cells and in culture fluid after infection with herpes simplex virus.

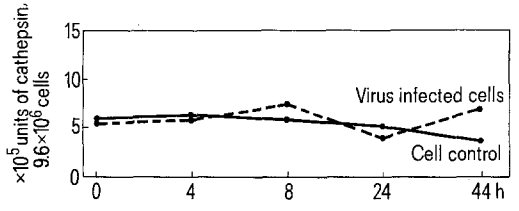

Fig. 2. The activity of cathepsin C in chicken embryonic fibroblasts infected with herpes simplex virus.

The tissue culture fluids from 2 bottles were pooled and the cells washed with a double amount of saline. Then the cells were frozen at −20 °C and thawed at room temperature. The cells were scrapped from the glass surface with a rubber policeman. The samples of the cells washed in distilled water were pooled and homogenized in a Potter-Elvejhems homogenizer. This suspension and the tissue culture fluids collected were assayed as 'enzymes'.

The activity of the enzymes was assayed in the cells and in the tissue culture fluids. Normal controls were treated by the same method and assayed simultaneously. The activity of cathepsins was measured by Ansons method using hemoglobin as substrate[11]. The cathepsin units were evaluated according to the dilution of the samples. Determinations were carried out with an UNICAM 500 SP spectrophotometer at 625 nm.

Results and discussion. The results (Figure 1) show changes of cathepsin C activity in the human embryonic kidney cell cultures and culture fluids infected with *Herpesvirus hominis*. The enzyme activity of infected cells compared with uninfected cells seems to be increased. This increase is marked in the cells at the 24th h after infection. The level of cathepsin is high, especially in the culture fluids of infected cells. Because in our experiments we could not detect cathepsin C activity in the tissue culture fluids of normal cells, we believe that it can be postulated that after the infection the activity of the enzymes increases first intracellularly and later in the tissue culture fluids.

This increased activity of cathepsins could not be detected (Figure 2) in fibroblasts of chick embryos infected with our strain of *Herpesvirus hominis*. This could be partly explained, as in this cell system the strain used does not replicate.

Our experiments indicate that increase of cathepsin C activity and cytopathogenic changes in our experimental system could be associated.

Zusammenfassung Die Aktivität des Kathepsins C ist in menschlichen embryonalen Nierenzellen nach Infektion mit *Herpes simplex* Virus erhöht. Ebenso ist die Menge des Kathepsins C in allen Zeitpunkten nach Infektion sehr gross im überstehenden Medium. Dagegen blieb die Aktivität des Kathepsins unverändert in Zellen, in denen keine Vermehrung von Herpesvirus stattfindet. Es ist möglich, dass in unserem Versuchsystem Kathepsin C einen der verantwortlichen Faktoren für die Cytopathogenität darstellt.

P. Schauer, H. Hren-Vencelj and M. Likar

Institute of Microbiology, Medical Faculty, 61105 Ljubljana (Yugoslavia), 27 April 1973.

[11] M. L. Anson, J. gen. Physiol. *22*, 79 (1939).

Cold Exposure: Effects on Hepatic Tryptophan Oxygenase and Tyrosine Aminotransferase, Plasma Tryptophan and Tyrosine, and Brain Monoamines

The activities of tryptophan oxygenase and tyrosine aminotransferase are affected by protein or food ingestion[1,2], adrenal hormones[3,4], environmental regimens[5,6], or dietary amino acids[7]. Recent investigations have been directed toward determining the relationship of the activity of these enzymes to the concentrations of tryptophan and tyrosine as well as serotonin and norepinephrine in peripheral and central tissues[8–10]. Hydrocortisone induction of hepatic tyrosine aminotransferase decreased both plasma and brain tyrosine[11], but did not alter the level of brain norepinephrine or serotonin. Alternatively, Curzon and Green demonstrated that immobilization stress[12] or hydrocortisone administration[13] induced hepatic tryptophan oxygenase, and concluded that