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.

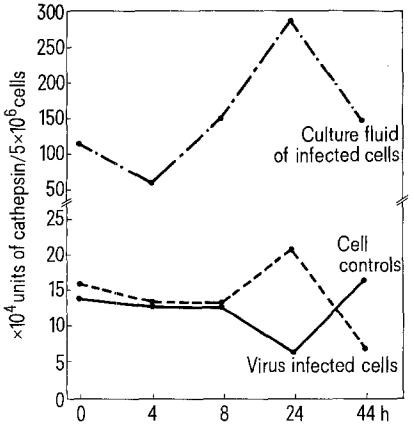

Fig. 1. The activity of cathepsin C in human embryonic kidney cells and in culture fluid after infection with herpes simplex virus.

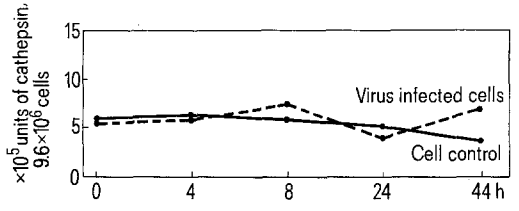

Fig. 2. The activity of cathepsin C in chicken embryonic fibroblasts infected with herpes simplex virus.

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.

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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

decreased brain serotonin was closely related to this induction. Recently, FERNSTROM and WURTMAN[14] reported that brain serotonin may be affected by physiological changes in plasma tryptophan levels.

We have documented increased activities of both hepatic tryptophan oxygenase and tyrosine aminotransferase in mice exposed to acute and chronic cold stress[6]. Accordingly, we hypothesized that cold exposure would offer an ideal experimental condition for investigating and clarifying the relationships between hepatic enzymes, plasma substrate levels, and concentrations of central monoamines.

Materials and methods. Adult, male (30–35 g) mice (Charles River, Wilmington, MA) were used in all studies. Control animals were housed in stainless steel, wire-bottom cages (4 per cage) in constant temperature (21–22 °C), windowless rooms with alternating light (06.00–18.00 h) and dark (18.00–06.00 h) periods. All animals had access ad libitum to Charles River Chow and fresh water throughout the experiment. Cold exposed (2–4 °C) animals were housed singly.

Sample collection. All animals were unanesthetized and were sacrificed at either 08.00 or 20.00 h. Blood was collected in heparinized tubes by cardiac puncture, centrifuged, and the plasma frozen and stored for later assay. Plasma assays were performed in triplicate from aliquots of pooled samples collected from at least 5 animals. Whole brains and livers were removed, frozen by immersion into liquid nitrogen, weighed, and stored at −20 °C for subsequent assay. Statistical analyses were performed by Student's *t*-test.

Assays. Tryptophan oxygenase was determined by the method of KNOX and AUERBACH[15] as modified by SEGLEN and JERVEL[16]; hepatic tyrosine amino-transferase after DIAMONDSTONE[17]. Tryptophan and tyrosine were assayed according to DENCKLA and DEWEY[18] and WAALKES and UDENFRIEND[19] respectively. The monoamines serotonin and norepinephrine were quantitated by the procedures of MAICKEL et al.[20] and CHANG[21].

Results. Figure 1 illustrates the effects of cold exposure on the specific activities of hepatic tryptophan oxygenase (TO) and tyrosine aminotransferase (TAT) in mice. In the cold stressed animals (broken line), disturbances in the daily periodicities of TO occurred primarily as a result of increases in the normally low AM (08.00 h) values. On day 4, TO activity remained significantly above control levels ($p < 0.01$), but by day 7 differences were not statistically different ($p > 0.05$). Tyrosine aminotransferase displayed a greater sensitivity to this environmental stress with increased rhythmic oscillations ($p < 0.02$ on day 28).

In Figure 2 is depicted, as percent change from respective control values, the effect of cold exposure on plasma tryptophan and tyrosine concentration. Mean control tryptophan levels ± SEM were 17.6 ± 0.7 µg/ml at 08.00 h ($n = 8$) and 20.1 ± 1.3 µg/ml at 20.00 h ($n = 3$); tyrosine concentrations measured 21.4 ± 1.5 µg/ml at 08.00 h ($n = 8$) and 21.2 ± 2.2 µg/ml at 20.00 hours ($n = 3$).

In Figure 3 are plotted the results for brain serotonin (solid line) and norepinephrine (broken line). The mean concentration of norepinephrine ± SEM in all control samples was 0.37 ± 0.02 µg/g at 08.00 h ($n = 46$) and 0.40 ± 0.03 µg/g at 20.00 h ($n = 16$); for serotonin, 0.59 ± 0.02 µg/g at 08.00 h ($n = 46$) and 0.64 ± 0.03 µg/g at 20.00 h ($n = 17$). Overall, both displayed similar patterns, i.e., initially gradual increases during the very early stages of cold exposures followed by sight decreases; thereafter, both of these constituents remained within ± 10% of control levels.

Discussion. MOIR and ECCLESTON[22] demonstrated that intraperitoneal administration of tryptophan rapidly increased both plasma and brain tryptophan levels, and

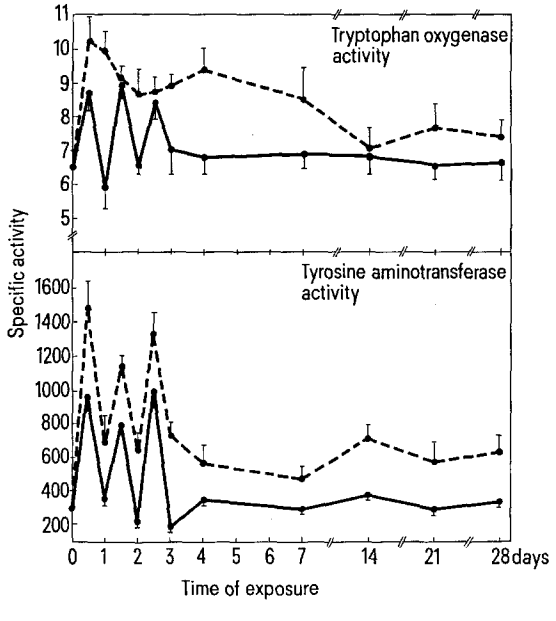

Fig. 1. Effect of acute and chronic cold exposure on hepatic tryptophan oxygenase (upper figure) and tyrosine amino transferase (lower figure). Each point represents the mean value ± S.E. for at least 5 animals. Broken line denotes activity from mice exposed to cold (2–4 °C); solid line connects control (21–22 °C) values. Specific activity units are in terms of µmoles kynurenic acid formed per hour/g liver wet weight for TO and µmoles P-hydroxybenzaldehyde formed per hour/g liver wet weight for TAT. All animals were sacrificed at 08.00 h except those at 0.5, 1.5, and 2.5 days, which were sampled at 20.00 h. The 0 values correspond to the specific activity (mean) of a group of six mice sacrificed at 08.00 h on the first day of the experiment.

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to a lesser extent brain serotonin. Other workers[11–13] correlated the effects of hepatic enzyme induction with central monoamine levels. More recently, CURZON et al.[23] reported that immobilization stress increased the levels of brain tryptophan and 5-hydroxyindolylacetic acid while brain serotonin was unaffected; however, food deprivation increased the levels of all three constituents.

Our experiments demonstrate that, although exposure of mice to acute cold stress results in a rapid induction of hepatic TO and TAT, there are no general correlations between increased activity of these catabolic enzymes and reduced levels of plasma tryptophan, tyrosine, and brain norepinephrine and serotonin. Although we did not measure their turnover, other stressors are effective in increasing the turnover rates of central monoamines[24–27], particularly norepinephrine. Our results indicate that if the rate limiting enzymes in serotonin and norepinephrine biosynthesis, tryptophan and tyrosine hydroxylases respectively[28], are stimulated by cold stress[29], then homeostatic conditions are maintained by increased levels of catabolic activity.

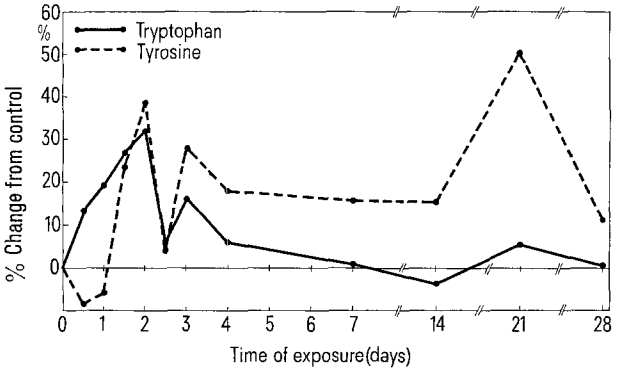

Fig. 2. Effect of acute and chronic cold exposure (2–4 °C) on plasma levels of tryptophan (solid line) and tyrosine (broken line) in mice. Each point represents the mean percent change from control values of a pool from at least 5 samples. All animals were sacrificed at 08.00 h except those at 0.5, 1.5, and 2.5 days, which were sampled at 20.00 h.

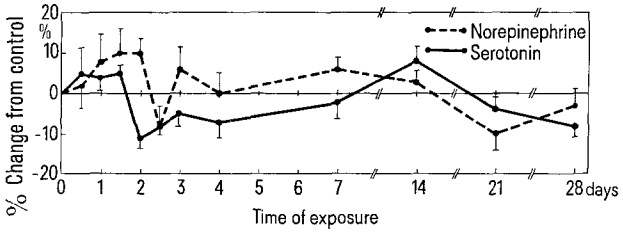

Fig. 3. Effect of acute and chronic cold exposure (2–4 °C) on the levels of brain serotonin (solid line) and norepinephrine (broken line) in mice. Each point represents the mean percent change from control values ± S.E.M. of at least 5 samples. All animals were sacrificed at 08.00 h except those at 0.5, 1.5, and 2.5 days, which were sampled at 20.00 h.

The increases noted in plasma levels of tryptophan, and particularly tyrosine, during cold exposure may be related to the process of cold acclimatization. Nonshivering thermogenesis, increased food consumption, prolonged survival in lethal cold, and augmented reactivity to norepinephrine administration are characteristic of both mice[30] and rats[31] when continuously cold-exposed[32]. Maximum sensitivity to exogenous norepinephrine is achieved with completion of the process of cold acclimatization, approximately 28 days, which in our experiments coincides with the decline of elevated tyrosine levels.

In conclusion, our results demonstrate significant effects of cold exposure upon hepatic enzyme activity, in spite of which we were unable to demonstrate the hypothesized correlations between such activity and plasma amino acid levels or central monoamine concentrations. Very recently, FERNSTROM and WURTMAN[33] have reported that brain serotonin content is primarily regulated by the ratio of tryptophan to other plasma neutral amino acids competing for uptake into the brain. Hence, it is possible that in our experiments the concentration of other plasma neutral amino acids may have contributed to the lack of relationship between plasma tryptophan and tyrosine levels and brain serotonin and norepinephrine.

Résumé. L'exposition des souris à une basse température a abouti à la production rapide de tryptophane oxygénase et de tyrosine aminotransférase. Malgré l'augmentation de l'activité enzymatique, les niveaux plasmatiques de tryptophane et de tyrosine furent augmentés d'une manière significative pendant le temps de l'exposition. Les niveaux de norepinéphrine et de sérotonine du cerveau n'en ont cependant pas été affectés.

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[35] In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care', as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences – National Research Council.