# Temperature-dependence of stress-induced hepatic autophagy[1]

M. Salas[2,3], B. Tuchweber[4], P. Kourounakis[5] and H. Selye[6]

*Institut de Médecine et de Chirurgie Expérimentales, Université de Montréal, Montréal (Québec, Canada H3C 3J7), 29 November 1976*

*Summary.* In rats, restraint for 48 h elicits hepatic glycogen depletion, autophagy and other ultrastructural changes (e.g. mitochondrial enlargement and rough endoplasmic reticulum disorganization) associated with marked hypothermia. By restoring the body temperature of these animals, all the hepatocytic alterations are abolished.

It is well-known that several stressor agents affect the liver ultrastructure causing such alterations as a decrease in the number of glycogen granules, an increase in the population of lysosomes and autophagic vacuoles, a moderate augmentation of lipid droplets, disorganization of rough endoplasmic reticulum and mitochondrial swelling[7–12]. However, these studies have neglected to consider the role of stress-induced hypothermia in the hepatic response. The effects of several acute stressors on the liver were compared in recent experiments[13], which revealed that enhanced autophagic vacuole formation and other major ultrastructural changes appear to be directly proportionate to the degree of hypothermia elicited by the stressing agents. In this communication, we examine the relationship between liver autophagy and hypothermia produced by restraint.

*Material and methods.* Female Charles River CD® rats, weighing 95–105 g, were divided into 6 groups, each consisting of at least 5 animals. 3 of these groups were housed at room temperature (24 ± 2 °C) while the other 3 were kept at 32 ± 2 °C (table). Groups 1 and 4 (absolute controls) were maintained ad libitum on Purina laboratory chow and tap water. Groups 2 and 5 (fasted controls) received neither food nor water for 48 h. Groups 3 and 6 were deprived of food and water for 48 h and simultaneously immobilized by taping their limbs to a metal board. Rectal temperature was monitored with a YS-1 Telethermometer® before autopsy, which was performed between 9.00 and 10.00 h, 48 h after the experiment was started. The organs were weighed fresh, and the stomachs were dissected along the greater curvature, rinsed and examined for ulcer formation. Blood was collected from the common trunk, and plasma corticosterone levels were measured by the technique of Guillemin et al., as modified by Mattingly[14]. For histology, fresh liver tissue was taken from the left lateral lobe and fixed in alcohol-formol. Sections (4–8 μm thick) were cut and stained by the periodic acid Schiff (PAS) technique for evaluation of glycogen content. For electron microscopy, immediately after sacrifice, a small portion of tissue was excised from the left lateral lobe of the liver and processed according to a routine technique described elsewhere[15].

The statistical significance of the biochemical results and of the incidence of gastric ulcers was evaluated by Student's t-test and the Fisher-Yates' exact probability test[16] respectively. Body: organ weights and rectal temperature were computed using the variance test.

*Results.* There were no significant differences in the body, thymus and liver weights of stressed rats at normal and at increased room temperatures; hence, these data are not reported in the table. At 24 °C, the body temperature of fasted and of restrained animals was significantly decreased when compared to the corresponding nonstressed controls. In contrast, rats kept at a temperature of 32 °C were virtually normothermic (table). Adrenal hypertrophy was observed in all stressed animals; however, this change was more pronounced in restrained rats at 32 °C than at 24 °C. The incidence of gastric ulcers was increased only after restraint at 24 °C, and these lesions were completely inhibited at 32 °C.

As expected, fasted and restrained rats exhibited higher levels of corticosterone than absolute controls, with the restrained group showing slightly lower values. Light histology revealed that, at normal room temperature (24 °C), there was a significant diminution of liver glycogen after fasting as well as restraint. However, at 32 °C, the magnitude of this decrease was less significant.

At normal ambient temperature, no ultrastructural alterations were noted in nonstressed controls. Fasted animals showed a slight increase in autophagic vacuole formation with significant glycogen depletion. There were striking changes in restrained rats, the most conspicuous being a marked proliferation of these vacuoles (figure 1). Glycogen was virtually absent from the hepatocytic cytoplasm. The mitochondria appeared to be enlarged and were occasionally surrounded by rough endoplasmic reticulum membranes. At an increased ambient temperature, nonstressed animals displayed abundant glycogen stores and occasional autophagic vacuoles. The fasted controls showed a moderate decrease of glycogen and a few autophagic vacuoles. Rats restrained at 32 °C exhibited a virtually normal ultrastructure (figure 2) in contrast to those restrained at 24 °C.

1 Supported in part by the Medical Research Council of Canada (Block Term Grant MT-1829) and the Conseil de la recherche en santé du Québec. A preliminary report of this work was presented at the 19th Annual Meeting of the Canadian Federation of Biological Societies, Halifax, 15–18 June 1976.

2 Recipient of a studentship from the Conseil de la recherche en santé du Québec.

3 Present address: Institut du Cancer de Montréal, Centre hospitalier Notre-Dame, Montréal, Québec, Canada H2L 4M1.

4 Present address: Département de nutrition, Faculté de médecine, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

5 Present address: Laboratory of Pharmaceutical Chemistry, Department of Pharmacy, University of Thessaloniki, Thessaloniki, Greece.

6 The authors thank Mr M. Audet, Mrs A. Perea and Miss F. Dionne, for their excellent technical assistance.

7 M. Bassi and A. Bernelli-Zazzera, Exp. molec. Path. *3*, 332 (1964).

8 D. B. Confer and R. J. Stenger, Am. J. Path. *45*, 533 (1964).

9 W. H. Glinsmann and J. L. E. Ericsson, Lab. Invest. *15*, 762 (1966).

10 J. L. E. Ericsson, Exp. Cell Res. *55*, 95 (1969).

11 H. David, I. Uerlings and M. Grupe, Exp. Path., Jena *5*, 2 (1971).

12 U. Pfeifer, Virchows Arch. Zellpath. *12*, 195 (1973).

13 M. Salas, B. Tuchweber and P. Kourounakis, Virchows Arch. Zellpath., submitted for publication.

14 A. Mattingly, J. clin. Path. *15*, 374 (1962).

15 B. Tuchweber, B. D. Garg and M. Salas, Archs Path. *100*, 100 (1976).

16 S. Siegel, in: Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York 1956.

Effects of maintaining normal body temperature on adrenal weight, incidence of gastric ulcers and plasma corticosterone in stressed rats

| Group | Treatment | Body temperature ± SEM (°C) | Adrenal weight ± SEM (mg/100 g b.wt) | Corticosterone ± SEM (µg/100 ml) | Gastric ulcers (positive/total) |
|---|---|---|---|---|---|
| | at 24 ± 2 °C | | | | |
| 1 | None | 37.0 ± 0.1 | 14.5 ± 0.3 | 15.00 ± 2.70 | 0/6[b] |
| 2 | Fasting | 35.9 ± 0.3** | 24.6 ± 0.5*** | 73.91 ± 3.26*** | 0/5 NS |
| 3 | Restraint[a] | 24.8 ± 0.1*** [***][c] | 19.3 ± 0.6* [*][c] | 55.61 ± 4.15*** [**][c] | 8/8*** [***][c] |
| | at 32 ± 2 °C | | | | |
| 4 | None | 37.1 ± 0.1 NS | 14.6 ± 0.4 NS | 10.62 ± 2.97 NS | 0/5 NS |
| 5 | Fasting | 36.2 ± 0.1** | 22.0 ± 2.4** | 64.92 ± 8.93*** | 0/5 NS |
| 6 | Restraint[a] | 36.4 ± 0.2* [NS][c] (***)[d] | 24.0 ± 1.3*** [NS][c] (**)[d] | 44.37 ± 3.36*** [NS][c] (NS)[d] | 0/8 NS [NS][c] (***)[d] |

[a]Groups 2, 3, 5 and 6 had no access to food or water for 48 h. [b]Total number of animals per group is shown in the denominator. [c]Significance with respect to group 2 or 5. [d]Significance with respect to group 3. NS = $p > 0.05$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

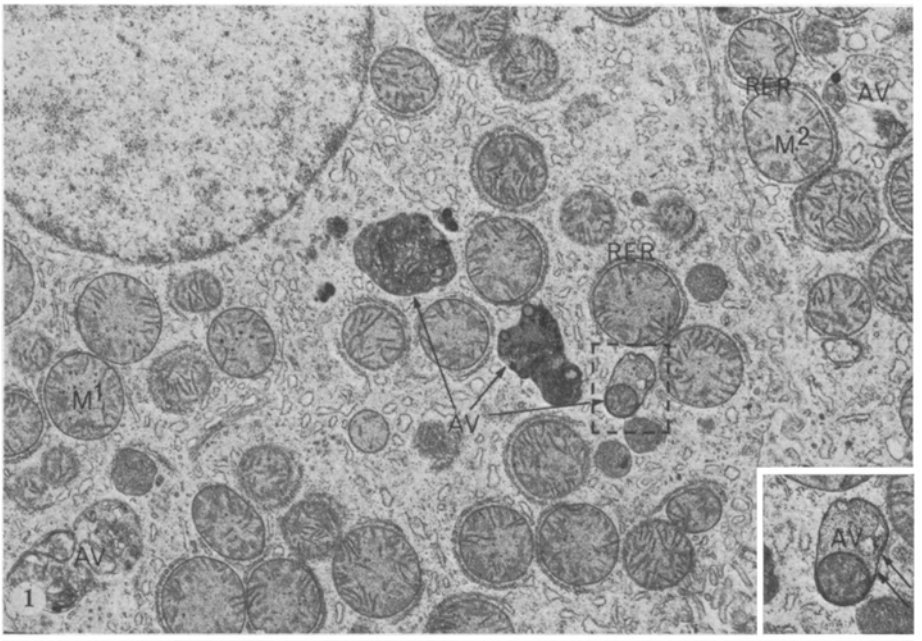


Fig. 1. Hepatocytes of fasted, restrained rat with a body temperature of 25 °C. The rough endoplasmic (RER) is fragmented. Single stacks of this organelle surround the mitochondria ($M^2$), which appear to be enlarged ($M^1$, $M^2$) and sometimes exhibit an abnormally pale matrix ($M^2$). A marked increase in autophagic vacuoles (AV, arrows), containing organelles in different stages of degradation, can be observed. ×10,500. Inset shows, in detail, an autophagic vacuole (AV) limited by possible endoplasmic reticulum membranes (double arrows); it contains a mitochondrion. ×13,600.

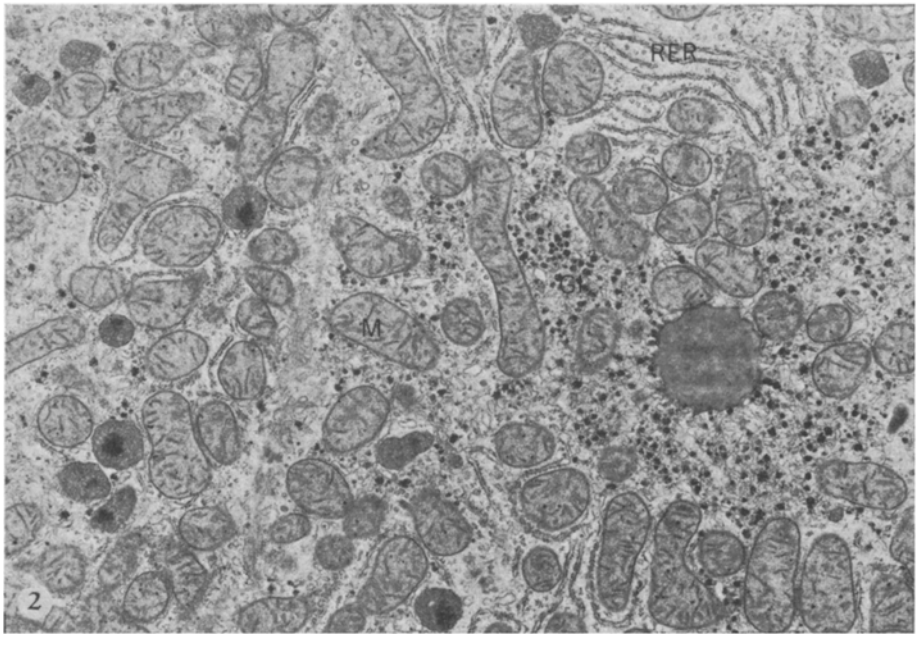


Fig. 2. Hepatocytes of fasted, restrained rat with a body temperature of 36.5 °C. The rough endoplasmic reticulum (RER) appears in parallel arrays, and the mitochondria (M) are normal. Abundant glycogen rosettes (Gl) can be seen. ×13,600.

*Discussion.* Temperature regulation is affected during acute stress[17]. However, the occurrence of hypo- or hyperthermia depends upon various factors, especially the animal species used. For instance, unlike man[18,19], the rat reacts to most acute stressors by lowering its body temperature[20]. Under our conditions, the hypothermia induced by stress was a decisive element in the production of hepatic ultrastructural changes, notably autophagy.

It is not known exactly through which mechanism the stress-induced hypothermia triggers autophagic vacuole formation and the other electron microscopic alterations in the liver. Perhaps the lowered body temperature during restraint elicits changes in carbohydrate metabolism (e.g. glycogen depletion, hypoglycemia) which in turn, enhance hepatic autophagy. Indeed, it has been proposed that acute glycogenolysis may be a stimulus for autophagic vacuole formation[21].

The present investigation validates the observation that stress-induced hypothermia plays an important role in experimental gastric ulcers[22]. However, the subcellular mechanism of protection is not yet understood. The question arizes as to why the other typical stress manifestations (adrenal hypertrophy, liver and thymus involution), which may be adaptive in nature, are not prevented by regulating the body temperature. It is well-known that numerous stress parameters (e.g. nervous arousal) can add to the total 'nonspecific' effect of a stressor[17] and this could partially explain why inhibition of hypothermia does not abolish the other signs of systemic stress.

17 H. Selye, in: Stress in Health and Disease. Butterworths, Reading, Mass. 1976.
18 M. Friedman, Ass. Res. Nerv. Dis. Proc. *29*, 433 (1950).
19 L. Yordanova and T. Gotsev, J. Physiol. *63*, 463 (1970).
20 J. Oyama, W. T. Platt and V. B. Holland, Am. J. Physiol. *221*, 1271 (1971).
21 F. F. Becker, Proc. Soc. exp. Biol. Med. *140*, 1170 (1972).
22 M. S. Martin, F. Martin and R. Lambert, Digestion *3*, 331 (1970).

## Demonstration by the Fink-Heimer impregnating method of a ventral mesencephalic-locus coeruleus projection in the rat

H. Simon and M. Le Moal

*Laboratoire de Psychophysiologie, Institut de Biologie Animale, Université de Bordeaux I, avenue des Facultés, F–33405 Talence Cédex (France), 12 October 1976*

*Summary.* With the help of the Fink-Heimer technique, we have demonstrated a ventral mesencephalic-locus coeruleus projection in the rat after lesions located in the region of the dopaminergic A10 and serotonergic B8 cells. This finding could help our understanding of the functional role of these structures.

While the locus coeruleus (LC) ascending efferences have been evidenced in many species including man, by using new anatomical techniques such as silver impregnating methods[1,2], fluorescence histochemistry[3,4], axonal retrograde transport techniques[5,6] and autoradiography[7], surprisingly, the descending pathways to this important structure involved in sleep regulation[8], learning processes[9] and self-stimulation[10,11], have not yet been anatomically demonstrated. In the present study we have demonstrated the existence of a mesencephalic-LC pathway. 2 factors led us to carry out this research. First, in 1970, using an electron microscope, Mizuno and Nakamura[12] noted, after a unilateral electrolytic lesion at the supramammillary area level, some electron dense degenerated synaptic profiles in the LC area. However, these degenerated synapses were rather small in number and they were absent when the lesions were more anterior in the hypothalamus. Unfortunately no degenerations were revealed by using silver impregnating methods; thus it was possible that the lesion did not reach massively the neurons projecting to the LC, and it was interesting to test the hypothesis of a ventral mesencephalic-coeruleus pathway whose origins could be located in a more posterior structure. Secondly, self-stimulation of the ventral mesencephalic tegmentum (VMT), lying just posteriorly to the mammillary bodies, provoked a) an important enhancement of the noradrenaline (NA) turnover at the level of the terminals of the dorsal noradrenergic bundle originating from the LC[13], and b) an alteration of the NA content of the locus coeruleus[14]. These results could not be explained by a direct stimulation of this NA bundle which runs far from the tip of the electrode[3,4]. A possible alternative was given by a transsynaptic activation of these NA neurons by a ventral pathway reaching the LC.

*Material and methods.* We examined, by the Fink-Heimer I technique[15], the degenerations produced after lesions in the VMT and in the median raphe nucleus (MRN) where high self-stimulation rates were obtained[16]. Male 90-day-old Sprague-Dawley rats were used. A bipolar iridium-platinum 240 µm wide electrode was chronically implanted in the VMT, or in the MRN under pentothal anesthesia. 9 VMT rats and 3 MRN rats which showed a stabilized self-stimulation behaviour and lever-press rates higher than 4000 per h were selected. Then they

1 D. C. German and D. M. Bowden, J. comp. Neurol. *161*, 19 (1975).
2 N. Shimizu, S. Ohnishi, M. Tohyama and T. Maeda, Exp. Brain Res. *20*, 181 (1974).
3 L. A. Loizou, Brain Res. *15*, 563 (1969).
4 T. Maeda and N. Shimizu, Brain Res. *36*, 19 (1972).
5 J. Kievit and H. G. J. M. Kuypers, Brain Res. *85*, 261 (1975).
6 M. Segal and S. C. Landis, Brain Res. *82*, 262 (1974).
7 V. M. Pickel, M. Segal and F. E. Bloom, J. comp. Neurol. *155*, 15 (1974).
8 M. Jouvet, Science *163*, 31 (1969).
9 G. M. Anlezark, G. W. Arbuthnott, J. E. Christie and T. J. Crow, Science *181*, 682 (1973).
10 T. J. Crow, Brain Res. *36*, 275 (1972).
11 S. Ritter and L. Stein, J. comp. Physiol. Psychol. *85*, 443 (1973).
12 N. Mizuno and Y. Nakamura, Brain Res. *19*, 160 (1970).
13 L. Stinus, A. M. Thierry, G. Blanc, J. Glowinski and B. Cardo, Brain Res. *64*, 199 (1973).
14 E. Miliaressis, N. B. Thoa, Y. Tizabi and D. M. Jacobowitz, Brain Res. *100*, 192 (1975).
15 R. P. Fink and L. Heimer, Brain Res. *4*, 369 (1967).
16 H. Simon, M. Le Moal and B. Cardo, C. r. Acad. Sci., Paris *277*, 591 (1973).