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.

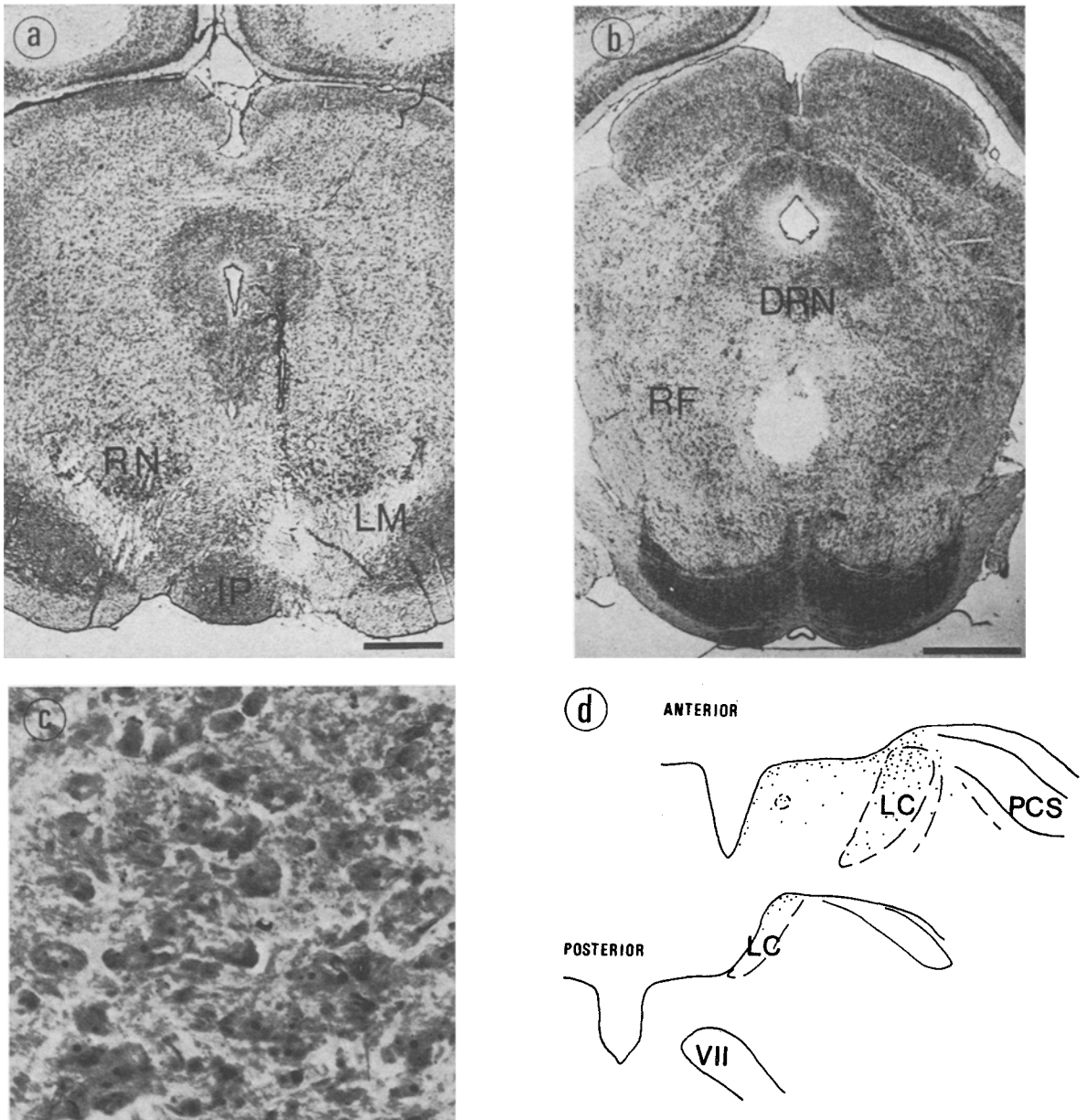
Material and methods. We examined, by the Fink-Heimer I technique[15], the degenerations produced after lesions in the VMT and in the median raphe nucleus (MRN) where high self-stimulation rates were obtained[16]. Male 90-day-old Sprague-Dawley rats were used. A bipolar iridium-platinum 240 µm wide electrode 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).

were anesthetized with ether and a radiofrequency lesion was made at the electrode tip. 3–5 days after, the rats were sacrificed by an intracardiac perfusion of 0.9% NaCl followed by 10% formalin solution. A post-operative survival time superior to 5 days does not permit the demonstration of terminal degenerations[15,17]. This is why the degenerations observed were directly in relation to the lesioned area, since those due solely to the electrode implantation had disappeared. The brains were quickly removed and stored in formalin for 6–12 weeks; 24 µm sagittal and frontal sections were made in a cryostat. The sections were impregnated by the Fink-Heimer technique and Nissl stain was used to determine the location and the size of the lesions.

Results. The degenerations observed after VMT or MRN lesions have been studied elsewhere; only the changes in the LC, observed for the first time in this present study, will be discussed. The Fink-Heimer technique showed bilateral degenerations in the LC after destruction

17 H. Simon, M. Le Moal, D. Galey and B. Cardo, Brain Res. *115*, 215 (1976).



A, *B* Microphotograph of brain frontal sections. Typical electrocoagulation made at the tip of the electrode of self-stimulation. Nissl stain. Bar calibration: 1 mm (*A*) – ventral mesencephalic tegmentum; unilateral lesion (*B*) – median raphe nucleus; medial lesion. Abbreviations: *DR* dorsal raphe nucleus, *IP* interpedoncular nucleus, *LM* lemniscus medialis, *RF* reticular formation, *RN* red nucleus (*C*, *D*). Microphotograph (*C*) and schematic drawing (*D*) of degenerations observed after interruption of a ventral mesencephalic-locus coeruleus pathway. Abbreviations: *LC* locus coeruleus, *PCS* pedunculus cerebellaris superior, *VII* facial nerve. Bar calibration: 20 µm.

of the VMT, particularly when the lesions were located in the area surrounding the interpeduncular nucleus (figure, A). Lesions in this area led to irregular and mostly finely impregnated terminals in the whole LC with a higher concentration in the anterodorsal part of the 2 nuclei (figure, C, D). Destruction of the MRN (figure, B) led to a similar pattern of bilateral degenerations in the LC, but the degenerations were more abundant after MRN lesions than VMT lesions.

Discussion. Our results indicate that a ventral descending pathway originates in the ventral medial tegmentum and reaches the LC bilaterally. However these results do not definitely demonstrate a MRN-coeruleus pathway since the MRN lesion can destroy fibres coming from the anterior VMT and reaching the LC by a medial way. Nevertheless, the degenerations in the LC are more abundant after MRN than after VMT lesion and it is possible that some of the degenerations are due to the destruction of raphe cells per se. A large part of these cells are serotoninergic[18], and it is interesting to note in this respect that relatively high concentrations of serotonin were evidenced in the LC[19]. If subsequent work confirms a MRN-LC pathway, the existence of LC rostral raphe relations[20] will be completed in the sense of a LCN-MRN loop. Such a loop could improve our understanding of sleep mechanisms.

The VMT lesions made in animals that showed a high rate of self-stimulation were located in the area of the dopaminergic A10 group cells[18]. Dopamine had been evidenced in the LC at levels which suggest the existence of dopaminergic terminals[21], and one could suggest the existence of terminals issued from the A10 nucleus. More generally our results can explain the paradoxical biochemical data obtained after VMT self-stimulation[13,14] and the role of the dopaminergic systems in self-stimulation obtained from the LC demonstrated by some investigators[22].

18 A. Dahlstrom and K. Fuxe, Acta physiol. scand. *62*, suppl. *232*, 1 (1964).
19 M. Palkovits, M. Brownstein and J. M. Saavedra, Brain Res. *80*, 237 (1974).
20 W. Kostowski, R. Samanin, S. R. Bareggi, M. V. Garattini and L. Valzelli, Brain Res. *82*, 178 (1974).
21 D. H. G. Versteeg, J. Van der Gugten, W. de Jong and M. Palkovits, Brain Res. *113*, 563 (1976).
22 B. R. Cooper, G. Ervin and G. R. Breese, Pharmacologist *16*, 108 (1974).

Reduced microbial transformation of bile acids in cystic fibrosis[1]

D. Lefebvre, S. Ratelle, L. Chartrand and C. C. Roy

Department of Pediatrics, Hôpital Sainte-Justine, The University of Montreal, 3175, chemin Sainte-Catherine, Montreal H3T 1C5 (Quebec, Canada), 11 October 1976

Summary. The microbial transformation of bile acids by incubates of stool homogenates from children with cystic fibrosis is decreased.

The human gastrointestinal tract is endowed with a microecological system in which the bacterial population is relatively stable[2]. There are many factors responsible for maintaining the balance between microorganisms and host. The anatomical and functional integrity of the intestine is essential. Stasis of intestinal contents leads to abnormal microbial growth[3]. The 'acid barrier' provided by the stomach[4] and a normal ileocecal valve[5] insure that the upper and lower small bowel remain relatively sterile in order to fulfill its absorptive role. Other important factors which prevent the bacterial contamination of the small bowel include intestinal motility[6], mucus[7], immunoglobulins[8], bacterial metabolic products[9] and bile acids.

Bile acids exert considerable antibacterial activity. In vitro, free bile acids are particularly effective[10]. In vivo, the administration of bile acids leads to a decrease of the ileal anaerobic flora in man[11]. The intestinal flora in turn significantly affects the concentration and composition of intestinal bile acids[12]. We have shown large losses of bile acids in the feces of patients with cystic fibrosis (CF)[13]. Qualitative patterns of fecal bile acids were similar to those in children with an ileal resection. A significant prevalence of bile acids which had not undergone microbial transformation were found in CF and in ileal resections, as compared to controls in whom 86.5% are secondary bile acids[14]. Hence, it seemed interesting to study the capacity of the CF fecal flora to transform bile acids.

Material and methods. The capacity of fresh stool samples to conjugate and dehydroxylate bile acids was studied in 13 CF patients and compared to that of 9 children free of any hepatic, pancreatic or intestinal disorder. The mean age of the CF group was 7.9 years (2–15.9 years) while that of controls was 8.6 years (2–12 years). None of these children had received antibiotics during the previous week. Among the children with CF, 4 had no clinical evidence of pancreatic insufficiency, the 9 others were on

1 This work was supported by grant MT 4433 from the Medical Council of Canada and from the Canadian Cystic Fibrosis Foundation.
2 S. L. Gorbach, Gastroenterology *60*, 1110 (1971).
3 G. Neal, D. Compertz, H. Schönsby, S. Tabaqchali and C.C. Booth, Am. J. Clin. Nutr. *25*, 1409 (1972).
4 B. S. Drasar, M. Shiner and M. B. McLeod, Gastroenterology *56*, 71 (1969).
5 W. O. Griffen, I. D. Richardson and E. S. Medley, Sth. Med. J. *64*, 1056 (1971).
6 G. M. Dack and E. Petran, J. infect. Dis. *54*, 204 (1934).
7 N. E. Goldsworthy and H. Florey, Br. J. exp. Path. *11*, 192 (1930).
8 W. R. Brown, D. C. Savage, R. S. Dubois, M. H. Alp, A. Mallory and F. Kern, Gastroenterology *62*, 1143 (1972).
9 M. R. Bryant, Am. J. clin. Nutr. *25*, 1485 (1972).
10 M. H. Floch, W. Gershengoren, S. Elliott and H. M. Spiro, Gastroenterology *61*, 228 (1971).
11 R. C. Williams, R. Showalter and F. Kern, Jr, Gastroenterology *69*, 483 (1975).
12 A. Mallory, F. Kern, Jr, J. Smith and D. Savage, Gastroenterology *64*, 26 (1973).
13 A. M. Weber, C. C. Roy, C. L. Morin and R. Lasalle, N. Engl. J. Med. *289*, 1001 (1973).
14 A. M. Weber, C. C. Roy, L. Chartrand, G. Lepage, O. L. Dufour, C. L. Morin and R. Lasalle, Gut *17*, 295 (1976).