as previously described[6]. Electrical potential difference (PD) is measured using a DC Differential Voltmeter (John Fluke MFG Co., Inc., Model 881A), connected to both sides of the epithelium through a pair of calomel electrodes and agar bridges. The short-circuit current (SCC) is measured using a device which is similar to the one described by Ussing and Zerahn[7]. The electrical resistance (R) is calculated by applying the Ohm law.

As shown in table 1 in all 3 isolated epithelia, the short-circuit current is much lower in torpid tortoises than in active ones. The potential difference increases during hibernation at the level of the small intestine but remains very low. In the colon no significant change of PD is observed. In contrast in the bladder there is an important drop in the values of PD recorded during the winter. In all 3 epithelia, these changes of PD and/or SCC bring about an increase of the transepithelial resistance.

This increase of the resistance is probably related to decreased passive permeabilities. Indeed the serosal border of small intestine and colon epithelia has been shown to be less permeable to inorganic ions during hibernation[2]. As to the decreased SCC values recorded during hibernation, one can assume it reflects a lower activity of the Na pump. This Na pump should be controlled by aldosterone. In a closely related species of land tortoise (*Testudo graeca*), it was shown that in in vitro experiments D-aldosterone was effective at the level of the bladder and perhaps of the colon, an increased SCC being recorded in active animals[8].

By radioimmuno-assay the aldosterone plasma level was studied in active and torpid tortoises (table 2). Despite tremendous individual variations, the aldosterone level is significantly lower in the torpid animals. But when the influence of aldosterone on the SCC of the 3 epithelia is tested, no effect is observed even after 7 h of incubation. Therefore it is tempting to conclude that lower SCC values recorded during hibernation are not the consequence of the lower aldosterone plasma level measured.

In the bladder, it is interesting to note that the water net flux is much lower in torpid animals than in active ones, and that it is controlled by antidiuretic hormones in torpid animals and not in active ones[9]. As to the SCC, it is also much lower in torpid animals, but it is controlled by D-aldosterone only in active animals and not in torpid ones.

Table 2. Aldosterone present in the blood plasma of active and torpid tortoises

	Activity	Hibernation
Aldosterone (pg/ml)	156.1 ± 198.8 (18) S	57.9 ± 55.8 (33)

Results are expressed as means ± SD. S, the change is significant at the 5% level.

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2 M. Gilles-Baillien, Archs int. Physiol. Biochim. *78*, 327 (1970).
3 M. Gilles-Baillien, Life Sci. *8*, 763 (1969a).
4 M. Gilles-Baillien, Archs int. Physiol. Biochim. *80*, 789 (1972).
5 M. Gilles-Baillien and C. Bouquegneaux-Tarte, Archs int. Physiol. Biochim. *80*, 563 (1972).
6 M. Baillien, Biochim. biophys. Acta *53*, 521 (1961).
7 H.H. Ussing and K. Zerahn, Acta physiol. scand. *23*, 110 (1951).
8 P.J. Bentley, Gen. comp. Endocr. *2*, 323 (1962).
9 M. Gilles-Baillien, Biochim. biophys. Acta *193*, 129 (1969b).

The sequential uptake of (^{14}C) deoxyglucose in brain after embolic stroke

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Summary. An i.v. bolus of (^{14}C) deoxyglucose was injected into 4 groups of rats which simultaneously were embolized through the internal carotid artery. 15 and 30 min post embolization there was a massive decrease in (^{14}C) deoxyglucose uptake in both deep and cortical structures. At 4 h a small zone of increased (^{14}C) deoxyglucose uptake persisted around each embolized microsphere.

Recent experiments have demonstrated that the technique of (^{14}C) 2-deoxyglucose (2-DG) autoradiography permits a pictorial and semi-quantitative representation of the relative rates of glucose uptake and/or utilization in functionally active neurons[1]. The technique is based upon the assumption that 2-DG is transported across neuronal cell membranes by the same carrier system utilized by glucose[2]. Once across the neuronal membrane it is phosphorylated to 2-DG-6-P but is not metabolized. The amount of glucose utilized by a particular neuronal population correlates with its activity and can be calculated from that population's 2-DG concentration, if: 1. adequate time has elapsed for

(^{14}C) 2-deoxyglucose in the embolized rat brain

	Embolized hemisphere				Non-embolized hemisphere			
	Cortex		Basal ganglia-thalamus		Cortex		Basal ganglia-thalamus	
	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold
A (15′)	0.34 ± 0.02	0.04 ± 0.01	0.32 ± 0.02	0.13 ± 0.01	0.30 ± 0.02	0.10 ± 0.01	0.18 ± 0.01	0.11 ± 0.01
B (30′)	0.38 ± 0.02	0.13 ± 0.01	0.27 ± 0.02	0.05 ± 0.01	0.20 ± 0.01	0.18 ± 0.02	0.12 ± 0.01	0.09 ± 0.01
C (30′)	0.29 ± 0.01	0.09 ± 0.02	0.28 ± 0.03	0.07 ± 0.02	0.25 ± 0.03	0.18 ± 0.03	0.16 ± 0.02	0.08 ± 0.02
D (60′)	0.20 ± 0.02	0.05 ± 0.01	0.18 ± 0.02	0.06 ± 0.01	0.18 ± 0.02	0.16 ± 0.02	0.16 ± 0.01	0.05 ± 0.01
E (240′)	0.18 ± 0.02	0.09 ± 0.01	0.15 ± 0.01	0.05 ± 0.02	0.16 ± 0.01	0.04 ± 0.01	0.09 ± 0.01	0.03 ± 0.01

2 DG utilization in the embolized and non-embolized hemisphere. Hot areas represent areas of increased glucose uptake whereas cold areas represent decreased uptake. The values recorded are the average of 5 OD readings taken from each representative area of increased or decreased glucose consumption in the embolized and non-embolized hemisphere. 95% confidence intervals were calculated for these values using the normal distribution.

tissue clearance of free isotope, and 2. the kinetics of transport, phosphorylation and turnover of both the free glucose and 2-DG pools are known[2,3].

Method. The experiments were performed with adult Wistar strain male rats, weighing between 300 and 400 g. Anesthesia was induced with ether and maintained with a mixture of 30% oxygen and 70% nitrous oxide. The right internal carotid artery was cannulated with PE-50 polyethylene tubing. 1 femoral artery was cannulated for intermittent blood gas sampling and for the injection of 2-DG (sp. act. 53 mCi/nmole). The bolus of isotope was calculated at 16 μCi/100 g.

4 groups of rats were embolized utilizing plastic microspheres 35 μm in diameter through the tubing of the cannulated right internal carotid artery. The animals were sacrificed at 15 min, 30 min, 1 h and 4 h after embolization. The brains were immediately frozen in situ with liquid nitrogen and chiseled from the skull[4]. They were sectioned at 16 μm thickness and then autoradiographed in the standard fashion. A photovolt densitometer with a 0.5 mm aperture was used to obtain OD values for areas of increased and decreased glucose uptake and consumption seen in both the embolized and non-embolized hemispheres.

Results. At 15 min (figure 1, A1 and 2) and 30 min (figure 1, B4 and C) post embolization it is evident that there is a marked decrease in 2-DG uptake in specific areas. These were in the distribution of the blocked vasculature as seen on H and E sections (figure 2). Coincident with the decrease of 2-DG glucose uptake in the center of 1 ischemic zone, surrounding areas showed a marked increase in uptake (figure 1, B3 and C6). This pattern appeared most prominent in the basal ganglionic and thalamic areas (figure 1, A2 and B4). Cortical zones where end arteries were blocked demonstrated markedly reduced uptake whereas adjacent zones had increased uptake (figure 1, C6). Quantitative measurement by means of photodensitometry corroborated the visual impression (table). At 15 and 30 min after embolization larger areas of decreased 2-DG uptake were seen than could be accounted for solely by blockage of the vasculature supply to these areas (figure 1A, B and C). At 1 h after ictus the areas of altered uptake shrank markedly to the immediate areas surrounding the regions of localization of the microspheres (figure 1, D7). By 4 h there appeared to be a further reduction in the size of the areas of altered 2-DG uptake (figure 1, E7). Altered 2-DG uptake was noted in the non-embolized

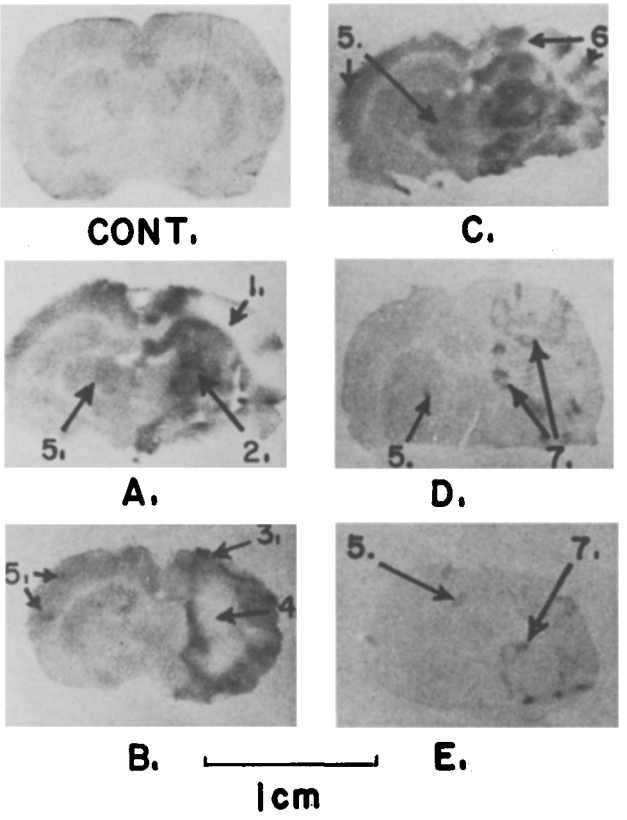

Fig. 1. Control. *A* (^{14}C) deoxyglucose uptake 15 min embolization. Note absence of (^{14}C) deoxyglucose in large areas of the cortex (1); increased uptake surrounding an ischemic zone in the deep circulation (2); an area of increased uptake in the cortex and deep circulation of the non-embolized hemisphere (5). *B* (^{14}C) deoxyglucose uptake 30 min after embolization. Note increased uptake in cortical area (3) concomitantly with a large area of increased uptake in the deep circulation (4). Several areas of increased uptake are evident in the non-embolized hemisphere (5). *C* (^{14}C) deoxyglucose uptake 30 min after embolization. Note the columnar pattern of increased uptake (6). *D* and *E* 1 and 4 h respectively after embolization. Note a zone of increased (^{14}C) deoxyglucose uptake immediately surrounding the embolized microspheres (7). It appears that there is slightly less (^{14}C) deoxyglucose uptake immediately surrounding the microspheres in 4 h than in 1 h. Note areas of increased utilization in the non-embolized hemisphere in all section (5).

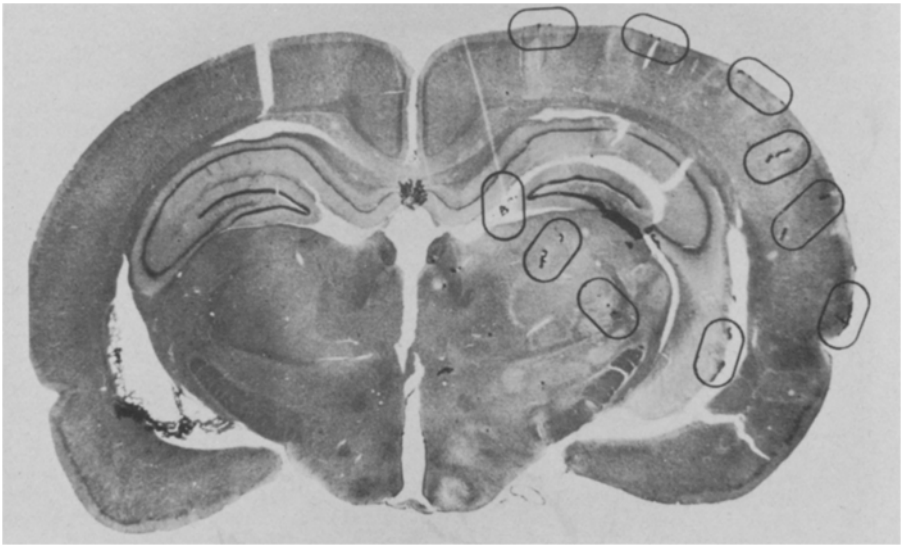

Fig. 2. H and E section demonstrating the typical distribution of microsphere emboli in the deep and cortical circulation (encircled).

hemisphere in the same section from each group of animals (figure 1, A5, B5, C5, D5 and E5).

Discussion. The simultaneous injection of the 2-DG isotope tracer and the microspheres was chosen to gain insight into the earliest metabolic events that occur in the whole brain following embolization.

Quantitative use of this method necessitates that at least 30 to 45 min have elapsed after isotope injection to insure that the 2-DG glucose in the unmetabolized brain pool has undergone conversion to 2-DG-6-P. Also a steady state must exist from the time of 2-DG administration to the time of the animal's sacrifice to attain a completely accurate representation of 2-DG utilization and uptake. Consideration of these 2 theoretical limitations of this technique makes quantitative interpretation of the 15 and 30 min autoradiographs difficult; however, they are so different from controls that they suggest that there is a massive early non-utilization of glucose in these time periods. EEG and evoked potential responses recorded from rats which have undergone similar carotid embolization demonstrate marked physiological changes in the entire hemisphere[5,7]. Recent experimentation in the cat has demonstrated similar alterations of glucose metabolism in both the deep and cortical circulation[6]. These present experiments clearly demonstrate shrinkage of zones of altered glucose utilization with time. I postulate is that the shrinkage of these zones is caused by collateral circulation which is stimulated by the glycolysis which occurs in an ischemic area[5,7]. The increased tracer accumulation seen at 30 min, 1 and 4 h is independent of increased blood flow due to collateral circulation unless this is also accompanied by an increased metabolic rate[3].

1 C. Kennedy, M.H. Des Rosiers, J.W. Jehle, M. Reivich, F. Sharpe and L. Sokoloff, Science *187*, 850 (1975).
2 L. Sokoloff, M. Reivich, C.S. Patlak, K.D. Pettigrews, M. Des Rosiers and C. Kennedy, Proc. 5th Meet. Am. Soc. Neurochem., p. 8, 1974.
3 M.H. Des Rosiers, C. Kennedy, C.S. Patlak, K.D. Pettigrews and L. Sokoloff, Neurology *24*, 389 (1974).
4 U. Ponten, R.A. Ratcheson, L.G. Salford and B.K. Siesjo, J. Neurochem. *21*, 1127 (1973).
5 K. Kogure, R. Busto, P. Scheinberg and O.M. Reinmuth, Brain *97*, 103 (1974).
6 M.D. Ginsberg, M. Reivich and A. Giandomenico, Neurology *26*, 346 (1976).
7 T.E. Duffy, D.C. Howse and F. Plum, J. Neurochem. *24*, 925 (1975).

Autonomic response of the fish to pyrogen

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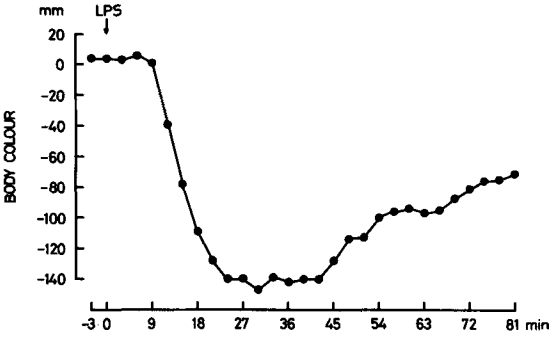
Summary. Lipopolysaccharide (LPS) applied to the anterior brainstem of the carp caused lightening of body colour. This indicates that an increase in set point temperature is responsible for increased cutaneous autonomic activity following LPS-administration.

Recently, pooled observations have clearly shown that the fish possesses the same central mechanism as that of homeotherms, for initiating temperature responses. Localized temperature displacement of the hypothalamus can elicite thermoregulatory behaviour by altering the thermopreferendum of the fish[2-5], and also induces changes in autonomic functions[6]. We have confirmed the existence of such central mechanisms in the fish by experiments in which the cyprinid fishes changed their heart rate and body colour in response to selective thermal stimulation of the spinal cord[7-9]. This functional similarity of the central nervous system in the fish and homeotherms has been further demonstrated by behaviourally-acquired fever in the fish following pyrogen administration; a pyrogen-injected fish increased its thermopreferendum presumably due to an increase in the set point temperature[10], as is actually the case for the homeotherms[11-14]. We report here that a pyrogen applied to the hypothalamus of the fish can also induce changes in cutaneous autonomic activity, besides an increase in behaviourally selected ambient water temperature.

We used gallaminized carp, *Cyprinus carpio*, of 24–27 cm length. Lipopolysaccharide (LPS) from *E.coli* UKT-B strain (0.02–0.5 µg in 0.1–0.2 ml fish saline) was applied manually to the anterior brainstem including the hypothalamus, while monitoring body colour of the tail region by means of a photoelectrical technique assessed to measure the intensity of reflected light from restricted body surface[9]. Body colour change of the carp is achieved by the pigmentary movements within the melanophores located in the skin areas. These pigmentary movements are controlled only through the single innervation by sympathetic efferents of the cutaneous region; excitation causes aggregation of the pigments, i.e. lightening, and inhibition causes dispersion of pigments within the melanophores, i.e. darkening of body colour[15]. Thus, body colour of the carp is an excellent indicator for examining the effect of pyrogen administration on the cutaneous sympathetic system.

LPS-administration to the anterior brainstem invariably caused body colour to be lightened (table and figure). Lightening began with a mean latency of 14.9 min, reached the maximal response after 48.0 min and recovered by 50%



Body colour lightening induced by LPS. Oridnate shows body colour measured every 3 min and abscissa shows time course. Negative values for body colour indicate lightening of body colour. The reference body colour level, 0 on the ordinate, was taken as the mean level during 4 min before LPS administration. Result from the carp numbered 3 in the table.