GAD activities in homogenates of brain regions of differentially-housed mice

	GAD activity			
	n-moles $^{14}CO_2$ formed/min/mg protein		n-moles [^{14}C] GABA formed/min/mg protein	
	Grouped mice	Isolated mice	Grouped mice	Isolated mice
Olfactory bulb	5.41±0.08 (21)	4.82±0.05 (21)[a]	4.81±0.08 (21)	4.25±0.08 (21)[a]
Cerebellum	4.03±0.05 (21)	3.63±0.06 (21)[a]	2.59±0.07 (21)	2.63±0.07 (21)
Hypothalamus	9.40±0.12 (6)	9.50±0.34 (6)	8.82±0.14 (6)	8.21±0.32 (6)
Striatum	5.08±0.09 (15)	4.88±0.09 (15)	3.80±0.10 (15)	3.50±0.10 (15)
Pons medulla	3.35±0.12 (6)	3.42±0.13 (6)	2.39±0.11 (6)	2.43±0.07 (6)
Midbrain	5.89±0.18 (6)	5.67±0.16 (6)	4.21±0.20 (6)	4.15±0.14 (6)
Hippocampus	2.91±0.19 (6)	2.89±0.10 (6)	2.10±0.11 (6)	2.07±0.06 (6)
Cerebral cortex	3.68±0.18 (6)	3.47±0.13 (6)	2.59±0.24 (6)	2.62±0.10 (6)

Means±SEM; numbers of determinations in parentheses (e.g., 12 determinations on 6 homogenates, or 6 determinations on 3 homogenates, each homogenate representing regions from 2 brains); [a] indicates $p<0.001$ when these values were compared with corresponding values for grouped mice (Student's t-test; 2-tailed).

activity was significantly lower in the whole forebrains of isolated mice than in those of grouped mice (3.87±0.09 vs 4.18±0.09 nmoles [^{14}C]GABA formed/min/mg protein (means±SEM; 12 determinations in each case; $p<0.02$). GAD activity (as measured by 2 different methods) of the olfactory bulb was also significantly lower in the isolated mice (table). GAD activity of cerebellum was lower for isolated mice, but only when the data were expressed in terms of CO_2 evolution (table).

In accord with previous results on GABA content[7,8] and GABA binding[6], the activities of cerebral GAD of male mice was shown to be sensitive to long-term changes in environment. The localization of these changes in the olfactory bulb further supports the contention that a hypoactive GABA-ergic system (lower GABA levels) might be responsible for muricidal behavior in rats[10,11]. Such a change in the olfactory bulb could also underlie the increased aggressiveness displayed by isolated mice. However, other brain regions not examined in the present study (e.g., thalamus) could also be involved in this behavioral change. In general, the results reported here further support the notion that central GABA-ergic systems are involved in behavioral adaptation and neuronal plasticity.

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Hypothermia induced in mice by enzyme-mediated depletion of serotonin[1]

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Summary. Injection of a purified serotonin (and tryptophan)-degrading enzyme into mice produced a pronounced hypothermia when the mice were maintained at 22 °C, but not at 30 °C. Brain levels of serotonin and tryptophan were strikingly depleted, but concentrations of norepinephrine and dopamine remained unchanged.

The regulation of body temperature in mammals is a complex process which is only beginning to be clarified. Numerous investigators have presented evidence inferring that either dopamine, norepinephrine, acetylcholine or serotonin is the neurotransmitter which is involved in thermoregulation[2]. Also, the sex of the test animal has been claimed to influence the effect of serotonin treatment[3]. We report here the attainment of hypothermia in mice at room temperature by administering a highly purified enzyme which attacks serotonin (and tryptophan), but which is inactive against the catecholamines, dopamine and norepinephrine. These results support the hypothesis that serotonin, or a structurally related indole compound, is involved in thermoregulation.

Female BDF_1 mice, weighing approximately 20 g, were injected i.p. with the indicated dose of highly purified indolyl-3-alkane α-hydroxylase (IAH), a newly-discovered bacterial enzyme which degrades tryptophan, serotonin and related 3-substituted indoles[4]. Rectal temperatures were monitored with a tele-thermometer (Yellow Springs Instruments) while the mice were maintained at room temperature (about 22 °C) or in a 30 °C incubator. At the indicated

The effect of IAH on body temperature in mice. Female BDF_1 mice were injected i.p. with saline (control), 400 or 600 units IAH/kg. Temperatures were measured rectally in the mice which were maintained either at room temperature (22 °C) or in a 30 °C incubator

Treatment of animals	Number of animals	Ambient temperature (°C)	Rectal temperature after 0	1	6	16	24 h
Saline	4	22	36.3	37.0	36.8	37.1	37.1
600 units IAH/kg	4	22	36.5	35.1	36.1	33.3	26.6
				4	7	17	24 h
Saline	2	22		37.0	37.5	38.0	37.3
400 units IAH/kg	3	22		36.0	37.3	33.0	30.7
Saline	2	30		38.0	38.0	37.5	37.8
400 units IAH/kg	3	30		36.8	37.3	36.0	36.3

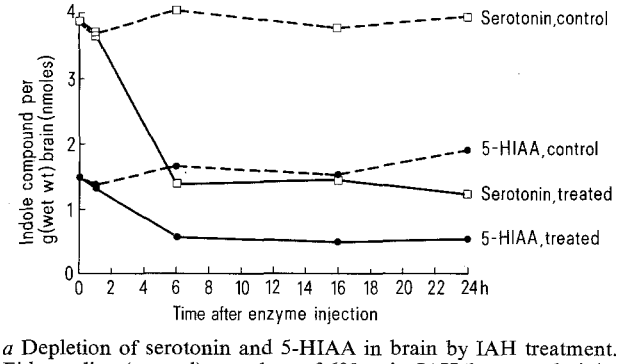
times, mice were sacrificed by cervical dislocation. Whole brains were quickly removed, rinsed in ice-cold saline phosphate to remove any blood, blotted and stored at −70 °C until assay. Serotonin, dopamine and norepinephrine were assayed fluorometrically[5] with a Perkin-Elmer fluorescence spectrophotometer, model MPF-44A. In some experiments altered serotonin levels, as revealed by the above assay, were confirmed by employing a completely different procedure, i.e., the enzymatic-isotopic microassay of Saavedra et al.[6]. Tryptophan was also assayed fluorometrically[7].

A single i.p. injection of 400 or 600 units IAH/kg into mice which were maintained at room temperature resulted in a progressive and pronounced drop in body temperature (see table). This effect exhibited a sharp threshold, since doses of 300 units/kg or less produced no temperature lowering. Significantly, when mice were kept in a 30 °C incubator after receiving 400 units IAH/kg, the hypothermia did not appear.

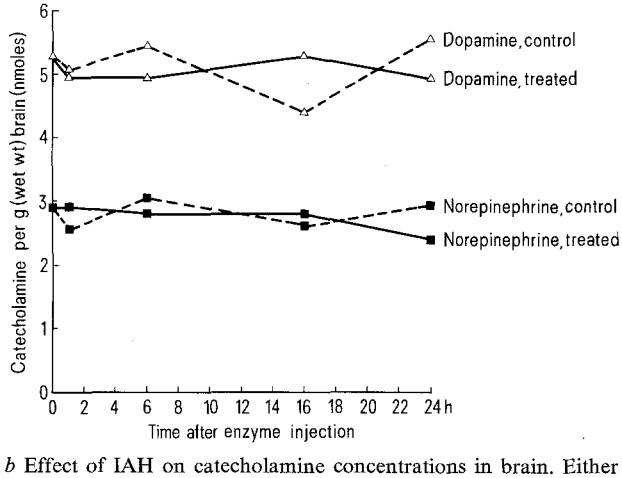
Since a dose of 400 units/kg was near the threshold value, it was decided to use 600 units/kg in most subsequent studies. Plasma tryptophan concentration in IAH-treated mice dropped 95% within 1 h and remained at the low concentration for at least 24 h. Concomitantly, whole brain tryptophan concentrations were diminished by 70% (data not presented). Since tryptophan is the precursor of serotonin, it was not surprising to discover that mice treated with IAH exhibited reduced levels of whole brain serotonin (figure, a). Little change was evident at 1 h, but serotonin dropped by 66% at 6 h and remained so for 24 h. These results were verified by employing a completely different assay, that of Saavedra et al.[6]. Similarly, 5-hydroxyindole-3-acetic acid (5-HIAA), an oxidation product of serotonin, showed a marked decrease over the same time span (figure a). It is noteworthy that brain serotonin was also strikingly depleted in IAH-treated mice which were maintained in a 30 °C incubator, although body temperature was not altered (table). Concomitant with these observations on serotonin (and 5-HIAA) were the important findings that whole brain levels of the catecholamines, dopamine and norepinephrine, were not significantly changed in IAH-treated mice maintained either at room temperature or at 30 °C (figure, b). The response of brain serotonin and catecholamines to administration of 400 units IAH/kg was essentially the same as with 600 units IAH/kg. At 7 h, serotonin decreased to 60% of the saline control, and had further decreased to 40% of the control at 24 h. During this entire period, the catecholamines did not exhibit any significant change in concentration.

The present study strongly suggests that serotonin or a closely related indole compound is involved in regulation of body temperature. Barofsky and Feldstein have suggested that the serotonin metabolite, 5-hydroxytryptophol, affected body temperature in mice[8].

There is ample evidence in the literature concerning the involvement of serotonin in thermoregulation. A major discrepancy is whether serotonin provokes hyperthermia or hypothermia. Early experiments of Feldberg and Myers[9] showed that, when injected into the cerebral ventricle of the cat, serotonin produced hyperthermia. Similar results were obtained in the monkey and dog[10]. On the other hand, hypothermia resulted when serotonin was injected either



a Depletion of serotonin and 5-HIAA in brain by IAH treatment. Either saline (control) or a dose of 600 units IAH/kg was administered i.p. The zero time and 24-h-values represent the average from 4 mice each, while the other values were each obtained from 3 mice.



b Effect of IAH on catecholamine concentrations in brain. Either saline (control) or 600 U IAH/kg was injected i.p., and dopamine and norepinephrine were assayed fluorometrically as outlined in the text. The number of mice used for each time interval is as described above.

i.p. or in the cerebral ventricle of the mouse[8]. Numerous other animal species, including rat, rabbit, sheep, goat and ox, exhibited qualitatively similar hypothermia (reviews by Preston and Schönbaum[11] and by Jacob and Girault[12]). A resolution of this dichotomy is difficult at present, but it must be realized that in some cases the reported alteration of body temperature was actually part of a multiphasic temperature response[8]. Also, even with microinjection techniques, it is difficult to avoid rapid diffusion of the test compound in the brain. For example, depending upon whether serotonin was injected into the posterior or anterior region of the hypothalamus, the pigeon exhibited hyperthermia or hypothermia, respectively[13]. Perhaps a partial explanation for the conflicting data may be found in the observation that in chick pineal gland in organ culture, endogenously formed serotonin and exogenously supplied serotonin entered into distinct metabolic pools, i.e., different metabolic products arose[14]. If this were generally true in other animal species, one would expect to see varying results in the literature, depending on the source of the serotonin. Also, this suggests that temperature regulation studies which are based on serotonin depletion may be more meaningful, physiologically, than experiments in which serotonin is administered. The present results would tend to favor the possibility that serotonin produces hyperthermia in mice. The observation that serotonin depletion was not accompanied by hypothermia if the mice were exposed to an ambient temperature of 30 °C is not without precedent. Francesconi and Mager[15] observed that injection of tryptophan (the precursor of serotonin) into rats maintained at cool ambient temperatures resulted in body temperature alteration, whereas no temperature change occurred if the ambient temperature was raised to 31 °C. A similar effect of ambient temperature was noted in the cat[16].

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Multiple esterase forms in isolated hepatocytes and Kupffer cells of partially hepatectomized rats

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Summary. The esterase patterns of isolated parenchymal liver cells of rats consisted of 6 bands of enzymatic activity, whereas the patterns of iron-loaded Kupffer cells showed 5 bands. Both patterns become simpler in the early prereplicative period of liver regeneration. During simultaneous replication of DNA, i.e. 24 h after partial liver removal, an additional band of esterase activity appears in patterns of hepatocytes and Kupffer cells. At the moment of maximum hepatocyte mitotic rate, i.e. 36 h after partial hepatectomy, both esterase patterns lose the single band of activity again. 2 or 3 days after surgery the initial esterase patterns in hepatocytes return whereas the patterns of Kupffer cells remain incomplete.

The patterns of multiple esterase forms may serve as markers of the state of maturity of isolated cells and cellular cultures[1]. By means of starch-gel electrophoresis of normal liver extracts 10 bands of esterase activity may be revealed. At the same time the data on the esterase patterns in isolated hepatocytes and Kupffer cells are scanty[2]; nothing is known about esterase patterns in regenerating liver and liver parenchymal and nonparenchymal cells. Here some results are presented showing the transient changes of multiple esterase patterns in hepatocytes and Kupffer cells from intact and regenerating liver.

Materials and methods. In all experiments, Wistar male rats, weighing 180–230 g were used. $^2/_3$ of their liver tissue was removed under urethan anesthesia[3]. Each operation was performed between 0900 and 1100 h. At each sampling time, 2,5, 24, 36, 48 and 72 h after surgery, 5–6 animals were sacrificed and the livers perfused in situ via the v.portae with 0.25 M cold sucrose solution containing 0.01 M EDTA. Kupffer cells were separated from hepatocytes in a magnetic field[4]. For the overloading of Kupffer cells, the rats received 1 ml of a 10% starch stabilized suspension of colloid carbonic iron (R-100F, Scientific Institute of Electroorganic Compounds, Moscow) 2 h before sacrifice, via the v.femoralis. To test the viability of

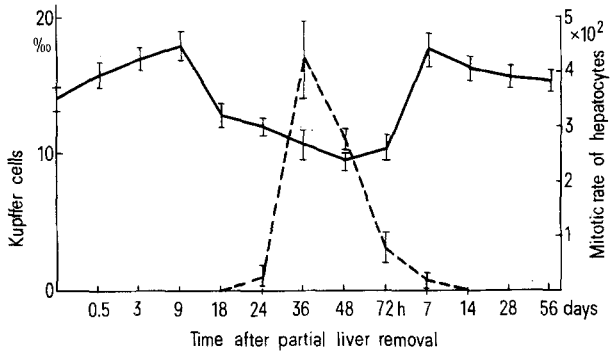

Fig. 1. The relative number of Kupffer cells per 1000 hepatocytes (——) and mitotic rate of hepatocytes in ‰ (– – –) in partially hepatectomized rats.