frogs. Since this decrease of glycogen content in the frog liver was observed in autumn (November), at a time when – in agreement with other authors – we have found a considerable glycogen accumulation in several experiments[6], we have repeated the experiment in Spring (April). In the repeated experiment, after orotic acid treatment a slight but not significant decrease of glycogen content in the liver was obtained.

The observation of some authors that insulin enhances the activity of glycogen synthetase[7–9], and the finding that alloxan diabetes can be normalized by orotic acid[10], raises the possibility that orotic acid may stimulate glycogen accumulation through insulin secretion as well. Since an important role is attributed to leucine in the production of insulin, and it is known that leucine influences the blood sugar level, even if its role in glycogen production is still discussed[11–17], we have investigated the effect of DL-leucine on the glycogen level of the animal species mentioned above, by giving DL-leucine alone and together with orotic acid.

As can be seen from the Figure, in almost all animal species DL-leucine influenced, in one way or in other, the effect of orotic acid. With rats and catfish DL-leucine depressed the liver glycogen level increased by orotic acid. On the other hand, glycogen content in the liver of mice augments considerably on combined orotic acid and DL-leucine treatment as compared to the liver glycogen content of animals treated only with orotic acid. It is also remarkable that in frogs DL-leucine moderates the decrease of glycogen level due to orotic acid. Usually, combined administration of orotic acid and DL-leucine exerts a contrary effect on liver glycogen of the species studied, as compared to the values obtained in controls and orotic acid treated animals. Therefore, it may be supposed that both substances have an influence on glycogen synthetase enzyme as well. It is interesting that generally the simultaneous administration of orotic acid and DL-leucine favours the accumulation of glycogen, even if this accumulation is not always of high degree.

Although DL-valine differs in one CH_2-group from DL-leucine, still it exerts a fundamentally different effect on liver glycogen in certain species, both in combination with orotic acid or given alone, than does DL-leucine.

From the results obtained it may be concluded that amino acids containing an SH-group may play an important role in the production of glycogen, probably as enzyme components. In addition to the stimulatory effect of L-cysteine on glycogen production (even with frogs the decrease of glycogen due to cysteine treatment is not significant), it also modifies the effect of orotic acid in almost all species studied.

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Is Serotonin or are its Metabolites Responsible for Induction of Hypothermia?[1]

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Summary. Serotonin per se, rather than its metabolites, was shown to produce hypothermia in mice. This effect was mediated within the CNS and could be attenuated by methysergide.

The involvement of the central serotonergic system in neural regulation of body temperature in mammals has been the subject of numerous reports (for review see MYERS[3]). Several points of controversy are readily apparent within the literature. The major point has been the lack of agreement as to whether serotonin acts in the CNS to produce a hypothermic or hyperthermic response in core temperature. Many of the studies aimed at elucidating this point have used intracerebral administration of serotonin and monitored rectal temperature[4–7], and lack of agreement between studies has been attributed to species differences[4,5], and locale and route of administration[6,7]. Another possible explanation for the controversial results has been introduced by BAROFSKY and FELDSTEIN[8]. Their studies indicate that the serotonin metabolite 5-hydroxytryptophol produces hypothermia in mice. The possibility thus arizes that 5-HT may produce hyperthermia while its metabolites produce hypothermia. The conversion in brain of exogenously administered 5-HT to a metabolite which also modulates temperature may be responsible for some of the conflicting results. Our studies were, therefore, aimed at elucidating whether the effects of 5-HT, administered into the CNS, were a result of the action of the amine or primarily due to its metabolites.

Materials and methods. C57Bl/6J male mice, 60–80 days old, were used for all experiments. For at least 6 days prior to injections, mice were housed in a controlled

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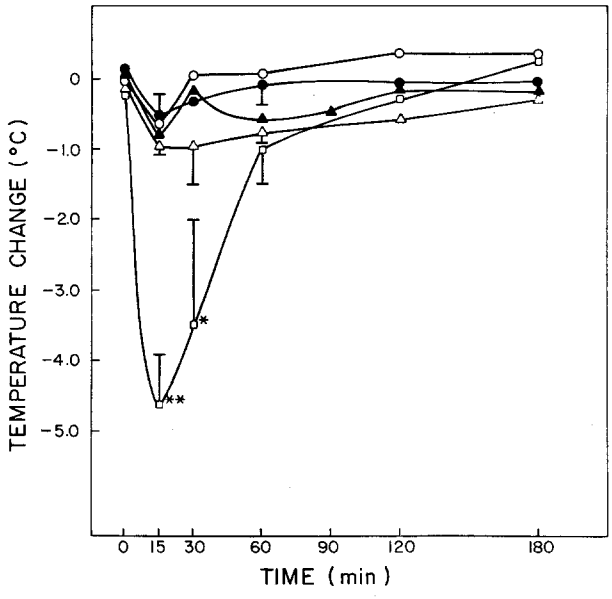

Fig. 1. Mice were injected intraventricularly with either artificial CSF (n = 6, ●—●) or artificial CSF containing 5-HT, 2.5 nmoles (n = 6, △—△) or 25.0 nmoles (n = 6, □—□) or 5-HIAA, 25.0 nmoles (n = 6, ○—○ o)r 5-HTOL 125.0 nmoles (n = 6, ▲—▲). Rectal temperature was recorded just prior to injection and at the various times thereafter. Results are expressed as change from preinjection temperature ± SD. Standard deviations are not included with certain points to facilitate the presentation of the data. *$p < 0.05$; **$p < 0.01$ compared to animals receiving only artificial CSF.

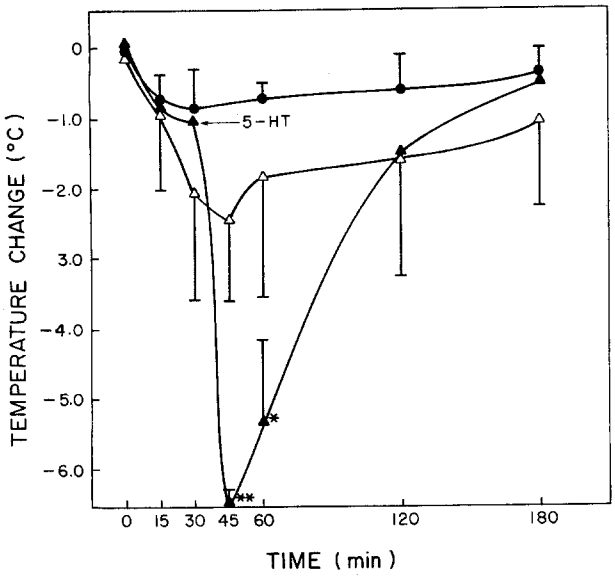

Fig. 2. In experiment I (n = 6, ●—●) serotonin 2.5 nmoles was injected intraventricularly at time 0. In experiment II (n = 6, △—△) pargyline 75 mg/kg was injected i.p. at time 0. In experiment III (n = 6, ▲—▲) pargyline, 75 mg/kg was injected i.p. at time 0 and 30 min following the injection of pargyline (↑), serotonin 2.5 nmoles was injected intraventricularly. Temperature was recorded immediately prior to injection and at various times thereafter. Results are expressed as change from the preinjection temperature ± SD. **$p < 0.001$; *$p < 0.01$ compared to animals treated only with pargyline.

environment with a 700–1900 h light cycle and at a temperature of 22 ± 1 °C with ad libitum access to food and water. All experiments were performed at 22 ± 1 °C and were initiated at 1000 h. All chemicals were of the highest commercially available quality. Serotonin creatinine sulfate, 5-hydroxyindoleacetic acid, and creatinine sulfate were obtained from Sigma Chemical Co. Regis Chemical Co. supplied the 5-hydroxytryptophol, and Sandoz Pharmaceutical Co. supplied the Sansert® (methysergide). Pargyline was kindly donated by Abbott Laboratories. Drugs for i.p. injection were dissolved in sodium chloride (USP), and administered in a volume of 0.1 ml per 10 g of mouse. Drugs administered intraventricularly were contained in artificial CSF, MERLIS[9] solution pH 7.4, and were administered in 10 µl total volume. Intraventricular injections were performed by methods used previously in our laboratories[10,11] and the site of injection was determined in a separate group of experiments by injection of methylene blue.

Rectal temperature was monitored using a Tele-Thermometer (Yellow-Springs Instrument Co.). The lubricated thermometer probe was inserted 2.0 cm into the rectum and the instrument was allowed to stabilize approximately 30 sec before the reading was noted. Assays for monoamine oxidase activity were performed as described by TABAKOFF and ALIVISATOS[12]. Statistical significance of the results was calculated using the students t-test and a p-value of 0.05 or less served as criterion for significance.

Results and discussion. Intraventricular injection of serotonin creatinine sulfate (5-HT) produced a dose dependent drop in the rectal temperature of the animals (Figure 1). The temperature returned to within the normal range in approximately 1 h after the administration of 5-HT. No significant change from preinjection temperature was noted in animals receiving intraventricular injections of MERLIS solution or MERLIS solution containing creatinine sulfate (25 n moles/10 µl). An i.p. injection of 25 nmoles 5-HT had no effect on rectal temperature indicating a central action of intraventricularly injected 5-HT. In contrast to the hypothermia produced by intraventricular injection of 5-HT, no change in rectal temperature was found when 5-HIAA (25 nmoles) or 5-HTOL in doses of 2.5, 25, or 125 nmoles per mouse were injected intraventricularly (Figure 1). Further evidence to implicate 5-HT, rather than its metabolites, as the agent responsible for the hypothermia was obtained by pretreating animals with a monoamine oxidase inhibitor, pargyline, prior to the intraventricular administration of the amine. Such pretreatment greatly enhanced the hypothermia produced by low doses of 5-HT (Figure 2). The administration of pargyline (75 mg/kg) i.p. 30 min before sacrificing the animal was found to result in a 91 ± 5% (n = 3) inhibition of brain MAO activity. Thus, a block in the major metabolic pathway for 5-HT potentiates rather than blocks the effect of intraventricularly administered 5-HT. The hypothermia produced by i.p. injection of pargyline may also reflect the accumulation of endogenous 5-HT within the temperature regulating centers of brain. Studies in our laboratory (TABAKOFF and MOSES, unpublished observations) indicate a linear

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increase in 5-HT levels in mouse brain, reaching 188% of normal levels 45 min after an i.p. injection of pargyline.

The administration of methysergide to mice (Figure 3) resulted in a prolonged hyperthermia which followed the injection artifact. This hyperthermia did not, however, reach statistical significance. On the other hand, when methysergide was injected intraventricularly concomitantly with 5-HT (25 nmoles) it significantly attenuated the hypothermia produced by 5-HT (Figure 3). The block of the hypothermic response to 5-HT by methysergide, which has been shown to be a 5-HT receptor blocker in the CNS[13], again indicates that the 5-HT induced hypothermia is probably a direct effect of this amine on central serotonergic receptors. The data obtained with methysergide would also weaken the argument that the effects of intraventricularly injected 5-HT would be mediated by a displacement of other amines (e.g., catecholamines) from storage sites. The possibility remains that the location of various receptors for 5-HT may play a primary role in determining whether the response to intracerebral administration of 5-HT is hypo- or hyperthermia. Several studies[14,15] have indicated that 5-HT injected into cerebral ventricles, reaches only a limited distribution in tissue surrounding the ventricular system in brain. Although most of the 5-HT sensitive thermoregulatory areas have been found to reside in the area of the third ventricle the diffusion gradient produced after intraventricular administration of 5-HT may produce results differing from those produced by instilling 5-HT directly into particular areas of the hypothalamus.

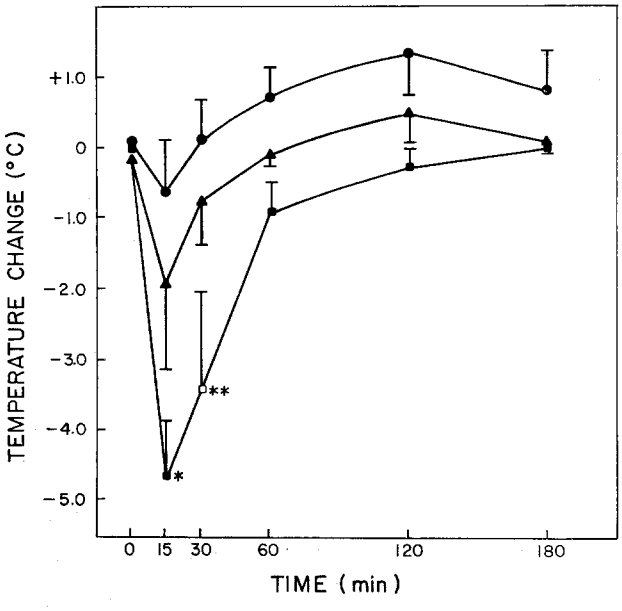

Fig. 3. Methysergide, 20 nmoles (n = 4, ●—●), or serotonin, 25 nmoles(n = 6, ■—■), or a solution containing both methysergide and serotonin at these same dose levels (n = ▲—▲) in artificial CSF, were injected intraventricularly in mice. Rectal temperature was recorded just prior to injection and at various times thereafter and results are expressed as change from preinjection temperature ± SD. **$p < 0.001$; *$p < 0.05$ as compared to animals receiving serotonin and methysergide.

The recent explanations[16,17] that changes in body temperature, after administration of drugs, are due to a block in the production of 5-HT metabolites in the CNS should, however, be reconsidered. The function of such metabolites (e.g., 5-HTOL) was postulated to be production of hypothermia. Our results do not support such a postulate, and indicate that these metabolites of 5-HT have no effect on body temperature even when administered into the CNS in doses well above the levels normally found in brain. Previous data[8] showing a hypothermic response to 5-hydroxytryptophol in doses of 0.8 or 1.2 g/kg may have been an expression of a more general phenomenon of alcohol induced lowering of body temperature[18]. Hydroxytryptophol, may have also been oxidized to 5-hydroxyindoleacetaldehyde which could have indirectly influenced body temperature. Our recent studies have indicated that this aldehyde inhibits brain ATPase activity[19].

Our current data strongly supports the direct involvement of 5-HT in mediating the temperature response produced after administration of this neurotransmitter into the CNS and indicates the presence of receptors for 5-HT in brain which, when activated, produce hypothermia in the mouse.

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Paroxysmal Discharges in the Electroencephalogram of the El Mouse

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Summary. The spike discharges in the EEG of the El mouse, a seizure-susceptible strain, were recorded during convulsive seizures. This fact provides evidence that those seizures are really epileptic convulsions.

Since an El mouse was found and developed by IMAIZUMI et al.[2,3], its seizure has been regarded as epileptiform and it has appeared to be a suitable model of a human genuine or hereditary epilepsy. Now it is available as a genetically pure strain; all the mice of this strain display epileptiform seizures, so far as the proper care is taken[2,4]. Whereas some neurochemical studies were carried out[4], neurophysiological efforts were not successful. There has been no evidence that an El mouse might be really epileptic, namely that it might have the disorders of paroxysmal cerebral dysrhythmia[5]. Recently, I was able to record the paroxysmal discharge patterns in the electro-