

150 min. BG increments following 2-DG showed reciprocal changes to those in BT; however, BG values significantly higher than in controls were still present at 150 min, when BT values had risen to equal those of controls. This centrally-evoked hypothermia could be suppressed by the concomitant central administration of glucose (2-DG 2 mg/10 μ l-glucose 2 mg/10 μ l). Glucose alone (2 mg/10 μ l) was without effect on either BG or BT. Central administration of 2-DG (2.5 mg/20 μ l) to hypophysectomized rats, 1 month following hypophysectomy, induced also a marked increase in BG and a striking decrease in BT, which were still present 120 min after the injection (data not presented). Thus, release of one or more anti-insulin factors by the anterior pituitary does not seem to play a part in the hyperglycemic response produced by central glucopenia, as previously suggested¹⁷.

Inhibition of glucose metabolism by central administration of 2-DG induces effects, i.e. hyperglycemia, inhibition of insulin secretion, hyperphagia and hypothermia, which cannot be explained by absorption of the substance into the systemic circulation. In the present experiments since 2-DG was injected into the lateral ventricle, it could have acted almost anywhere in the brain adjacent to the ventricular system to produce its action. However, electrophysiological studies have indicated that neurons responsive to 2-DG reside both in the ventromedial and the ventrolateral nuclei¹⁸. In addition it has been reported that the effective locus for 2-DG stimulation of gastric acid secretion¹⁹, growth hormone²⁰ and food intake¹² is in the ventrolateral hypothalamus. It cannot be excluded a priori that 2-DG is active at more than one CNS locus; to solve this problem it will be necessary to inject the drug directly into the specific brain loci and/or produce selective destruction of such loci. The different physiological effects resulting from central glucoprivation probably reflect the behavioural and reflexual effort to satisfy the

blocked 'glucoprivic' receptors. This teleologic scheme can explain the hyperglycemia unaccompanied by increased insulin secretion and the feeding behaviour. The hypothermic effect, on the other hand, might be interpreted in term of BROBECK's thermoregulatory theory in relation to feeding²¹. However, before drawing any further conclusion from these results the question must be answered as to whether the different effects elicited by glucoprivation are independent functions of the glucosensitive area of the brain or whether they are closely interrelated.

Riassunto. Il 2-desossi-D-glucosio somministrato nel ventricolo laterale del cervello del ratto provoca iperglicemia, inibizione della secrezione di insulina, iperfagia ed ipotermia a dosi che sono inefficaci per via sistemica.

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Phenylethanolamine-N-Methyl Transferase Activity in the Ground Squirrel (*Citellus citellus*)

There are many data on the seasonal changes in the adrenal catecholamines content in hibernators examined in normothermic state, as well as in hibernation (see KAYSER¹). There is some evidence that in autumn, before the hibernation, in normothermic animals, an accumulation of catecholamines in adrenals occurs. ALLARA² found that the adrenal medulla of the active hedgehog, examined in the middle of October, is particularly rich in adrenaline. KAYSER and ARON³ observed that the amount of adrenaline in normothermic hamster depends on the environmental temperature at which animals are kept. The highest level of this amine was found in animals kept in summer at 27°C. The amount of adrenal catecholamines in the normothermic ground squirrel kept in the cold, is significantly higher in autumn, in the period prior to hibernation, than in summer (PETROVIĆ and DAVIDOVIĆ^{4,7}). This accumulation of catecholamines in adrenals of the active ground squirrel in autumn might be the consequence of the higher rate of synthesis of these amines, or of its decreasing secretion. In order to contribute to the solution of this problem, we examined in September-October adrenal phenylethanolamine-N-methyl transferase activity in the normothermic ground squirrel under different experimental conditions.

Material and methods. Male ground squirrels weighing 200–250 g were used for experiment. Animals were divided into 3 groups, each consisting of 6 to 10 animals:

1 group was active and kept at 20–25°C, the 2nd was active and kept in a cold room at 6–8°C and the 3rd one was in hibernation some days before the experiment. Before sacrificing, hibernating animals were removed from the cold room and placed at 25°C for about 30 min. They were sacrificed when rectal temperature reached 36–37°C. After killing, both adrenals were dissected free of fat, weighed and homogenized in 10 ml of chilled isotonic potassium chloride solution. The homogenate was centrifuged for 30 min at 12000 g. A portion of the supernatant fluid was assayed for PNMT activity by the method of WURTMAN and AXELROD⁵, modified by GRIPOIS and PARVEZ⁶. This method consists of the incubation of adrenals extract with S-adenosyl methionine ¹⁴C, in the presence of normetanephrine, and of the measurement of the radioactivity of epinephrine formed in this process. Incubation was performed at 37°C for 60 min. Results are expressed in counts per min per pair or per mg of tissue and presented in the Table.

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Experimental conditions	Body temperature (°C)	No. of ground squirrels	Weight of adrenals pair (mg)	Adrenal PNMT activity	
				cpm/pair of adrenals	cpm/mg of adrenals
Active at 20–25°C	36–37	8	46.53 ± 4.73 ^c	2886 ± 109 ^a	6497 ± 596
Active at 6–8°C	36–37	6	62.93 ± 8.79 ^c	4555 ± 381 ^{a, b}	7533 ± 642
Active, after arousal from hibernation	36–37	10	59.75 ± 8.34	3202 ± 266 ^b	5830 ± 487

^a $p < 0.001$, ^b $p < 0.01$, ^c $p < 0.01$.

Results and discussion. PNMT activity in the normothermic ground squirrel kept at 20–25°C was the lowest compared with the 2 other groups. Significantly higher PNMT activity was found in adrenals of active animals kept at 6–8°C ($p < 0.001$), indicating an intensification of methylation of noradrenaline to adrenaline. The weight of adrenals in the group kept at 6–8°C was higher than in the first one ($p < 0.01$). Higher adrenal PNMT activity found in the ground squirrel kept in September–October at 6–8°C permits to conclude that a high level of adrenaline found in the same experimental condition by PETROVIĆ and DAVIDOVIĆ⁷ was the consequence of the increasing biosynthesis of this amine. An increased adrenocortical activity, as evaluated by the augmentation of plasma 17-OHCS and decreased adrenals ascorbic acid levels, was also found in active ground squirrel kept at the same temperature and examined in October (PETROVIĆ and JANIĆ⁸). As glycocorticoids are involved in the activation of adrenal PNMT in the rat (WURTMAN et al.⁹), the augmentation of adrenocortical activity found in the ground squirrel in the same experimental conditions, seems to be responsible for higher PNMT activity. The consequence of this alteration in the synthesis was the higher level of adrenal catecholamines, especially adrenaline found in the active ground squirrel in the period prior to hibernation.

Activity of PNMT in adrenals of animals examined immediately after the arousal from hibernation was lower than in active ones kept at the same environmental temperature ($p < 0.01$). The weight of adrenals in this group, however, was about the same as that in the previous group. Lower PNMT activity found in adrenal of post-hibernating animals may be explained by the depressed adrenocortical activity in general, found in hibernating animals (see KAYSER¹).

Summarizing the results of this study together with our previous finding, we may conclude that in autumn

before hibernation in normothermic ground squirrel kept in the cold, an intensification of the methylation of noradrenaline to adrenaline occurs. As in the same experimental conditions a transitive augmentation in adrenocortical activity was found, it seems that glycocorticoids are involved in the increasing PNMT activity in autumn. The consequence of this alteration in the synthesis is expressed in the higher level of adrenals catecholamines, especially adrenaline, found in the active ground squirrel examined in the period prior to hibernation.

Résumé. L'activité de la phényléthanolamine-N-méthyltransférase (PNMT) a été mesurée dans l'homogénat des surrénales chez le *Spermophile*, soit actif, exposé aux températures extérieures de 20–25°C ou de 6–8°C, soit réveillé pendant l'hibernation. Chez les animaux actifs, l'effet du PNMT a été le plus faible. Une augmentation significative de l'activité enzymatique a été constatée chez les animaux actifs maintenus à la température de 6–8°C ($p < 0.001$). L'activité du PNMT diminue immédiatement chez les *Spermophiles* réveillés pendant leur hibernation et ceci par rapport aux animaux actifs maintenus à la même température extérieure ($p < 0.01$).

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Effects of Sexual Hormones on Gonadotrophin Secretion in Prepuberal Female Rats¹

Sex steroids can exert a positive and a negative feedback effect on LH and FSH secretion in puberal and prepuberal rats². Since the different effect of sex steroids on gonadotrophin secretion during prepuberal state could be conditioned by the neural maturation of different hypothalamic areas, it seems of interest to study the gonadotrophin response to estradiol and testosterone in prepuberal female rats at different ages. These studies were also performed in female rats, with hypothalamic alterations due to the administration of a single dose of testosterone soon after birth.

Material and methods. Female prepuberal rats were injected 3 to 4 days after birth with either 1 mg testoster-

one propionate (androgenized rats) or peanut oil (other groups). Testosterone was administered s.c., dissolved in 0.1 ml peanut oil.

Series I: Control and androgenized rats were injected at 20 days old with estradiol (1 µg/100 g body wt.) or with testosterone (100 µg/100 g body wt.) for 3 days. Series II: A similar schedule of treatment to that described

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