ure 3). However, in case of the highest dose of SRBC, adjuvant effectiveness of lipids was not observed. Further experiments showed that lipids from listeria organisms played an important role not only in primary, but also in secondary response (Table). The lipids administered to mice together with SRBC 40 days before a second challenge with SRBC, resulted in a very high number of PFC.

Discussion. The data accumulated at the cellular and humoral levels indicate that lipids from *Listeria monocytogenes* used in appriopriate dose may be regarded as a strong adjuvant. The depressive action of high doses of listerial lipids probably depends on the damage of immunocytes or disorder of antigen-processing[6,7]. The results give evidence that the adjuvant action of these lipids depends on interaction in the early stages of immune response, probably when antigen is being processed by macrophages or recognized by antigen-sensitive cells[8,9]; the lipids injected after the antigenic stimulus did not show an adjuvant effect. According to some authors the lipids of *Listeria monocytogenes* influence the phagocytic activity of macrophages[10], cause an elevation of wet spleen weight[1] and increase the number of circulating monocytes[2]. It may be concluded that they act by increasing the number of memory cells during the primary response. Similar action of other adjuvants of bacterial origin (*Bordetella pertussis*, endotoxins from Enterobacteriaceae) was shown by FINGER et al.[11,12].

Résumé. Nos expériences ont démontré que les lipides de *Listeria monocytogenes* ont un fort effet adjuvant aux niveaux cellulaire et humoral. Comme antigène, on a utilisé des èrythrocytes de mouton. On a déterminé le nombre de cellules de la rate produisant des anticorps ainsi que le titre de l'hémolysine d'après la méthode de l'hémolyse radiale en gélose.

P. JAKONIUK, J. BOROWSKI, A. SZPAK and A. JARZYNA[13]

Department of Microbiology, University Medical School, PL-15089 Białystok 8 (Poland), 8 January 1973.

[6] H. FINGER, I. FÖLMER, L. PLAGER and M. HENSELING, Experientia *28*, 562 (1972).
[7] A. TANAKA, K. TANAKA, D. HAGIMOTO and K. SUGIYAMA, Int. Arch. Allergy *32*, 224 (1967).
[8] H. FINGER, P. EMMERLING and E. BRÜSS, Infect. Immun. *1*, 251 (1970).
[9] H. FINGER, P. EMMERLING and M. BÜSSE, Can. J. Microbiol. *16*, 623 (1970).
[10] A. JARZYNA, Thesis (Bialystok 1971).
[11] H. FINGER, P. EMMERLING and E. BRÜSS, Can. J. Microbiol. *15*, 814 (1969).
[12] H. FINGER, P. EMMERLING and M. BÜSSE, Int. Arch. Allergy *38*, 598 (1970).
[13] Supported by grants from Polish Academy of Science.

Central Glucoprivation: Some Physiological Effects Induced by the Intraventricular Administration of 2-Deoxy-D-Glucose

2-deoxy-D-glucose (2-DG), an unmetabolizable analogue of glucose, blocks glycolysis and produces a 'metabolic paradox' of intracellular glucopenia associated with hyperglycemia[1]. As a result of glucoprivation, systemic administration of large doses of 2-DG induces feeding behaviour[2], inhibition of insulin secretory response to insulin secretagogues[3] and an influence on growth hormone and corticosteroid release[4]. Systemic administration of 2-DG induces also a striking increase in epinephrine secretion, which is the usual response of the organism to glucose unavailability[5,6]. The fact that systemic administration of 2-DG is coupled with cerebral symptoms characteristic of hypoglycemia (drowsiness, stupor and ataxia, hunger and sweating) despite marked hyperglycemia, suggested that both cerebral and peripheral glucoprivation occurred in animals[2] and humans[5]. We have recently been interested in determining whether 2-DG was capable of exerting physiological effects by an action on the central nervous system (CNS) independent of its inhibitory[3] or stimulatory effects[2,4] at the peripheral level. This report summarizes part of our recent work on the effects of the intraventricular (IVT) administration of 2-DG in the unanesthetized rat.

Material and methods. Intact Sprague-Dawley (SD) female rats, 150–200 g or SD female rats, hypophysectomized at 26 days of age, were used in the experiments. Food was withdrawn at the start of each experiment whereas access to water was permitted throughout. A small polyethylene cannula (PE 10) was implanted into the lateral ventricle of the brain under light pentobarbital anesthesia[7] and the rats were then placed in individual cages. After allowing 2–3 days for recovery, the substance was injected through the implanted cannula without anesthesia in a volume of 20 µl.

Blood glucose. A sample of blood was collected for blood glucose (BG) determinations (glucose oxidase) immediately before and at various time intervals (see Results and discussion) following 2-DG or pyrogen-free saline administration[8].

Plasma insulin. Plasma levels of insulin (I) were determined according to a radioimmunoassay (RIA) method previously described[9].

Food Intake. Rats bearing an IVT cannula were individually housed and for 2 consecutive days their spontaneous food intake was measured accurately every hour, beginning at 10.00 h and for the next 6 h. 2-DG (2,5 mg) was injected IVT on the day of the experiment at 10.00 h. Control rats were injected with saline. Food intake ingested hourly on the experimental day was compared to the mean food intake ingested during the same time on the 2 days prior to the experiment. Tap water was available during the experiment, but water intake was not measured.

Body temperature (BT). These experiments were performed in an ambient temperature of 24 °C at 10.00 h. Animals were injected IVT with 2-DG (2,5 mg) or pyrogen-free saline. Core temperature was measured by means of a

[1] A. N. WICK, D. R. DRURY, H. NAKADA and J. B. WOLFE, J. biol. Chem. *224*, 963 (1957).
[2] G. P. SMITH and A. N. EPSTEIN, Am. J. Physiol. *217*, 1083 (1969).
[3] S. J. GOLDSMITH, R. S. YALOW and S. A. BERSON, Diabetes *19*, 453 (1970).
[4] G. P. SMITH and A. W. ROOT, Endocrinology *85*, 963 (1969).
[5] B. HÖKFELT and S. BYDGEMAN, Proc. Soc. exp. Biol. Med. *106*, 537 (1961).
[6] J. LASZLO, W. R. HARLAN, R. F. KLEIN, N. KIRSHNER, E. H. ESTES JR. and M. D. BOGDANOF, J. clin. Invest. *40*, 171 (1961).
[7] F. B. ALTAFFER, F. DE BALBIAN VERSTER, S. HALL, G. J. LONG and P. D'ENCARNACAO, Physiol. Behav. *5*, 119 (1970).
[8] C. RERUP and I. LUNDQUIST Acta endocr. copenh. *52*, 357 (1966).
[9] L. A. FROHMAN and L. L. BERNARDIS, Endocrinology *82*, 1125 (1968).

rectal probe, read intermittently at 30 min intervals for 150 min. Blood was also collected at the same time intervals for BG determinations. BG and BT values were expressed as increments or decrements of baseline values (see Results and discussion). Student's *t*-test was used to determine the significance of the differences between groups.

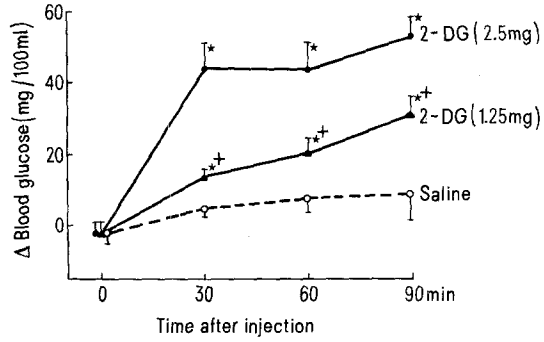

Fig. 1. Effect of IVT adminstration of 2-DG on blood glucose. Shown are the mean ± S.E. Asterisks indicate significate differences between 2-DG groups and their saline-injected controls. Crosses indicate significant differences between low (1.25 mg) and high (2.5 mg) 2-DG doses. Initial BG leveels were 73 ± 0.78 ± 1 and 78 ± 2 mg/100 ml in saline (8) 2-DG (1.25 mg) (8) and 2-DG (2.5 mg) (8) treated rats, respectively.

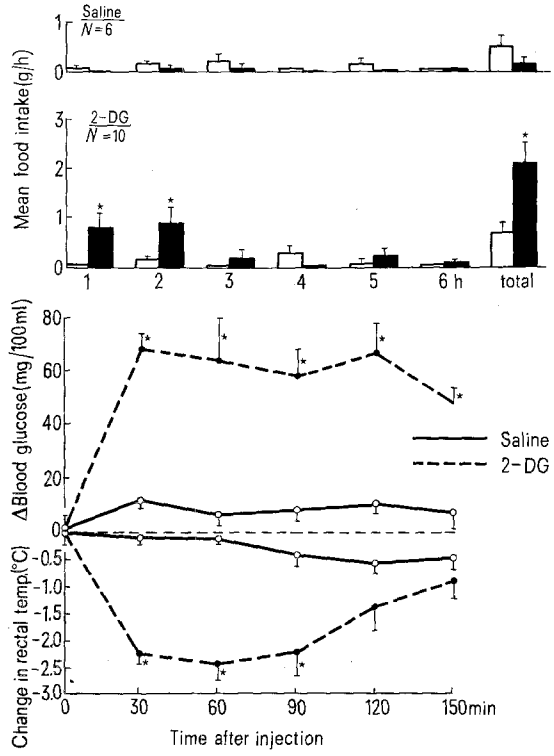

Fig. 2. Upper panel, mean hourly food intake and total food intake ± S.E. during the 6-h period after the IVT injection of saline (20 µl) or 2-DG (2.5 mg). Open histograms represent baseline intake on days when no injections were made. Asterisks indicate significant differences from baseline intake. N = number of animals. Lower panel, effect of 2-DG (2.5 mg IVT) on body temperature and blood glucose. Shown are the mean ± S.E. Initial BT was 38.1 ± 0.1 and 37.9 ± 1 °C and BG levels were 67 ± 3 and 74 ± 2 mg/100 ml in 2-DG (7) and saline (8) treated rats, respectively. Asterisks indicate significant differences between the 2-DG group and the saline-injected control.

Results and discussion. Blood glucose. Figure 1 shows that IVT administration of 2-DG induced a marked hyperglycemic response both at 2.5 and 1.25 mg (2-DG-2.5 mg vs saline – $p < 0.001$ at 30,60 and 90 min.; 2-DG – 1.25 mg vs. saline – $p < 0.01$ at 30 min, $p < 0.02$ at 60 min, $p < 0.05$ at 90 min). The hyperglycemic effect was dose-dependent: 2-DG – 2.5 mg vs. 2-DG 1.25 mg $p < 0.05$ at 30 min, $p < 0.02$ at 60 min, $p < 0.01$ at 90 min. Twice the higher dose of 2-DG (5 mg) administered i.p. had no effect on BG. Equally ineffective was the IVT administration of hypertonic saline (2.4% – 20 µl), the osmolarity of which was equal to that of the higher dose of 2-DG.

Plasma insulin. In an experiment similar to the one previously described, the effect of the IVT injection of 2-DG (2 mg) on BG and plasma RIA-I was studied (data not presented). Despite the marked hyperglycemia (mean BG rise in 2-DG treated rats greater than 30 mg/100 ml at each time period), there was no increase in plasma insulin levels at any time. Twice this dose of 2-DG (4 mg), given i.p., was without effect on either BG or plasma insulin. Thus, it appears that the central mechanism responsible for 2-DG hyperglycemia is associated with an inhibition of the I response.

Food intake. The presence of glucoreceptors in the ventromedial hypothalamus and extroventromedially has been established and their contribution to food intake regulation repeatedly emphasized[10,11]. The glucoreceptors would sense change in glucose utilization and would act to signal the initiation or termination of feeding. To determine the function of the hypothalamic glucoreceptors in feeding behaviour, 2-DG was given IVT and its effect on food intake was studied. As shown in Figure 2 (upper panel) central 2-DG significantly increased food intake during the first 2 h after the injection. The total amount of food ingested in the 6-h period of the experimental day was also significantly higher than the total baseline intake. Central administration of saline did not significantly affect food intake. In accordance with the present findings, a central site for the hyperphagic action of 2-DG has already been reported[12,13].

Body temperature. There is ample evidence for multiple factors affecting feeding behaviour[14,15]. Brobeck proposed a regulatory mechanism based upon the close relationship between BT and food intake. According to this 'thermostatic' theory, an increase in BT will stimulate the satiety centre and inhibit the feeding center. A decrease in tempearature will induce apposite changes in activity in these areas. More recent studies have suggested that the interaction between food intake and BT may not reflect a conscious appreciation of peripheral temperature but a 'central heating' of the cells in the hypothalamic preoptic area. Warming this area increased feeding while reflexively decreasing core temperature[16]. Since we had previously shown increased feeding to be a result of central administration of 2-DG, it was felt to be of interest to see whether or not changes in BT might also be present following central glucoprivation.

As shown in Figure 2 (lower panel), central 2-DG induced a striking decrease in BT which was maximum at 60 min and still present after 90 min. Administration of saline induced only a very slight decline in BT at 90, 120 and

[10] J. Mayer, Physiol. Rev. *33*, 472 (1953).
[11] R. W. Reynolds and J. Kimm, J. comp. Physiol. *60*, 438 (1965).
[12] S. Balagura, M. Kanner, Physiol. Behav. *7*, 252 (1971).
[13] R. R. Miselis and A. N. Epstein, Physiologist *13*, 262 (1970).
[14] In *Neural Regulation of Food and Water Intake* Ed. P. J. Morgane; Ann. N.Y. Acad. Sci. *157*, 531–1216 (1969).
[15] B. G. Hoebel, A. Rev. Physiol. *33*, 533 (1971).
[16] S. P. Grossman, Fedn. Proc. *27*, 1349 (1968).

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 µl-glucose 2 mg/10 µl). Glucose alone (2 mg/10 µl) was without effect on either BG or BT. Central administration of 2-DG (2.5 mg/20 µl) 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 suggested[17].

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 nuclei[18]. In addition it has been reported that the effective locus for 2-DG stimulation of gastric acid secretion[19], growth hormone[20] and food intake[12] 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 feeding[21]. 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.

E. E. MÜLLER, A. PANERAI, DANIELA COCCHI, L. A. FROHMAN and P. MANTEGAZZA[22]

2nd Chair Department of Pharmacology, University of Milan, Via Vanvitelli 32, I-20129 Milano (Italy),
4 December 1972.

[17] N. KOKKA and R. GEORGE, Neuroendocrinology *6*, 1 (1970).
[18] T. DESIRAJU, M. G. BANERJEE and B. K. ANAND, Physiol. Behav. *3*, 757 (1968).
[19] D. G. COLIN-JONES and R. L. HIMSWORTH, J. Physiol., Lond. *206*, 397 (1970).
[20] R. L. HIMSWORTH, P. W. CARMEL, A. G. FRANTZ, Endocrinology *91*, 217 (1972).
[21] J. R. BROBECK, Yale J. Biol. Med. *20*, 545 (1947-48).
[22] Misses F. FERRARI, S. BIANCHI and L. SCAPELLATO provided skilful technical assistance. This investigation was supported by Grant No. 70.01788.04 115.2380 of the Consiglio Nazionale delle Richerche, Italy.

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 KAYSER[1]). There is some evidence that in autumn, before the hibernation, in normothermic animals, an accumulation of catecholamines in adrenals occurs. ALLARA[2] found that the adrenal medulla of the active hedgehog, examined in the middle of October, is particularly rich in adrenaline. KAYSER and ARON[3] obeserved 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 AXELROD[5], modified by GRIPOIS and PARVEZ[6]. This method consists of the incubation of adrenals extract with S-adenosyl methionine ^{14}C, in the presence of normetanephrine, and of the measurement of the radioactivity of epinephrine formed in this processus. 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.

[1] CH. KAYSER, *The Physiology of Natural Hibernation* (Pergamon Press, London 1961), vol. 8, p. 241.
[2] E. ALLARA, Bull. Soc. Biol. sper. *33*, 259 (1957).
[3] CH. KAYSER and M. ARON, Archs Anat. Histol. Embryol. *33*, 21 (1950).
[4] V. M. PETROVIĆ and V. DAVIDOVIĆ, J. Physiol. *57*, 678 (1965).
[5] R. J. WURTMAN and J. AXELROD, Science *150*, 1464 (1965).
[6] D. GRIPOIS and M. PARVEZ, Biochimie *54*, 413 (1972).