we have measured the daily excretion of one of the urinary metabolites of catecholamines, namely VMA. As can be seen in Figure 3, α-MpT-methyl ester did not reduce the excretion of VMA but, on the contrary, markedly increased the latter.

Discussion. Administration of α-MpT to guinea-pigs[2] or rats[3] has been shown to result in a sharp decrease of the heart, spleen and brain NE levels. From these experiments, it was postulated that this decrease was a consequence of NE synthesis inhibition. On the other hand, the *meta* isomer of α-MpT, α-MmT, a potent inhibitor of dopa-decarboxylase[12], has been reported to increase the release of catecholamines[13]. In the same conditions, our experiments show that administration of either α-MpT or α-MmT is followed by a drastic rise in the urinary excretion of E and NE. It seems, therefore, that the effects of these drugs described above are not only related to their inhibitory action on catecholamine biosynthesis, but may also be the consequence of a modification of their release, their oxidation, as well as on their uptake.

SPECTOR et al.[2] have tested the possibility that α-MpT may increase the tissular release of NE. As α-MpT is methylated in vivo and transformed into α-methylated amines which are known to induce the release of NE, these authors have assayed methyl tyramine and α-methyl norsynephrine in both heart and brain of guinea-pig after the administration of α-MpT. Since these assays were unsuccessful, SPECTOR et al. concluded that the NE tissular decrease induced by α-MpT was unrelated to an effect on catecholamine release, and was only the consequence of tyrosine-hydroxylase inhibition.

Another mechanism which could explain the increase of urinary excretion of catecholamines is an inhibitory effect of α-MpT on the catabolism of catecholamines. As shown by the increase of the urinary excretion of VMA, it can be assumed that α-MpT does not inhibit the oxidation of catecholamines.

The effects of α-MpT or α-MmT on the excretion of E and NE could also be attributed to an interference of these compounds with the mechanism involved in the catecholamine uptake. As a matter of fact, HESS et al.[12] have shown that α-MmT decreases the ability of tissues to take up and bind exogenous NE. The same effects were obtained with α-MpT by BRALET et al.[5], who concluded that a decrease in the re-uptake of NE enhances the release of NE for a few hours. Our present data showing that α-MpT and α-MmT also increase the excretion of catecholamines therefore support this finding.

From our results, it is not possible to determine precisely the mechanism by which α-MpT enhances the urinary excretion of catecholamines. However, we can postulate, like ENNA et al.[14], that the inhibition of tyrosine-hydroxylase is not the only action of α-MpT. We can furthermore conclude that this drug stimulates the tissular release of E and NE into the blood, the part taken by adrenal medulla in this release being negligible[15].

Résumé. Une injection à des rats d'α-méthyl-*para*-tyrosine (α-MpT), d'α-MpT-méthyl ester ou d'α-méthyl-*méta*-tyrosine (α-MmT), aux doses de 200 mg/kg, produit une augmentation rapide et importante de l'excrétion urinaire de l'adrénaline et de la noradrénaline, ainsi que de l'acide vanyl-mandélique. La surrénalectomie ne modifie pas l'augmentation de l'excrétion urinaire de noradrénaline produite après injection d'α-MpT-méthyl ester. L'élévation des taux des catécholamines urinaires résulte vraisemblablement de leur libération tissulaire, sous l'action de l'α-MpT ou de l'α-MmT.

M. BEAUVALLET, J. ROFFI, Y. GIUDICELLI and M. LEGRAND-TISSOT

Institut de Pharmacologie, Université de Paris VI, 21, rue de l'Ecole de Médecine, F–75270 Paris (France); Laboratoire d'Endocrinologie, Université de Paris XI, Bâtiment 491, F–91405 Orsay (France), and Laboratoire de Biochimie, C.H.I. de Poissy, F–78303 Poissy (France), 31 January 1975.

[11] J. J. PISANO, J. R. CROUT and D. ABRAHAM, Clin. chim. Acta *7*, 285 (1962).
[12] S. M. HESS, M. OZAKI and S. UDENFRIEND, Pharmacologist. *2*, 81 (1960).
[13] S. M. HESS, R. H. CONNAMACHER, M. OZAKI and S. UDENFRIEND, J. Pharmac. exp. Ther. *134*, 129 (1961).
[14] S. J. ENNA, R. L. DORRIS and P. A. SHORE, J. Pharmac. exp. Ther. *184*, 576 (1973).
[15] Acknowledgments. We wish to thank Dr. LE DOUAREC (Scientific Director of Merck-Chibret Institute, France) for the generous gift of α-methyl-*para*-tyrosine.

Amphetamine-Induced Changes in Body Temperature and Glycogen Content of the Encephalon in the Chicken[1]

Thirty min after an i.p. injection of D-amphetamine, 5 mg/kg, the concentration of glycogen in the mouse brain decreased by 30%; this depletion was closely associated with an increase in phosphorylase a, and the subsequent marked resynthesis of the polysaccharide seemed achieved by the conversion of glycogen synthetase D to I[2,3]. In these conditions, the depletion of glycogen may occur in glial cells in response to the release of catecholamines[4,5]. On the other hand, injection of amphetamine in the rat, the NMRI-strain mouse and the rabbit produced an increase in body temperature, though in the C3H-strain mouse no change appeared[6–8]. The possible role of dopamine in the amphetamine-induced hyperthermia was suggested[7,9,10]. We may expect that some connection exists between cerebral glycogenolysis and body hyperthermia, which is mediated by the level of biogenic amines

[1] This work was supported by grants from the Centre National de la Recherche Scientifique (E.R.A. No. 331) and from the Fondation pour la Recherche Médicale Française.
[2] D. A. HUTCHINS and K. J. ROGERS, Br. J. Pharmac. *39*, 9 (1970).
[3] S. R. NAHORSKI and K. J. ROGERS, J. Neurochem. *23*, 579 (1974).
[4] J. J. SCHILDKRAUT and S. S. KETY, Science *156*, 21 (1967).
[5] S. R. NAHORSKI and K. J. ROGERS, J. Neurochem. *21*, 679 (1973).
[6] J. SCHEEL-KRÜGER and E. HASSELAGER, Psychopharmacologia *36*, 189 (1974).
[7] S. CACCIA, G. CECCHETTI, S. GARATTINI and A. JORI, Br. J. Pharmac. *49*, 400 (1973).
[8] A. HORITA and H. F. HILL, in *The Pharmacology of Thermoregulation* (Eds. E. SCHÖNBAUM and P. LOMAX; S. Karger, Basel 1973), p. 417.
[9] C. MATSUMOTO and W. GRIFFIN, J. Pharm. Pharmac. *23*, 710 (1971).
[10] H. F. HILL and A. HORITA, J. Pharm. Pharmac. *23*, 715 (1971).

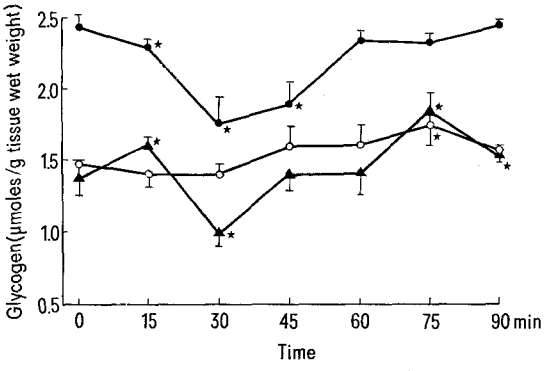

Fig. 1. Effect of D-amphetamine sulphate, 5 mg/kg, injected i.p., on the glycogen content of cerebral hemispheres (▲—▲), optic lobes (○—○) and cerebellum (●—●) of the 30-day-old chicken. Each point is the mean of 3 to 21 experiments ± S.E.M. The differences between control and experimental animals were assessed by *t*-test. * $P < 0.05$.

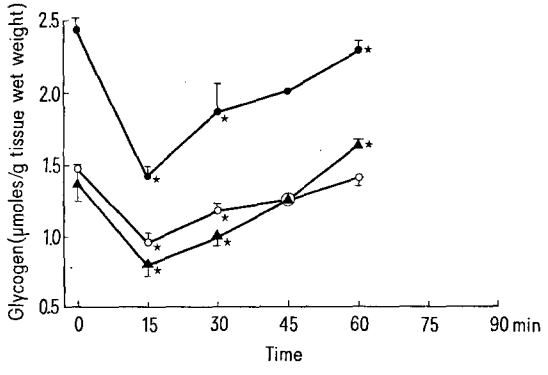

Fig. 2. Effect of D-amphetamine sulphate, 7.5 mg/kg, injected i.p., on the glycogen content of cerebral hemispheres (▲—▲), optic lobes (○—○) and cerebellum (●—●) of the 30-day-old chicken. Each point is the mean of 3 to 21 experiments ± S.E.M. * $P < 0.05$.

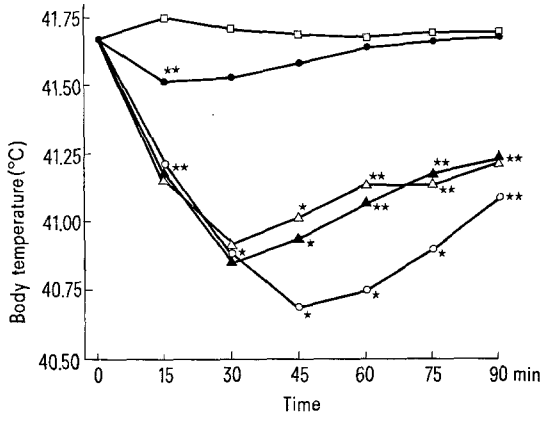

Fig. 3. Mean cloacal temperatures of 30-day-old chickens injected i.p. with D-amphetamine sulphate, 2.5 mg/kg (●—●), 5 mg/kg (△—△), 7.5 mg/kg (▲—▲) and 10 mg/kg (○—○) and with saline (□—□). Each point is the mean of 2 to 6 experiments. * $P < 0.05$; ** $P < 0.01$.

[11] C. EDWARDS and K. J. ROGERS, J. Neurochem. *19*, 2759 (1972).
[12] F. N. LE BARON, Biochem. J. *61*, 80 (1955).
[13] H. U. BERGMEYER and E. BERNT, in *Methods of Enzymatic Analysis* (Ed. H. U. BERGMEYER; Academic Press, New York 1965), p. 123.

in the central nervous system. Since the effect of amphetamine on the metabolism of glycogen in the avian brain was never investigated, we have performed a study on the glycogen content in the encephalon, and correlatively on the body temperature, of the adult chicken submitted to the drug.

Materials and methods. 30-day-old male and female chickens (*Gallus domesticus*) from a Plymouth × Rhode Island Red cross, weighing about 500 g, were used. The birds were allowed free access to food and water and were kept in groups in breeding cages, at an environmental temperature of 21 ± 1 °C and under diurnal light-dark cycle, until the start of the experiments. Body temperature was recorded, the chicken being placed in a restraining apparatus at an environmental temperature of 21 ± 1 °C, in a Gilson polygraph equipped with a S.E. 21 type modulus and a A-4062 type bridge by means of a thermistor (Yellow Springs Instruments Co., Yellow Springs, Ohio) inserted 5 cm into the cloacal cavity. D-amphetamine sulphate, dissolved in isotonic sodium chloride, was injected i.p. in a volume of 1.0 ml; control animals received 1.0 ml isotonic sodium chloride. For the experiments on the estimation of brain glycogen, the birds, which were kept in individual cages at an environmental temperature of 21 ± 1 °C, were sacrificed at regular time intervals between 15 and 90 min after the injection. All the experiments were made in the morning, between 09.00 and 11.00 h; this period, in the circadian rhythm, corresponds to a relatively stable concentration of glycogen in the normal adult mouse [2] and young chicken brain [11]. The birds were decapitated and their head was instantaneously immersed in liquid nitrogen; afterwards, it was kept at −30 °C and the cerebral hemispheres, optic lobes and cerebellum were dissected out and weighed whilst in the frozen state. Glycogen was isolated from each of the 3 encepahlic tissues by a modification of the method of Le BARON [12], the estimation of glucose being made by a glucose oxidase-peroxidase spectrophotometric method [13]. The concentration of glycogen was expressed in μmoles of anhydroglucosyl units per g wet weight of tissue.

Results. The concentration of glycogen in the cerebral hemispheres, optic lobes and cerebellum of the 30-day-old intact chicken is respectively 1.35 ± 0.10, 1.47 ± 0.03 and 2.44 ± 0.08 μmoles per g. 30 min after an injection of 2.5 mg/kg D-amphetamine, an increase occurs in the concentration of glycogen in the 3 encephalic tissues, the percentage of which is 37, 12 and 9 respectively in the cerebral hemispheres, optic lobes and cerebellum, and at 45 min the values are the same as those evaluated in the control animals. Figure 1 illustrates the evolution of the concentration of brain glycogen, after an i.p. injection of D-amphetamine, 5 mg/kg. In the cerebral hemispheres, after an initial increase of 18% at 15 min, the glycogen content decreases by 73% at 30 min and finally it increases by 36% at 75 min. In the optic lobes, the concentration of the polysaccharide remains practically constant during the first 30 min, then it increases by 19% at 70 min. In the cerebellum, the concentration of glycogen decreases regularly by 28% at 30 min, then it gradually rises to the value estimated in the control chickens. Another series of experiments was made with 7.5 mg/kg D-amphetamine (Figure 2). In the 3 encephalic tissues analyzed, a rapid fall occurs in the concentration of glycogen at 15 min after the injection of the drug, reaching 42% in the cerebral hemispheres and cerebellum and 36% in the optic lobes. In the cerebral hemispheres, this depletion in the concentration of glycogen is followed by its enhanced resynthesis, since at 60 min the concentration of the polysaccharide increases by 20%. After treatment with 10 mg/kg D-amphetamine, the fall in the concentration of

glycogen in the 3 encephalic parts reaches a value which is very close to that estimated with 7.5 mg/kg amphetamine; however, the subsequent resynthesis of glycogen is always faster.

The time course of the variation of body temperature of the chicken after administration of amphetamine is represented in Figure 3. D-amphetamine, 2.5 mg/kg, induces a slight hypothermia at 15 min. But by increasing the dose of the drug from 5 mg to 10 mg/kg, there occurs a gradually increasing hypothermia, the mean value of which does not exceed 1 °C at 45 min after the injection of amphetamine.

Discussion. In the 30-day-old chicken, the concentration of glycogen estimated in the cerebellum is about 81% greater than that estimated in the cerebral hemispheres or the optic lobes. Although the rate of depletion of the polysaccharide, after the administration of 5 mg/kg D-amphetamine is approximately the same in the cerebral hemispheres and the cerebellum, it does not significantly produce any change in the level of glycogen in the optic lobes. Since the depletion of glycogen induced by amphetamine in the mammalian brain would be mediated by the release of central catecholamines[5,14], it may be of interest to note that the highest amount of noradrenaline in the chicken encephalon was found in the optic lobes[15]. The relative stability of the store of glycogen in the optic lobes could be explained either by the low sensitivity of their catecholaminergic nerve endings towards amphetamine, or by the slow rate of uptake of released catecholamine transmitter by glial cells, thus affecting glycogenolysis in these cells[5]. But presumably, as was shown recently in the rat brain[16], the localization of amphetamine, and possibly its metabolites, in the various parts of the chicken encephalon differs. Consequently, if a threshold appears for the cerebral glycogenolytic effect of amphetamine, this threshold may reflect the heterogeneous distribution of the drug in the brain.

Amphetamine induces a hypothermic effect in the chicken organism. Such a hypothermic effect was also previously obtained in the male Swiss albino mouse after an i.p. injection of 1 to 5 mg/kg D-amphetamine, administration of a larger dose, as 10 mg/kg, resulted in a hyperthermia followed by a hypothermia[17]. At a low dose, amphetamine may induce a hypothermia by a direct action on the thermoregulatory structures in the anterior hypothalamus, whereas the hyperthermia recorded at a high dose of the drug could be possibly correlated with some peripheral events[17–19]. If in the male albino mouse, some relation seemed to exist between cerebral glycogenolysis and body hyperthermia, after an i.p. injection of 5 mg/kg D-amphetamine[2], we have demonstrated that in male and female chickens the drug administered at the same dose induced a cerebral glycolysis which was associated with body hypothermia. Our results are in accordance with the previous observations proving that the effect of amphetamine on body temperature is dependent not only on the species but also on the strains of the animals used in the experiments[20].

Résumé. L'injection i.p. de sulfate de D-amphétamine, à la dose de 2,5 à 10 mg/kg, chez le poulet âgé de 30 jours, est suivie d'une diminution de la concentration en glycogène dans l'encéphale; cette diminution est particulèrement importante dans les hémisphères cérébraux et le cervelet, mais elle l'est nettement moins dans les lobes optiques. La drogue provoque une hypothermie corporelle qui n'excède pas 1 °C.

T. K. HEVOR, P. R. LEHR and J. GAYET

Laboratoire de Physiologie Générale, Université de Nancy I, C.O. No 140, F–54037 Nancy (France),
27 January 1975.

[14] D. A. HUTCHINS and K. J. ROGERS, Br. J. Pharmac. *48*, 19 (1973).
[15] K. KOBAYASHI and S. EIDUSON, Devel. Psychobiol. *3*, 13 (1970).
[16] A. JORI and S. CACCIA, J. Pharm. Pharmac. *26*, 748 (1974).
[17] D. O. MCCULLOUGH, J. N. MILBERG and S. M. ROBINSON, Br. J. Pharmac. *40*, 219 (1970).
[18] G. L. GESSA, G. A. CLAY and B. B. BRODIE, Life Sci. *8*, 135 (1969).
[19] C. MATSUMOTO and W. N. SHAW, J. Pharm. Pharmac. *23*, 387 (1971).
[20] E. DOLFINI, S. GARATTINI and L. VALZELLI, Eur. J. Pharmac. *7*, 220 (1969).

Effect of Drug-Induced Increase of Brain GABA Levels on Penicillin Focus

The role of γ-aminobutyric acid (GABA) in epilepsy is still not clear[1]. One way to elucidate this problem is by closely correlating neurochemical analyses with electrophysiological studies. It was previously reported that GABA concentrations decreased significantly in the spiking penicillin focus during the inter-ictal stage[2]. However, no further decrease in GABA was observed with the transition from inter-ictal spiking to seizures. The purpose of this work was to study the inhibitory effect of GABA on penicillin-induced seizures. This was tested by elevating the endogenous concentrations of GABA in the brain by amino-oxyacetic acid (AOAA) and di-*n*-propylacetate (DPA), known to inhibit the enzyme α-keto-glutarate-GABA transaminase[3,4].

Methods. Cats of either sex weighing from 2.5 to 3.5 kg and Charles River rats were used. Technical details were described previously[2]. Cerveau isolé sections in cats or exposure of the cortex in rats were done under ether anaesthesia, but after infiltration of the pain areas with Novocain, ether was no longer administered. The animals were paralyzed with gallamine triethiodide and received artificial respiration. Before taking brain samples for analysis, dry ice was applied directly on the cortex and frozen samples were cut and homogenized in ice-cold 80% ethanol. GABA was determined after separation by paper chromatography and staining with ninhydrin.

Results and discussion. Effect of AOAA on penicillin focus. In the first series of experiments, an epileptic focus was produced by applying on the cerebral cortex a 1cm² cotton pledget imbibed in a solution containing 500,000 IU of Na-penicillin/ml. Electrocorticogram recordings were taken continuously from the vicinity of the penicillin area. 30 min after the appearance of the first penicillin spikes, the animals were administered i.p., through an implanted cannula, with 20 mg/kg of AOAA in saline. Seizures lasting a few seconds to 10 sec appeared 1 h later. Brain cortex samples from the focal area were taken for GABA determination at that time. The treatment with 20 mg/kg

[1] E. ROBERTS and K. KURIYAMA, Brain Res. *8*, 1 (1968).
[2] Z. GOTTESFELD and Z. ELAZAR, Nature, Lond. *240*, 478 (1972).
[3] D. P. WALLACH, Biochem. Pharmac. *5*, 323 (1961).
[4] Y. GODIN, L. HEINER, J. MARK and P. MANDEL, J. Neurochem. *16*, 869 (1969).