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.

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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

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Table I. Effect of AOAA on GABA concentrations in penicillin focus of cat cerebral cortex

Treatment		Duration of treatment (h)	GABA (μmoles/g)	P^b	P^c
Penicillin	AOAA (mg/kg)				
–	–	–	1.43 ± 0.07 (8) [a]		
–	20	1	2.02 ± 0.20 (5)	< 0.001	
+	20	1	1.12 ± 0.10 (3)	< 0.01	< 0.001
–	30	6	4.25 ± 0.38 (6)	< 0.001	
+	30	6	3.40 ± 0.10 (5)	< 0.001	< 0.01

[a] Mean ± SEM; number of animals in parentheses. [b] Significant difference compared to untreated controls; Student *t*-test. [c] Significant difference compared to AOAA-treated animals.

of AOAA resulted in elevated GABA concentration in cat cerebral cortex by 41% compared to control, whereas in the focus GABA was decreased (Table I). It appears that the decrease of GABA in the spiking focus[2] cannot be prevented by the treatment of AOAA.

A second series of experiments was designed to test whether a greatly increased GABA level, caused by 30 mg/kg of AOAA, would inhibit the appearance of seizures in the focus. Penicillin was applied 5 h after AOAA injection and inter-ictal spikes appeared, as usual, minutes later, followed by seizure activity 30 min thereafter (Figure 1). Thus, the increased GABA (Table I) did not prevent the development of seizure in the focus[5].

Doubts about relationship between the increased GABA level produced by AOAA and its anticonvulsant effect were expressed by other workers[1,6]. ROBERTS and KURIYAMA[1] pointed out that AOAA may have effects on other neuroactive substances with convulsive action. Ammonia, which has convulsant properties[8,9], is increased after AOAA treatment[7]. LUX et al.[9] found that i.v. administration of ammonium salts in dosages comparable to concentrations of ammonia found in the brain in convulsive states, produce considerable reduction of hyperpolarizing potentials. It was shown that AOAA in large dosages was convulsant[7,10], and we also observed epileptiform patterns in the EEG after AOAA (Figure 1A). These uncertainties prompted us to try the effect of another drug, di-*n*-propylacetate (DPA), also known to increase GABA content in the brain.

Effect of DPA on penicillin focus. DPA produces significant increases in GABA content of the cerebral cortex in rats (Table II and also ref. [4] and [11]). Penicillin was applied first on the cortex and when seizures apeared, a dose of 200 mg/kg DPA was injected i.p. (5 rats). The focus activity did not change for 2 h thereafter. On the other hand, a dose of 400 mg/kg of DPA had a weak anticonvulsant effect.

Seizures activity before (Figure 2, upper trace) and 60 min after (Figure 2, lower trace) DPA administration remained essentially unchanged and continued to be long-lasting. However, the amount of total seizure after DPA treatment was decreased. The total seizure activity is defined as the sum of high frequency discharges lasting more than 5 sec, counted during 30 min. Indeed, 30 min before DPA administration, total seizure activity (TSA) amounted to 750 sec, whereas after the treatment TSA it was 490 sec during the first 30 min and 475 after the following 30 min.

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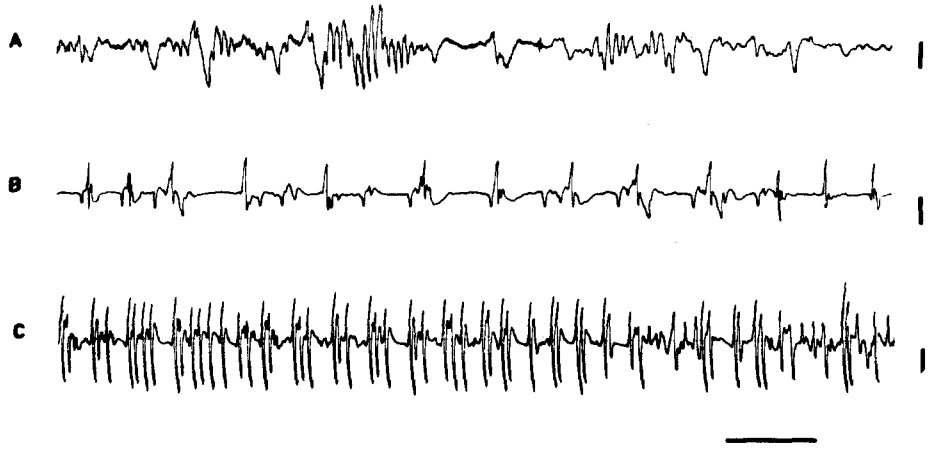

Fig. 1. EEG recording from a single cat. a) 3 h after injection of 20 mg/kg of AOAA. b) Appearance of inter-ictal spikes $5^1/_2$ h after AOAA and 30 min after penicillin application. c) Seizure activity 6 h after AOAA and 1 h after penicillin. Calibration: Vertical-500 μV; horizontal – 1 sec.

Table II. Effect of DPA on GABA concentrations in rat cerebral cortex

DPA (mg/kg)	Duration of treatment (min)	GABA (μmoles/g)	P^b
—	—	1.97 ± 0.12 (8) [a]	
200	40	2.53 ± 0.09 (8)	< 0.001
400	60	3.06 ± 0.22 (8)	< 0.001

[a] Mean ± SEM; number of animals in parentheses. [b] Significant difference compared to untreated controls; Student's *t*-test.

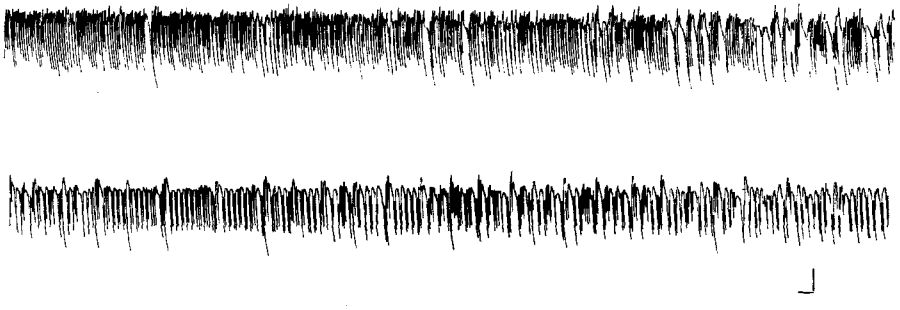

Fig. 2. EEG recording from the penicillin focus of a rat. Upper trace: Seizure, before DPA administration. Lower trace: Seizure, 60 min after the injection of DPA. Calibration: Vertical – 500 μV; horizontal – 1 sec.

It was found in rats that 200 mg/kg of DPA provided effective protection against convulsions induced by electroshock and pentylenetetrazole[12,13] and against audiogenic seizures[11]. In cats, DPA was effective against cobalt-induced focal seizures but was without effect against alumina gel focal seizures[14]. In human, DPA was found to be an effective anticonvulsant drug in several forms of epilepsy[15,16], but lack of effect was noticed in cases with focal sharp waves[17].

It appears that increased GABA after AOAA or DPA has no, or a very weak, antiepileptic effect on a penicillin spiking focus. However, it was reported that AOAA increased inhibitory responses to iontophoretically applied GABA and to synaptic inhibitory stimulation[5]. In the present work, when a solution containing 1% of GABA was applied directly onto the focus, it abolished seizure activity for some time, whereas drug-induced increase of endogenous GABA was not clearly effective. The possibility exists that GABA inhibitory mechanism is unable to overcome the excessive excitatory processes located in the focus under these conditions. Similarly, a strong excitatory process could be found in foci of certain patients in which DPA was ineffective. It was reported that in penicillin foci[18], and also in cerebral tissue involved in electrically produced after-discharge[19], neurons became less sensitive to iontophoretically applied GABA. Moreover, during electrical after-discharges many neurons responded to applied GABA by an increase in discharge[19]. Since neurons in the penicillin focus are strongly depolarized[18], a certain amount of hyperpolarization produced by GABA could not be sufficient to inhibit discharges. It is also possible that a certain amount of hyperpolarization could bring the neurons to a level below depolarization block but still be hyperexcitable. The effectiveness of GABA elevation by drugs depends on the intensity of the excitatory processes in an epileptic focus. Possibly, for an intense focus like the penicillin focus, much higher increase of GABA level is needed in order to get antiepileptic effects, but both AOAA and DPA are toxic at higher doses. The cobalt focus is probably less intense since DPA was effective[14]. Similarly in human patients, those forms of epilepsy in which DPA was found to be effective, are probably produced by less intense or more diffuse excitatory processes.

Résumé. L'acide amino-oxyacétique ou le di-*n*-propylacétate entraînent une élévation du taux de GABA dans le cerveau chez le rat et le chat. Nous avons étudié l'influence de ces substances sur les décharges épileptiques induites par la pénicilline. En dépit de l'élévation du taux de GABA, aucune protection importante n'a pu être mise en évidence.

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