17-hydroxycorticosteroids were determined by following the method of SILBER and PORTER[2] as modified by PETERSON[3]. After the extraction, the supernatant was saved for the determination of the conjugated forms. ½ cm³ of 2 *M* phosphate buffer pH 6·2 and 400 units of bacterial β-glucuronidase (Σ) were added. The re-extraction was performed after 48-h incubation at + 37°C.

Recovery of added hydrocortisone from human milk

Amount of added hydrocortisone (γ/5 cm³)	Recovery %
½	83
½	87
1	87
1	92
2	89
2	97

The suitability of the method for the estimation of 17-hydroxycorticosteroids from milk was checked by a recovery study of added hydrocortisone (Table).

Results. The values of the *free* 17-hydroxycorticosteroids before the administration of cortisone or hydrocortisone acetate ranged from 0 to 2·6 γ% in the eight cases studied. These concentrations are so low that the accuracy of the method used is no longer reliable. The single intramuscular injection of 100 mg hydrocortisone acetate produced a very slight elevation in the PORTER-SILBER reacting material. 6 h after the injection the values ranged from 2·2 to 4·7 γ%, and after 24 h from 1·5 to 3·0 γ%. The increase in the PORTER-SILBER reacting material after the injection of cortisone acetate was still smaller.

The level of the *conjugated* 17-hydroxycorticosteroids in the eight human milks studied ranged from 0·2 to 3·4 γ%. The single intramuscular injection of 100 mg cortisone or hydrocortisone acetate produced a slight elevation in the values of the conjugated 17-hydrocorticosteroids only in three instances in the samples drawn 6 h after the injection. All the values were near zero 24 h after the injections.

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Kivelä Hospital and The Womens Clinic of the University of Helsinki, July 8, 1956.

Zusammenfassung

Der Gehalt an freiem und gebundenem 17-Hydroxycorticosteroid wurde an 8 Muttermilchentnahmen nach einer Modifikation der Methode von SILBER und PORTER geprüft. Der erhaltene Wert war nichtsignifikant niedrig. Auf einmalige intramuskuläre Injektion von 100 mg Cortison oder Hydrocortison erfolgte kein signifikanter Anstieg des Steroidwertes.

[2] R. H. SILBER and C. C. PORTER, J. biol. Chem. *210*, 923 (1954).

[3] R. E. PETERSON, J. B. WYNGAARDEN, S. L. GUERRA, B. B. BRODIE, and J. J. BUNIM, J. clin. Invest. *34*, 1779 (1955).

The Effect of LSD-25 upon the Cerebral Blood Flow and EEG in Cats

The object of the present study was to determine whether LSD-25 (*d*-lysergic acid diethylamide[1]) has any effects upon the cerebral blood flow, the EEG and the blood pressure in anaesthetized and non-anaesthetized (*encéphale isolé*) cats. In recent experimental studies[2], a sympathomimetic action of LSD-25 in man and experimental animals has been reported. PURPURA[3] has analyzed the effects of LSD-25 upon cortical electrical activity. Recently, SOKOLOFF *et al.*[4] found no changes from LSD-25 in cerebral blood flow, vascular resistance, oxygen consumption, glucose utilization or respiratory quotient in man (normals and schizophrenics). The present experiments have been performed with the method of INGVAR and SÖDERBERG[5] which provides a continuous registration of the cerebral blood flow with a high degree of accuracy.

In cats under pentothal or of the *encéphale isolé*-type, the venous outflow from the cannulated superior sagittal sinus was continuously measured with an electrical drop counter. Anastomoses between diploic veins and the sinus were interrupted by a longitudinal craniotomy. The bony defect was filled with dental acrylate cement. EEG was recorded from rostral and parietal parts of the cortex.

Injected intravenously, LSD-25 in doses of up to 10 μg/kg had no effects upon the functions studied. In larger doses up to 100 μg/kg, however, a small transient cerebral vasoconstriction was observed which coincided with a transient 'activation' of the EEG. Sometimes a small cutaneous vasoconstriction was also noted[6].

Injections into the carotid artery of LSD-25 were also carried out in doses of up to 50 μg/kg. It was then observed that the EEG effects were very pronounced in the homolateral hemisphere. Figure 1 shows a successful example in which 100 μg of LSD-25 were injected into the right carotid artery (*encéphale isolé*-preparation). There was an immediate transient effect of the 'activation' type in both leads from the right hemisphere which, however, was soon followed by a general reduction of the amplitude, an increase in the numbers of spindles and – bilaterally – a generalized decrease in frequency. In about 4 min, the EEG changes had almost disappeared, but a right-sided reduction of the amplitude was still retained.

The flow record shows an immediate vasodilatation followed by a long period during which the flow was practically unchanged in spite of the fact that the blood pressure gradually increased about 30 mm Hg in 5 min. This is interpreted as due to a vasoconstriction in the cerebral vessels. A long-lasting increase of the cerebral vascular resistance was, in fact, the most characteristic effect of LSD-25 seen in the experiments.

In Figure 2 (*encéphale isolé*) another example is given of the effects of 100 μg LSD-25 into the left carotid

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[3] D. P. PURPURA, Arch. Neurol. Psychiat. *75*, 122 (1956); *75*, 132 (1956).

[4] L. SOKOLOFF, S. PERLIN, C. KORNETSKY, and S. S. KETY, Fed. Proc. *15*, 174 (1956).

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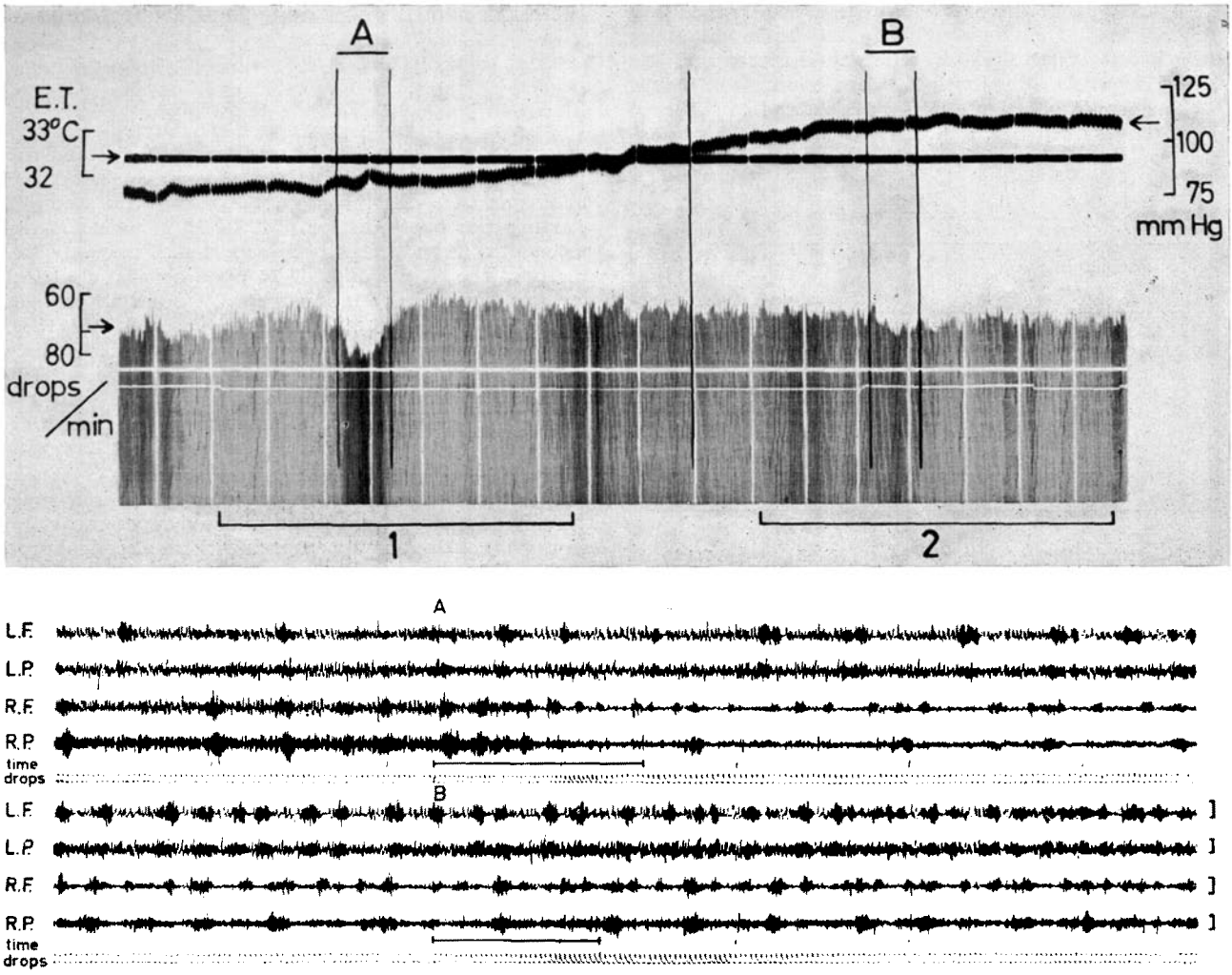

Fig. 1.—Cat, *encéphale isolé*-preparation. *Upper diagram*: Records of ear temperature, blood pressure and cerebral blood flow. Interruptions of diagram every 30 s. Height of vertical lines in flow record is proportional to time interval between blood drops from the cannulated superior sagittal sinus. Thus, a curve joining upper ends of the vertical lines demonstrates variations in cerebral blood flow. *Lower diagram:* Bipolar EEG leads on left frontal (*L.F.*), left parietal (*L.P.*), right frontal (*R.F.*) and right parietal (*R.P.*) parts of the cortex. EEG samples from periods marked 1 and 2 in upper diagram. *A* Injection into right carotid artery of 100 μg of LSD-25 in 1 ml solution. Note initial transient vasodilatation followed by a period with increasing blood pressure with almost constant cerebral blood flow, indicating vasoconstriction. EEG records demonstrate initial unilateral effects with depression. In sample 2 bilateral EEG effects with increase in number of spindles. *B:* Control injection into carotid artery of 1 ml Ringer solution.

artery. There was a marked ipsilateral activation period in the EEG while the spindle pattern of the opposite hemisphere remained almost unifluenced. In this case there were no significant changes in the cerebral blood flow.

In deeply anaesthetized preparations LSD-25 did not change the EEG or the cerebral blood flow even in doses of up to 100 μg/kg given intravenously or into the carotid artery.

The present results have confirmed the findings of SOKOLOFF *et al.*[4] that doses comparable to those with clinical effects (4 μg/kg body weight) have no observable effects upon the EEG. Larger doses were, however, found in the present investigation to exert an evident effect upon the cerebral blood flow as well as upon the EEG and systemic circulation. From the results obtained after injection into the carotid artery, it may be reasonable to assume that an important part of the action of LSD-25 is exerted directly upon the cortex, since a brain stem action would have given bilateral effects[7]. MORUZZI and MAGOUN[8], however, observed that unilateral electrical stimulation of the reticular activating system of the brain stem may sometimes give desynchronization in the homolateral hemisphere only.

The EEG effects found are in general agreement with the electrophysiological findings of PURPURA[3], who demonstrated a facilitatory action of smaller doses and a depressant action of larger doses upon certain central synaptic events.

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Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm, Sweden, August 13, 1956.

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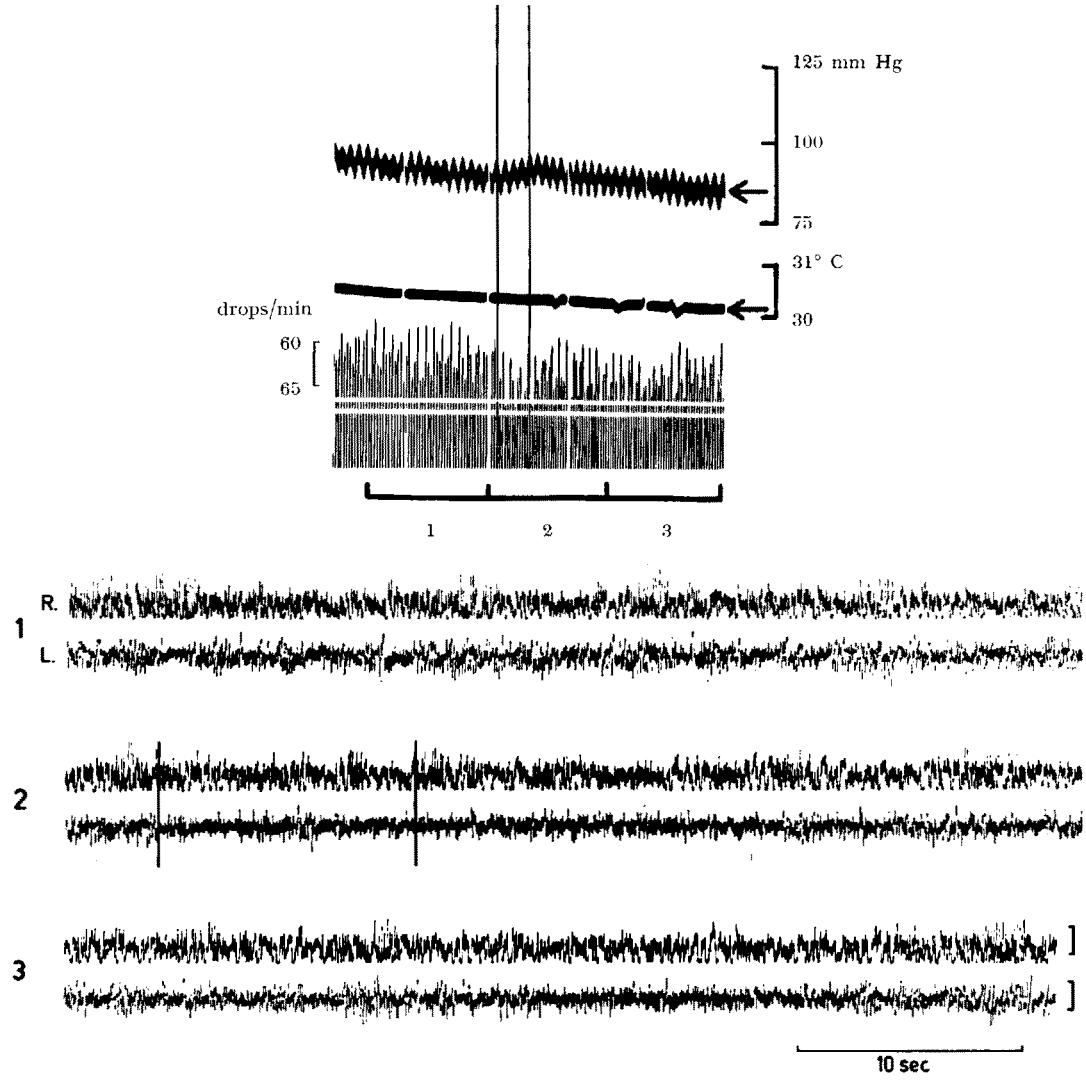

Fig. 2. – Cat, *encéphale isolé* preparation. *Upper diagram:* Blood pressure, ear skin temperature and cerebral blood flow (*Cf.* Fig. 1). *Lower diagram:* EEG tracings from right (*R*) and left (*L*) cruciate region during periods marked 1 to 3 below upper diagram.

Between two vertical lines injection of 40 µg/kg of LSD-25 into the left carotid artery. Note activation pattern in EEG-record from left hemisphere.

Zusammenfassung

Es wurde gezeigt, dass LSD-25 (*d*-Lysergsäure-diäthylamid) in Dosen von 30 bis 100 γ/kg Körpergewicht bei der nichtnarkotisierten Katze (*encéphale isolé*) meistens eine Erhöhung des zerebralen Gefässwiderstandes herbeiführte. In die Carotis injiziert, gab LSD-25 in denselben Dosen nach vorübergehender, halbseitiger Aktivierung eine Depression des Elektroenzephalogrammes. Kleinere Dosen gaben im EEG eine Aktivierung oder waren ohne Wirkung.

Relationship Between Methionine and Aromatic Amino Acids in *Escherichia Coli*

It was previously reported from this laboratory[1] that the inhibition of *Escherichia coli* by chloromycetin, aureomycin, terramycin and 5-fluorotryptophan, could be alleviated, within limits, by methionine, and by tryptophan, phenylalanine and tyrosine. This appeared to indicate that there is a relationship between the aromatic amino acids and methionine. To confirm this relationship, the problem was approached from the opposite angle, i.e., to see whether the aromatic amino acids play a role in the metabolism of methionine.

On the basis of extensive work carried out in recent years, it has generally been considered that the methionine analogues, ethionine, methionine sulfone, methionine sulfoximine and methoxinine are specific antagonists of methionine metabolism both in bacteria and animals (for a general review see [2]).

The present work with *E. coli* shows, however, that the inhibition caused by the antimetabolites of methionine can be reversed not only by methionine, but by tryptophan, phenylalanine and somewhat by tyrosine

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[2] G. J. Martin, *Biological Antagonism* (The Blakiston Co. Inc., New York 1951), p. 118.