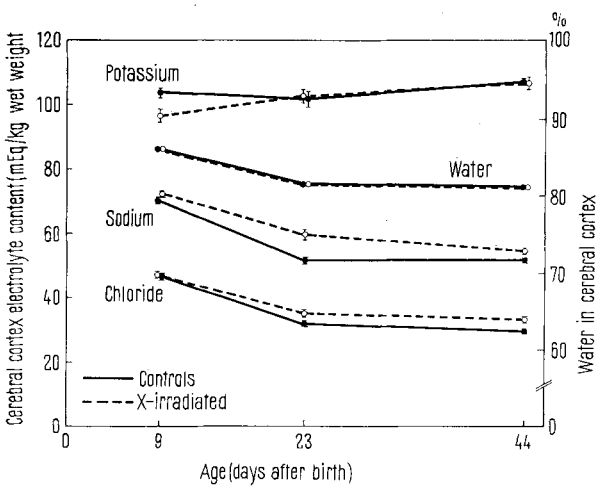
brain maturation the volume of the glial compartment increases, and those of the interstitial and neuronal compartments decrease. According to these authors, the increase in the glial compartment and decrease in interstitial compartment account for the decrease in Na and Cl, since the concentrations of these ions are lower in the glial than in the interstitial compartments. The greater decrease in these ions in the irradiated groups suggests a further increase in the glial compartment induced by prenatal X-radiation. Further evidence for an increase in glial cells after prenatal X-radiation has been reported by HICKS and D'AMATO[12]. These authors found a marked increase in glial cells in the outer parts of the cortex of animals irradiated at 18 days of gestation with 30 r.

Changes induced by prenatal X-radiation in the electrolyte distribution may lead to an altered extraneuronal environment which together with cytoarchitectural alterations[12] could influence the functional development of the CNS.



Changes in water and Na, K, and Cl content of the cerebral cortex in offspring of rats exposed to 100 r whole-body X-radiation at 14 days of gestation. Points with bracketed lines represent means and standard errors (standard errors for water values are less than 0.1% and are not shown in the Figure). *Abscissa* is age in days after birth, *left-hand ordinate* is electrolyte content (mEq/kg wet weight) and *right-hand ordinate* is % water.

Zusammenfassung. Bei in utero röntgenbestrahlten Rattenembryonen wurden die Elektrolyte im Gehirncortex bestimmt. Während der K-Gehalt unverändert blieb, war derjenige von Na und Cl bei 9 Tage alten Tieren erniedrigt. Zytologische Veränderungen, durch pränatale Bestrahlung verursacht, waren mit diesen Befunden korreliert.

THEONY VALCANA, ANTONIA VERNADAKIS, and PAOLA S. TIMIRAS

Department of Physiology, University of California, Berkeley (California, USA), April 18, 1966.

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On the Origin of Homovanillic Acid in the Cerebrospinal Fluid

In the cerebrospinal fluid of man, homovanillic acid (HVA; 3-methoxy-4-hydroxyphenylacetic acid) occurs normally[1] and appears also after i.v. injection of ^{14}C-dihydroxyphenylalanine (DOPA)[2]. The origin of the acid in the cerebrospinal fluid is not clear. A possible source may be the blood which probably takes up HVA formed in the extracerebral tissues like liver, heart, etc. HVA may, however, also originate in the central nervous system which is known to transform DOPA into dopamine and subsequently into acidic metabolites. Therefore, the concentration of HVA in the cerebrospinal fluid might be an indicator of the metabolic activity of the central nervous system.

In order to clarify the origin of HVA in the cerebrospinal fluid, cats were administered either L-^{14}C-DOPA or ^{3}H-HVA and the radioactivity of the HVA fraction was compared in blood, cerebrospinal fluid and brain.

Experimental. In cats of 1.8–2.5 kg, fasted for 16 h and anaesthetized with Nembutal, L-^{14}C-DOPA[3] (labelled in 2-position of the side chain; specific activity 103.5 μC/mg) or ^{3}H-HVA[3] (labelled in 5-position of the ring; specific activity 284 μC/mg) were administered i.v. At various time intervals thereafter, arterial blood was withdrawn and supplemented with 1/10 vol versene (5%). At the end of each experiment, cerebrospinal fluid was obtained by occipital puncture. Plasma and cerebrospinal fluid were deproteinized by addition of equal volumes of perchloric acid, 7 and 3.5% respectively. The total brains were homogenized in 7% perchloric acid.

The determination of the unconjugated HVA fraction was carried out according to a previously described procedure[4]. Thereby, the supernatants of plasma, cerebrospinal fluid and brain homogenates were passed through columns of Dowex 50 · 4 in order to remove the amines and the amino acids. The effluent containing the phenolcarboxylic acids was adjusted to pH 1.5, saturated with NaCl and extracted 5 times with a double volume of peroxide-free ethylether. After evaporation of the ether (previously dried with Na_2SO_4), the residue was dissolved in 0.75 cm^3 methanol-water (3:1) and submitted to paper chromatography (Whatman No. 1; solvent system: propionic acid-benzene-H_2O, 2:2:1). The radioactivity of the HVA spot was measured with a Packard radiochromatogram scanner and expressed in % of the values in the plasma after 10 min (=100%). Recoveries of ^{3}H-HVA carried through the whole procedure were about 40–50%.

Results. (1) The radioactivity of the HVA fraction of the plasma which appears subsequent to i.v. injection of ^{14}C-DOPA progressively declines between 10 and 120 min,

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whereas the ^{14}C-HVA of the cerebrospinal fluid continuously increases during this time period. After 120 min, the ^{14}C-HVA of plasma and of cerebrospinal fluid amounts to 30 and 65% respectively of the ^{14}C-HVA present in plasma at 10 min (Figure 1).

(2) The ^{3}H-HVA fraction in the plasma following injection of ^{3}H-HVA decreases more rapidly, i.e. to levels as low as about 5% after 120 min. The radioactivity of the ^{3}H-HVA fraction in the cerebrospinal fluid reaches only low levels, attaining a maximum of about 10% after 10 min. Subsequently, the ^{3}H-HVA slowly declines to about 6% after 120 min (Figure 1).

(3) The radioactivity of the HVA fraction of the brain is about 15–50 times higher after L-^{14}C-DOPA than after ^{3}H-HVA. No significant differences can be seen at 30, 60 and 120 min (Figure 1).

(4) During a continuous infusion of ^{3}H-HVA, the ^{3}H-HVA fraction of the plasma rapidly rises and reaches values as high as about 150%, which persist for between 30 and 60 min. The ^{3}H-HVA in the cerebrospinal fluid and the brain increases only slowly, attaining maximal values of 20 and 5% respectively after 60 min (Figure 2).

Discussion. The above experiments show that the increase of radioactive HVA in the cerebrospinal fluid of cats (as compared to the plasma values at 10 min) is considerably higher after i.v. injection of L-^{14}C-DOPA than after ^{3}H-HVA (maximal values of 65% versus 10%). This difference might be due to differences in the blood levels of radioactive HVA, since the decrease of this acid is slower after L-^{14}C-DOPA than after ^{3}H-HVA. Experiments with continuous HVA infusion show, however, that even with persistent relatively high plasma content of the acid, only minor amounts of ^{3}H-HVA (maximally 20%) are to be found in the cerebrospinal fluid. The differences of the HVA content of the cerebrospinal fluid after i.v. injection of L-^{14}C-DOPA and ^{3}H-HVA are therefore probably not only due to differences in the plasma levels of the acid. In consequence, it may be assumed that at least part of the HVA appearing in the cerebrospinal fluid after L-^{14}C-DOPA is not derived as such from the blood. It might therefore originate in the brain, since in this organ the increase of the radioactive HVA is much more marked after i.v. L-^{14}C-DOPA than after ^{3}H-HVA.

No indications regarding the cerebral sites in which ^{14}C-HVA is formed can be obtained from the present results. According to some histochemical findings in mice and rats, DOPA hardly penetrates the cerebral capillaries and might be metabolized within their endothelial cells[5]. In other histochemical investigations with mice, catechol derivatives were, however, demonstrated to accumulate in neurones of various brain areas after injection of DOPA[6]. Furthermore, biochemical experiments with large as well as with small doses of DOPA showed that in rats and rabbits the amino acid is preferentially metabolized in brain areas known to be rich in noradrenergic and dopaminergic structures, e.g. the hypothalamus and the caudate nucleus. Other brain parts, e.g. the cerebellum and the cerebrum, transform only little DOPA into dopamine and phenolcarboxylic acids[7,8]. Since the capillaries show the same histochemical behaviour in all the brain areas[5], at least part of the administered DOPA is probably metabolized in neuronal structures. It cannot be excluded that some DOPA might also undergo metabolic transformation in still other structures, e.g. the chorioid plexus.

In conclusion, the concentration of ^{14}C-HVA after L-^{14}C-DOPA and possibly also of the endogenous HVA in the cerebrospinal fluid might, at least in part, reflect a metabolic process which occurs in the brain.

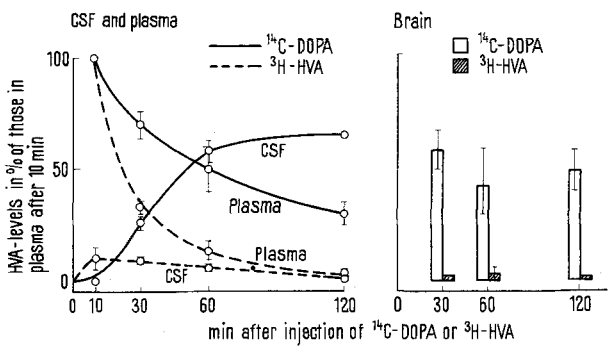

Fig. 1. Levels of radioactive homovanillic acid (HVA) per cm^3 cerebrospinal fluid (CSF), per cm^3 plasma and per g brain of cats after i.v. injection of 75 μC/kg L-^{14}C-DOPA or 500 μC/kg ^{3}H-HVA. Each value represents an average of 2–4 experiments $\pm$ S.E. The values are expressed in % of the levels of radioactive HVA found per cm^3 of plasma after 10 min.

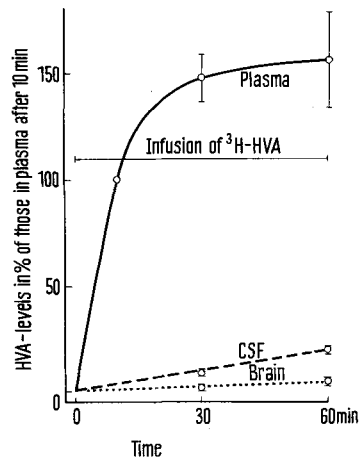

Fig. 2. Levels of ^{3}H-homovanillic acid (^{3}H-HVA) during constant i.v. infusion of ^{3}H-HVA 5.4 or 8.9 μC/kg/min. The values represent an average of 2–4 experiments $\pm$ S.E. and are expressed in % of the levels of ^{3}H-HVA found per cm^3 of plasma after 10 min.

Zusammenfassung. In Occipitalliquor und Gehirn von Katzen ist der Gehalt an radioaktiver Homovanillinsäure (HVS) bezogen auf Plasma nach i.v. Applikation von L-^{14}C-DOPA erheblich höher als nach i.v. ^{3}H-HVS. ^{14}C-HVS, welche nach L-^{14}C-DOPA im Liquor erscheint, entsteht deshalb wahrscheinlich mindestens zum Teil im Gehirn.

G. BARTHOLINI, A. PLETSCHER, and R. TISSOT[9]

Medizinische Forschungsabteilung der F. Hoffmann-La Roche & Co. AG, Basel (Switzerland),
June 13, 1966.

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