The Chemoarchitectonics in the Forebrain of Reptiles

It is obvious from many paleontological surveys that mammals are descended from reptiles, though their relationship to the recent reptiles is exceedingly remote. In other words, the reptilian order is closest to the mammals. Moreover, it has been proved by comparative neuromorphology that the forebrain of reptiles reveals in a more schematic form the basic division of the forebrain, which is much more complicated in mammals, owing to the secondary features appearing at a higher stage of development. Several histochemical studies have recently been carried out concerning the distribution of some enzymes in the forebrain of mammals. The present authors attempted to study the distribution of monoamine oxidase and succinic dehydrogenase in the reptilian forebrain, in order to help to understand the chemoarchitectonics of the highly differentiated forebrain of mammals. No histochemical studies of these enzymes in the reptilian forebrain have yet been published.

The animals used were *Caretta caretta* and *Geoclemmys reevesi*, collected in September, which are among the more primitive reptiles, that is, an early side branch of the stem reptiles. The fresh and unfixed brains were cut into serial frontal sections of 30 μ by means of a cryostat. The Glenner method was utilized for demonstrating monoamine oxidase, and the Nachlas method was applied to show succinic dehydrogenase.

In both turtles, the monoamine oxidase activity (Figures 1 and 2) is relatively strongly positive in the hippocampus, septal part, preoptic area, hypothalamus, habenula, nucleus reuniens and archistriatum, compared with other parts of the forebrain. On the other hand, the succinic dehydrogenase reaction (Figures 3 and 4) is relatively marked in the neostriatum, paleostriatum and nucleus rotundus of the dorsal thalamus. The succinic dehydrogenase activity is, in general, weak in the areas which are strongly positive for the monoamine oxidase, excepting the hippocampus which shows relatively intense reaction for these both enzymes.

From these results, the monoamine oxidase activity is intensely recognizable in the hippocampus and the septal part which occupy the dorsomedial towards the medial wall of the telencephalon. As for the striatum, the archistriatum homologous to the amygdaloid body of mammals is relatively strongly positive in the monoamine oxidase reaction, in comparison with the neostriatum and paleostriatum. It is noted that these monoamine oxidase intense-positive portions correspond to the limbic system of mammals. In the divisions from the telencephalon medium to the diencephalon, the preoptic area, hypothalamus, nucleus reuniens and habenula show more intense activity of monoamine oxidase than other portions of the diencephalon. From the fibre anatomy[1], the

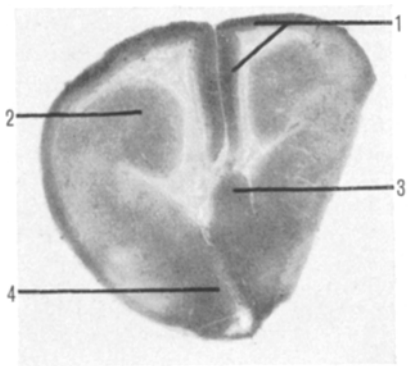

Fig. 1

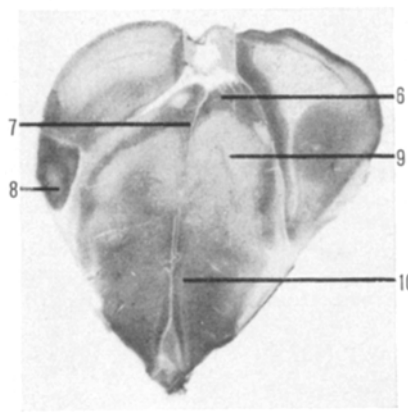

Fig. 2

Fig. 1 and 2. Transverse sections of the brain of *Caretta* stained with monoamine oxidase reaction (× 4). 1 = hippocampus; 2 = neostriatum; 3 = septum; 4 = preoptic area; 5 = paleostriatum; 6 = habenula; 7 = nucleus reuniens; 8 = archistriatum; 9 = nucleus rotundus; 10 = hypothalamus.

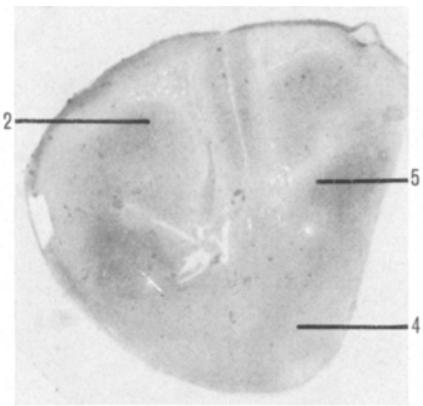

Fig. 3

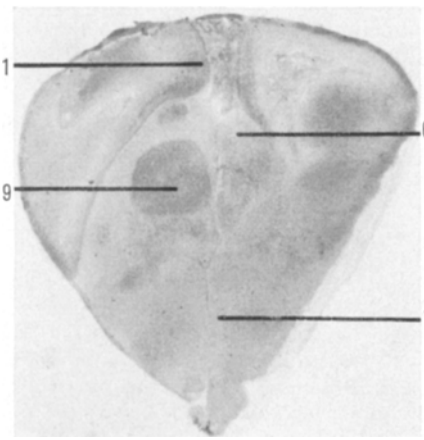

Fig. 4

Fig. 3 and 4. Transverse sections of the brain of *Caretta* stained with succinic dehydrogenase reaction (× 4.2).

[1] C. U. Ariëns Kappers, G. C. Huber, and E. C. Crosby, *The Comparative Anatomy of the Nervous System of Vertebrates, Including Man* (The Macmillan Company, New York 1936).

preoptic area and the hypothalamus are related to the limbic system of the telencephalon by the medial forebrain bundle and the stria terminalis, and the habenula is connected with the limbic system by the stria medullaris. The area in which the monoamine oxidase activity is strongly positive shows a weak reaction of succinic dehydrogenase participating in aerobic respiration.

This is similar to the results obtained in chick embryo[2] by the present authors in that the activity of monoamine oxidase metabolizing the chemical transmitters as catecholamines and serotonin is intensely revealed in the limbic system, preoptic area and hypothalamus, which play an important role in the visceral functions of the reptilian brain. The present data can be used to explain clearly the distribution of monoamine oxidase in the mammalian forebrain[3].

Zusammenfassung. Ausgehend vom Vorderhirn der Reptilien als schematischer Typus für Säugerverhältnisse, wurden histochemische Untersuchungen von Monoaminoxydase und Succinodehydrogenase im Vorderhirn der Schildkröten ausgeführt. Die Monoaminoxydasereaktion ist in den visceralen Abteilungen, dem limbischen System, Area preoptica und Hypothalamus stark, während die Succinodehydrogenasereaktion im Neostriatum, Paleostriatum und Nucleus rotundus des Thalamus dorsalis stärker zu sein scheint. Die Befunde lassen die Verteilungen der Enzymaktivität im kompliziert gebauten Vorderhirn der Säuger befriedigend erklären.

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Department of Anatomy, Yokohama City University School of Medicine, Yokohama (Japan),
April 29, 1966.

[2] H. MASAI, T. KUSUNOKI, and H. ISHIBASHI, Experientia *21*, 572 (1965).
[3] P. H. HASHIMOTO et al., Med. J. Osaka Univ. *12*, 425 (1962).

Incorporation of a Metabolically Inert Amino Acid into the Central Nervous System

As part of a more general study of the distribution of various amino acids between plasma, cerebrospinal fluid and nervous parenchyma, we have employed α-amino isobutyric acid (AIBA) as representative of an inert amino acid which is not metabolized by the nervous tissue. In this way, other metabolic factors which might modify the basic mechanisms of transport of amino acids were eliminated.

Studies related specifically to AIBA in its relationship between blood and nervous system in vivo, are those of KUTTNER et al.[1] and GORDON et al.[2], who postulated the existence of active transport mechanisms capable of concentrating the amino acid in the brain up to values twice its plasmatic level in a lapse of 20 h. This process is more restricted than for liver or kidney. In these studies a single injection of the amino acid was given, and tissue concentration was determined simultaneously with blood levels at different intervals. The tissue concentration of an injected substance is the result of complex processes such as rates of influx, local metabolism and efflux. In the case of AIBA, the important metabolic factor is eliminated as a cause of variation, but there still remain those concerned with the entrance and output of the compound. In the brain, the existence of the blood-brain barrier, which also appears operative for this amino acid[1], is another factor which influences the kinetics of passage in both directions (entrance and efflux). In such conditions, the blood values could decay at a rate different to that in the tissues, consequently the kinetics of penetration and the resultant tissue concentration could be better appreciated if steady levels in the plasma compartment were maintained throughout the experimental period. This approach has been used by REDDY and KINSEY[3] to study AIBA transport from blood to ocular fluids and lens, and it was also followed in the present work.

Materials and methods. Unanaesthetized adult rabbits, 2–3 kg in weight, were used throughout. The AIBA solution was infused into the ear vein during 120 min by means of an infusion pump with adjustable speed. The rate of infusion was adjusted after preliminary trials to maintain the amino acid plasma at a constant level. Blood samples were taken from the vein of the other ear or occasionally from the heart after 6, 15, 30, 60, 90 and 120 min. Experiments in which any sample showed more than 20% deviation over the mean values of the series were discarded. Both kidneys were removed in 2 previously anaesthetized animals, and then a single intravenous AIBA injection was given. In this way it was possible to maintain steady levels of AIBA in the blood for longer periods. After 25 h the rabbits were killed and the AIBA of the tissues under study was determined.

1-^{14}C-α-amino isobutyric acid, 4.6 mc/m*M* (Calbiochem) at a concentration of 1 μc/ml was used. On occasion ^{12}C-AIBA was added as carrier. The AIBA determinations were carried out in plasma, cerebrospinal fluid (CSF), aqueous and vitreous humor, brain, spinal cord, and muscle. At the end of the infusion period, the animal was anaesthetized and CSF was collected by cisternal puncture. Aqueous humor was taken by puncture of both eyeballs; then the eyes were enucleated to obtain the vitreous humour[4]. The rabbit was decapitated and the brain from the tentorium cerebelli frontwards was removed. The tissue was conveniently disintegrated in a Potter-Elvehjem homogenizer with a mixture of Triton X-100 and toluene (1:2). Spinal cord and muscle were subjected to the same treatment and with the biological fluids were prepared for scintillation counting in a Tri-Carb liquid scintillation spectrometer.

[1] R. KUTTNER, J. A. SIMS, and M. W. GORDON, J. Neurochem. *6*, 311 (1961).
[2] M. W. GORDON, J. A. SIMS, R. K. HANSON, and R. E. KUTTNER, J. Neurochem. *9*, 477 (1962).
[3] D. V. N. REDDY and V. E. KINSEY, Invest. Ophthal. *1*, 41 (1962).
[4] L. BITO and H. DAVSON, Expl Eye Res. *3*, 283 (1964).