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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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.

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Results. In Table I it can be seen that there is a restriction for the incorporation of AIBA in the nervous tissue, thus confirming a barrier effect between blood and the central nervous system. The values for muscle are 3 to 4 times higher than those for brain, which could give a quantitative appreciation of the blood-brain barrier if the muscle is taken as representative of the extra neural tissue interrelations for the amino acid studied. The values for CSF are lower than those for the nervous parenchyma, which means that the blood-CSF barrier is stronger for AIBA than the blood-brain tissue barrier. The CSF presents some similarities with the aqueous humor, especially in relation to its production and drainage. The comparison of the penetration of AIBA into both fluids shows that there is a restriction for the passage into the CSF in relation to the ocular fluid. The values obtained for the ocular fluids are in accordance with those of REDDY and KINSEY[3]. In 2 nephrectomized animals there is a drop in the blood values of the amino acid, but after approximately 5 h the decrease is much slower and the mean blood value from 5 h on can be taken as the steady concentration level (± 15%) during the last 20 h of the experiment. 25 h after the injection, AIBA attains equilibrium in the brain in relation to blood values in one animal, while in the other a low concentrative process is apparent. The barrier phenomenon in brain is evident in comparison with muscle, in which the process of concentration reaches a value of 2.4 for the muscle/plasma ratio in the 25 h period. For aqueous and vitreous humor the values are in accordance with those of REDDY and KINSEY[3] for 24 h.

Discussion. Our results show that in the central nervous system there exists a restriction to the passage of AIBA from blood when compared with muscle and for CSF in relation to aqueous humor.

In one experiment, the rate of disappearance of AIBA from blood after a single i.v. injection was measured. After 120 min the blood values were 20% of the initial concentration. The brain and spinal cord were removed from this animal to determine the AIBA tissue levels in this condition. The tissue/plasma · 100 ratio, calculated with the plasma value at 120 min, gives a value of 20.0 (brain/plasma · 100 = 21.7; spinal cord/plasma · 100 = 18.4). This value is approximately twice that obtained when steady levels are maintained in blood. Using only the 120 min blood level, the ratio is similar to that pre-

Table I. Tissue/plasma · 100 and fluid/plasma · 100 ratios for α-amino isobutyric acid (AIBA) after 120 min of steady blood offer

Brain	Spinal cord	Cerebrospinal fluid	Muscle	Aqueous humor	Vitreous humor	
					Anterior	Posterior
14.4	12.4	7.7	–	45.1	6.0	5.4
10.3	7.4	3.7	36.3	41.1	–	–
9.7[a]	11.1	3.5	40.8	56.7	–	–
10.6[a]	6.5	3.7	45.0	–	5.8	2.7
12.0	–	6.0	53.6	39.5	4.8	2.0
14.4[a]	16.0	9.0	28.8	30.4	5.2	2.8
11.9 ± 1.0[b]	10.7 ± 1.9	5.6 ± 1.0	40.9 ± 4.6	42.6 ± 4.8	5.4 ± 0.1	3.2 ± 0.9

The values refer to the water volume of each compartment: brain 78%; spinal cord 66%[5]; cerebrospinal and ocular fluids 99%[6]; plasma 94%[7]; muscle 75% (average of 6 determinations). Each horizontal line corresponds to one animal. [a] In these experiments 1-^{14}C-AIBA was infused with ^{12}C AIBA 1 *M* as carrier, obtaining an AIBA plasmatic level between 10 and 15 m*M* throughout the experimental period. No saturation effect was observed with respect to the values without carrier. [b] Mean ± standard error.

Table II. Radioactivity and tissue/plasma · 100 ratios of AIBA after 25 h of its injection in 2 nephrectomized rabbits[a]

	Plasma		Brain	Spinal cord	CSF	Muscle	Aqueous humor	Vitreous humor	
								Anterior	Posterior
Rabbit No. 1	5 min	12.5							
	6 h	4.0							
	19 h	3.7 (3.7)	4.5	3.5	0.7	9.2	1.5	0.6	0.5
	25 h	3.5							
	tissue/plasma · 100		121.6 (108.0)	94.4	18.9	248.6	40.5	16.2	13.5
Rabbit No. 2	5 min	15.5							
	5 h	5.9							
	9 h	5.1 (5.1)	7.9	7.6	1.3	12.0	3.6	1.5	1.4
	19 h	4.8							
	25 h	4.7							
	tissue/plasma · 100		154.9 (151.5)	149.0	25.5	235.3	70.6	29.4	27.4

[a] Values represent radioactivity in counts · 10^3/min/ml referred to the water content of each tissue. Values in parenthesis are means of the data of the respective column.

sented by KUTTNER et al.[1]. It is possible that the kinetics of efflux of AIBA from the central nervous system is slower than its entrance, in which case it could give the impression of a concentrative process when the plasmatic levels are falling. This type of effect can also be appreciated for thiocyanate[10]. After single intraperitoneal injection of AIBA it was shown that the plasma levels decrease rapidly[8,9].

The AIBA levels in the CSF are lower than in the aqueous humor, indicating a stronger barrier effect for the blood-CSF penetration than for that of the blood-aqueous. In the eye, the barrier effect is obscured by the concentrative process of AIBA by the lens[3-11], and it is possible that the aqueous levels of AIBA are a reflection of the lens-humor relations superimposed on the plasma-humor equilibrium[12].

Résumé. Le passage de l'acide α-amino isobutirique (AIBA) du sang vers le système nerveux central a été étudié en situation constante des niveaux sanguins. Un phénomène de barrière hémato-nerveux est rendu évident par rapport aux autres tissus. On n'a pas observé d'accumulation de l'acide aminé au-dessus des niveaux sanguins après une période de 25 h.

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Investigations on the Effects of Chronically Administered Small Amounts of DDT in Mice

Owing to the relative chemical stability and cumulative property of DDT (*p,p'*-dichlor-diphenyl-trichlorethane) the question permanently arises as to whether the long-term presence of DDT in the organism is injurious to health or not. In connection with this question, the Expert Committee of FAO-WHO[1], among others, found the study of the possible blastomogenic effect of DDT to be of great importance.

Our investigations on the harmful effects of DDT were started in 1963. The plan of work was to give orally small amounts of DDT to animals of a genetically isogenous species for several generations.

Experimental. From the Institute's inbred BALB/c mice strain, 15 bigamous families were selected as P generation. These families were kept together for 6 months. From the descendants of each P generation again 15 bigamous families were selected as F_1 generation. The breeding of further generations was carried out similarly. The animals selected during the 6 month breeding periods were kept isolated according to sex and generation. From July 1963 till August 1965, the breeding of 5 mouse generations had been accomplished. Until this time the experiments were performed with a total of 3766 mice (1797 experimental animals, Group 1; and 1979 controls, Group 2). In September 1965 we isolated 1089 mice: 683 animals originated from Group 1, and 406 animals from Group 2. In both groups, males and females of the 5 generations were represented approximately in the same ratio.

Both mouse groups received the same feed and tap-water ad libitum. The chow of the 2 groups differed only in the DDT content. To that of Group 1, 2.8–3.0 ppm DDT was mixed homogeneously, whereas to that of Group 2, no DDT was added. In the diet of Group 2, DDT was found in amounts of 0.2–0.4 ppm as unavoidable contamination, since at present the natural components of feed contain DDT traces all over the world. Thus the animals of Group 1 consumed on the average 0.3–0.6 mg of DDT/kg body weight (the equivalent of $^1/_{500}$ to $^1/_{1000}$ of the oral LD_{50}), and those of Group 2 0.03–0.05 mg.

The DDT content of the diet was regularly supervised by the method of SCHECHTER and HALLER[2] and that of KOVÁCS[3]. In the organs of the mice, DDT determination was performed by the same methods.

For the determination of the erythrocyte and leucocyte count and the hemoglobin content of the blood, for the preparation of the peripheral blood picture, blood was taken from the tail vein of the animals. The bone marrows were obtained from the femurs. All animals were subjected to biopsy.

For supervision of the isologous homogeneity of our BALB/c strain, the method of skin grafting was used.

Results. During the experiment, started in October 1965, in several animals of the experimental group hematological disorders resembling leukemia were observed. Later on, malignant tumours of different localization and structure also appeared.

Table I shows that in the experimental group of 684 mice, 24 animals (3.51%) developed leukemia and 37 (5.41%) developed tumours. At the same time malignant disorders could be observed only in 5 (1.22%) of 406 control animals. In Tables I and II it can be seen that cases of leukemia as well as tumours were of very different origin and structure. According to Table III, malignant processes in the experimental group developed mainly in the animals of the F_4 and F_5 generations.

The DDT content of fatty tissue of the animals in Group 1 and Group 2 was 7.0–11.0 mg/kg and 1.8–2.2 mg/kg respectively.

Discussion. Evaluating our results, it is necessary to mention that in BALB/c mice spontaneous leukemia is unknown. BALB/c mice are, however, susceptible to

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