SHIMIZU[8] has reported the occurrence of variable quantities of glycogen in the area postrema of the mammalian brain located in the intercellular fibrillar structures around small vessels.

KAPPERS[9] describes in Amblystoma mexicanum the presence of glycogen in the cytoplasm and in the long thin processes of the ependymal gliocytes of the ventricular wall, especially in the lateral ventricle. The endings of these processes, which make up the external limiting membrane, also contain glycogen. He thinks that the paraphysis cerebri secretes glycogen in the ventricular cavity in Amblystoma. Such glycogen would then be adsorbed by the ependymal gliocytes and carried along their processes into the nervous parenchyma.

The transporting capacity of ependymal gliocytes of Amblystoma[9] and of guinea-pigs[10,11] has been demonstrated by inoculating India ink into the ventricles.

The glycogen deposited in the nervous parenchyma would have both a metabolic (as an energy source) and a plastic function.

The evidence reported for amphibians and mammals suggests that the accumulation of glycogen in the ependymal cells and in the surrounding neuropile of the bud, of the medulla oblongata and pons in birds can be correlated with the transport of glycogen, formed either by direct production by these cells or by adsorption.

This transport can be considered as a trophic function with regard to the nervous parenchyma. There is expression of a prevalence of anaerobic metabolism[12] in this area at the time of neuron differentiation, when a large energy supply is needed. Since anaerobic glycolysis contributes a low energy yield, a large glycogen reserve is required to satisfy the energetic needs.

Riassunto. Viene condotto uno studio sulla distribuzione del glicogeno nel romboencefalo dell'embrione di pollo durante lo sviluppo. I risultati delle indagini condotte hanno messo in evidenza una caratteristica localizzazione del glicogeno nell'abbozzo del ponte e del bulbo più evidente in embrioni dal 7° al 13° giorno di incubazione. Tale reperto viene discusso nel suo significato.

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Intracellular Localization of Glutamine-Aminohydrolase in Normal and Malignant Tissues

Glutamine aminohydrolase has been reported to be a mitochondrial enzyme in normal tissues[1-4] and is present in various organs of the animal body[5]. Glutaminase in the tumour tissue has been found to be a dialysable enzyme[6,7] and a possible leakage of this enzyme from the transplanted tumour into the blood stream of the animal has also been postulated[8]. This lead us to investigate the intracellular localization of this enzyme in tumour and the host tissues of the tumour transplanted animals.

Materials and methods. A mouse fibro-sarcoma (MFS)[9], Ehrlich's ascites tumour and Sarcoma 180 strains used, were maintained in Swiss mice in our laboratories. A 10% (w/v) tissue homogenate was prepared in 0.25 *M* sucrose in cold and centrifuged at 3000 *g* for 10 min using Sorvall RC-2B centrifuge. The supernatant thus obtained was recentrifuged at 12,000 *g* for 15 min to obtain mitochondrial and supernatant fractions. The supernatant was collected and mitochondrial pellet was resuspended in 0.25 *M* sucrose and centrifuged at the same speed for 10 min to get a washed mitochondrial preparation. Glutaminase assays were done, as described by BRAGANCA et al.[10]. Incubation mixtures used were as described by HOROWITZ and KNOX[11] for the liver and kidney type glutaminases. The incubation mixture used for tumour enzyme assay was as used for the kidney[11]. Protein estimations were carried out according to the method of LOWRY et al.[12]. Succinic dehydrogenase was assayed at 400 nm by the method of SLATER and BONNER[13], using a Zeiss spectrophotometer. S.c. injection of Actinomycin-D (100 γ/kg body wt.) was started 30 min after the transplantation of tumour and was continued on every alternate day until the tumour attained maximum size. The animals were sacrificed 24 h after every injection of Actinomycin-D.

Results. It can be observed from Table I that the glutaminase activity was mainly found to be present in the supernatant fraction in both types of tumours studied. There was, however, some difference in the 2 tumours, in that the enzyme activity was completely

Table I. Distribution of glutaminase activity in tumour cell-fractions

Tumour type	Homogenate	Mitochondria	Supernatant
MFS	3.0	0.0	3.3
Ehrlich's solid tumour	4.5	1.0	3.8

All values expressed as μmoles ammonia produced/mg protein/min and are an average of 4 experiments.

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Table II. Distribution of glutaminase activity in cell-fractions of liver and kidney

Tissue	Animal type	Homogenate	Mitochondria	Supernatant
Liver	Control	0.32	0.34	0.0
	MFS-bearing Mice	0.6	0.0	0.7
	EAC-bearing mice	1.6	0.43	1.2
	Sarcoma-180 bearing mice	1.75	0.32	1.2
Kidney	Control	0.18	0.14	0.0
	MFS-bearing mice	2.2	0.25	1.8
	EAC-bearing mice	1.1	0.05	0.8

All values expressed as μmoles ammonia produced/mg protein/min and are an average of 4 experiments.

Table III. Succinic dehydrogenase activity in cellfractions of liver, kidney and tumour tissues

Fractions		Mitochondria (ΔOD/min/mg)	Supernatant (ΔOD/min/mg)	
Liver	Normal	0.034 ± 0.006	0.007 ± 0.002	$p < 0.05$
	EAC-bearing mice	0.052 ± 0.015 $p < 0.4$	0.003 ± 0.005 $p < 0.02$	$p < 0.30$
Kidney	Normal	0.045 ± 0.01	0.0076 ± 0.0014	$p < 0.1$
	EAC-bearing mice	0.19 ± 0.024 $p < 0.02$	0.017 ± 0.0038 $p < 0.1$	$p < 0.05$
Tumour	Ehrlich (solid)	0.015[a]	0.002[a]	
	MFS	0.012[a]	0.001[a]	

[a] Values are not significant.

Table IV. Effect of Actinomycin-D on the glutaminase activity in liver cell-fractions of EAC-transplanted mice

	Glutaminase activity (μmoles NH_4^+/mg/min)						
	Before injection	After 1st inj.	After 2nd inj.	After 3rd inj.	After 4th inj.	After 5th inj.	7 days after last injection
Homogenate	0.096	0.1	0.012	0.007	0.09	0.001	0.011
Mitochondria	0.29	0.003	0.02	0.006	0.008	0.001	0.5
Supernatant	0.0	0.0	0.002	0.0	0.0	0.0	0.016

absent in the mitochondrial fraction of the MFS, but the Ehrlich's solid tumour showed about 25% of the homogenate activity in the mitochondrial fraction.

Having obtained the above results, it was of interest to see whether there was any change in the intracellular localization of this enzyme in the host tissues of tumour-bearing animals. The results obtained show that, like in tumours, the host liver and kidney also show a maximum activity in the supernatant fraction (Table II), whereas mitochondria contain most of the enzyme activity in control animals.

Since, a shift was obtained in the host liver and kidney, the question naturally arose whether, during the process of isolation of mitochondria from tumour or host tissues, there was either a breakage or leakage of glutaminase into the supernatant fraction. With this idea, another mitochondrial enzyme, viz. succinic dehydrogenase, was also estimated as a marker enzyme. The results obtained show that, like the normal tissues, the tumour as well as host liver and kidney show the presence of this enzyme only in the mitochondria (Table III). Experiments were then carried out to see if there was a synthesis or preferential leakage of glutaminase into the supernatant fraction. Hence, animals were injected with actinomycin-D. It may be observed from Table IV that the appearance of this enzyme in the supernatant fraction is completely inhibited.

Discussion. The results presented here indicate that the intracellular localization of glutaminase in tumours is different from that reported earlier from normal tissues[3, 14, 15]. It seems quite evident from the data obtained that there is also a shift of glutaminase activity from mitochondria to the supernatant fraction of the host liver and kidney, due to the presence of tumour in the body of the animal. That the difference in the location of glutaminase in either the tumour or host liver and kidney, as compared with the normal tissues, is not due to the fragility of mitochondria, but is indicated by the results obtained for the succinic dehydrogenase. If there was a rupture of mitochondria, then the succinic dehydrogenase also would have been obtained in supernatant fraction, but the results indicate otherwise. The problem then arises whether this may be due to a preferential leakage of glutaminase from the mitochondria to the supernatant or a new enzyme is being synthesized, with the development of the tumour. The results obtained with actinomycin-D experiments indicate that there is no appearance of glutaminase in supernatant fraction of the liver obtained from the tumour-bearing host. The reason for the Actinomycin-D not having any effect on the growth of tumour could be due to the fact that a much higher dose of the antibiotic is needed for the inhibition of tumour growth[16].

Résumé. On a comparé l'activité de la glutamine-aminohydrolase dans les fractions de divers tissus cellulaires de souris normales avec celles des tissus à partir de souris ayant des tumeurs. On a constaté cette activité surtout dans la fraction surnageante des tissus de l'hôte, tandis que dans les tissues des animaux normaux elle se manifeste en majeure partie dans la mitochondrie. Dans les tissus même de la tumeur, toute l'activité est limitée à la fraction surnageante.

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