was no agreement in the observers' opinions. The largest vascular profiles were detected in cases of gliobastoma and meningioma, but also in 3 lung carcinomas (oat-cell type), 1 prostatic carcinoma, 1 leukemia, and 1 lymphoma in a patient in whom no involvement of the CNS had been detected. Only 1 of the 26 controls showed a markedly positive vascular response. This patient had a cerebrovascular accident. These results, when statistically analyzed (χ^2 Yates) were considered significant (p 0.001). There was no correlation between vascular development and age or sex of the patients.

Discussion. These results suggest the occurrence of an angiogenic factor in the CSF of patients with meningioma and gliobastoma multiforme, in whom an angiogenic factor has been demonstrated in tissue culture[11]. Positive results were also obtained in other malignancies with no clinical signs of CNS involvement. Widespread micrometastases accounting for the occurrence of an angiogenic factor in the CSF can not be excluded in these cases. Alternatively, the presence of a diffusible plasmatic factor is yet to be verified. The need for more accurate techniques is however apparent from the high proportion of doubtful cases.

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Influence of lodgement site on the proliferation-kinetics of tumor cells

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Summary. This paper describes the influence of lodgement site on the proliferation-kinetics of rat ascites hepatoma AH7974. It was demonstrated that there was a difference in labeling indexes between tumor cells in the brain and in the choroid plexus in both single and continuous administration of tritiated thymidine.

Concerning the mechanism of organ specificity in cancer metastasis, a 'seed and soil' hypothesis has been advocated for a long time[1,2]. Though results supporting the hypothesis have been reported by some workers[3-5], there is no information about the difference in the proliferation-kinetics among tumor cells lodging in different organs. In the present paper, the proliferation-kinetics of rat ascites hepatoma AH7974 cells arrested in the blood vessels of the brain and choroid plexus was examined by using tritiated thymidine (^{3}H-TdR) autoradiography.

Materials and methods. Female Donryu strain rats weighing about 160 g were used. The tumor used was rat ascites hepatoma AH7974. This tumor is an island-forming strain containing cell aggregations of up to 10 cells. After washing the ascites fluid with physiological saline solution, 1 ml of tumor cell suspension containing 1×10^7 cells was injected into the carotid artery. The kinetic parameters of this tumor in the ascites[6] and the distribution pattern of tumors after the intracarotid injection[7,8] have been described in previous papers. The flash labeling index with ^{3}H-TdR of AH7974

Table 1. Labeling index of AH7974 cells with ^{3}H-TdR

	Time after injection	Labeling index of tumor cells			
		Brain		Choroid plexus	
		Mean	SD (n)	Mean	SD (n)
Flash labeling	6 h*	23.8	11.2 (3)	57.9	3.0 (3)
	1 day*	43.6	2.6 (3)	61.8	5.3 (3)
	2 days*	50.5	1.8 (2)	63.0	5.3 (2)
	3 days*	51.2	4.0 (3)	74.6	8.4 (3)
	4 days*	50.0	4.3 (2)	67.2	0.7 (2)
	5 days*	46.5	1.5 (2)	64.3	9.5 (2)
	7 days	22.5	0.8 (2)	-	
Continuous labeling	6 h*	68.4	0.6 (2)	85.4	3.7 (2)
	12 h*	74.0	9.8 (3)	94.1	1.5 (3)
	1 day*	86.6	0.4 (2)	96.9	1.5 (3)
	2 days*	89.5	3.5 (2)	99.0	1.0 (2)
	3 days	96.6	3.7 (2)	100.0	0.0 (3)

* There was a statistical significance in the labeling indexes between tumor cells in the brain and in the choroid plexus ($p < 0.01$).

cells in the ascites is in the range from 60 to 70%. Flash labeling experiments were performed as follows: 1 μCi of ^{3}H-TdR (New England Nuclear) per g of the animal was administered into the peritoneal cavity (i.p.) at 6 h, 1, 2, 3, 4, 5, and 7 days after the tumor injection. 90 min after the administration the animals were sacrificed with ether anesthesia. Continuous labeling experiments were performed as follows: the first i.p. administration of ^{3}H-TdR (1 μCi per g of the animal) was made at 10 min after the tumor injection, and the successive administration of the same dose of ^{3}H-TdR was repeated at time intervals of 6–9 h. The animals were sacrificed with ether anesthesia at 90 min after the last administration of ^{3}H-TdR. Autoradiographs of 100 sections of an animal were obtained by the dipping technique (Sakura NR-M2). 2–3 animals were used for every time point.

Results and discussion. When ^{3}H-TdR was administered at 6 h after the tumor injection, the labeling index of tumor cells in the brain vessels was in the range from 10 to 30%. During 1–5 days, the labeling indexes were in a range from 41 to 55% and at 7 days was about 20%. In multiple injection-groups, the labeling indexes of tumor cells in the brain increased progressively with the lapse of time after the tumor injection and reached about 90% at 24 h after the tumor injection (table 1).

In contrast to the case of the brain, the higher labeling indexes of tumor cells in the choroid plexus were demonstrated. In the flash labeling experiments, labeling indexes of tumor cells at every time examined were in a range from 54 to 79%, and in the continuous labeling experiments the labeling index reached 96% at 24 h after the tumor injection (table 1). Since in the case of the brain the lower labeling indexes were demonstrated, further studies were made on the comparison of flash labeling indexes of the tumor cells existing in the blood vessels and constituting tumor nodules in the brain parenchyma at times of 2, 3, and 4 days after the tumor injection. As shown in table 2, there were no significant differences between them ($p < 0.05$).

These results indicate that there was a difference in labeling indexes between tumor cells in the brain and in the choroid plexus with both single and continuous administration of ^{3}H-TdR. From the results indicated in tables 1 and 2, it is apparent that such differences in the labeling index are independent of whether the tumor cells exist in the blood vessels or not. Since the kinetics of the labeling index with ^{3}H-TdR are generally considered to reflect the state of the cell cycle in the cell, it could be said that the AH7974 cells proliferate better in the choroid plexus than in the brain. In the present study and the previous examination[7], the difference of tumor growth between the 2 sites was apparent: in the choroid plexus the growth was already a mass of tumor nodules consisting of several tumor cells 3 days after the injection, and forming tumors of 1–2 mm in diameter 2 weeks after the injection, while in the brain small nodules consisting of 2–3 tumor cells were formed after 3 days, and even after 2 weeks the small nodules could only be detected under the microscope.

The investigations presented here seem to support the 'seed and soil' hypothesis on the mechanisms of organ specific distribution of cancer metastasis.

Table 2. Labeling index of AH7974 cells existing in the blood vessels and constituting tumor nodules in the brain parenchyma

Time after injection	Labeling index of tumor cells*			
	Blood vessels		Brain parenchyma	
	Mean	SD (n)	Mean	SD (n)
2 days	50.0	1.8 (2)	51.5	1.3 (2)
3 days	55.3	2.7 (3)	49.4	4.2 (3)
4 days	51.2	(1)	49.6	(1)

* There was no statistical significance in labeling indexes between tumor cells in the blood vessels and in the parenchyma ($p < 0.05$).

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Increase in acid phosphatase activity during fertilization of a teleost egg

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Summary. Acid phosphatase activity was localized at the periphery of the yolk globules in the egg of the teleost, *Catostomus commersoni*. In addition, a 3-fold increase in acid phosphatase activity was associated with fertilization and pronucleus formation.

Considerable evidence indicates that changes in phosphorylation of egg components play important roles in regulating events in oocyte maturation, egg activation and fertilization[2,3]. A variety of phosphatases have been described in the sea urchin egg and early embryo[4,5]. Similarly, phosphatase activity has been observed in amphibian[6] and teleost[7] eggs. The present cytochemical and biochemical study of acid phosphatase was carried out using the egg of the teleost *Catostomus commersoni*. This study was designed to determine if acid phosphatase activity changed during fertilization and to localize any acid phosphatase activity within the egg.

Eggs and sperm of *Catostomus commersoni* were obtained and in vitro fertilization was carried out as previously described[8,9]. Eggs were fixed in either 2.5% gluteraldehyde or 5% calcium-formaldehyde at 4 °C at various times after addition of sperm and filtered stream water. Egg and sperm activation, including progression of pronuclear development, was verified histologically[8,9] in eggs from batches used in this study. All experiments reported here were repeated at least twice with eggs from several different females.

After a 1-h fixation period, eggs were transferred to 0.1 M Na cacodylate buffer (pH 7.4) containing 0.25 M sucrose. For cytochemistry, fixed eggs were frozen at −30 °C in Lipshaw M-1 embedding matrix and cut at 15 μm using a Lipshaw 1700 cryostat microtome. Frozen sections were picked up on albumin coated slides and allowed to dry for