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

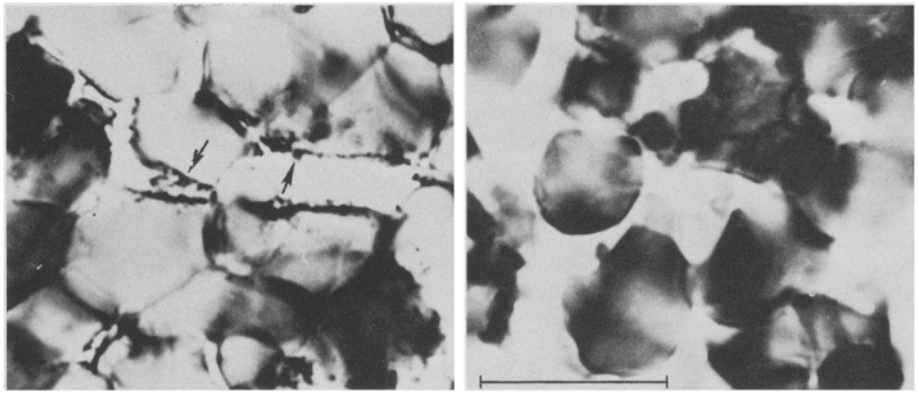

Fig. 1. Localization of acid phosphatase activity in frozen sections of *Catostomus* eggs. Arrows indicate reaction products at yolk globule periphery. Photomicrograph of control at right shows absence of reaction products. Bar 20 µm. × 1000.

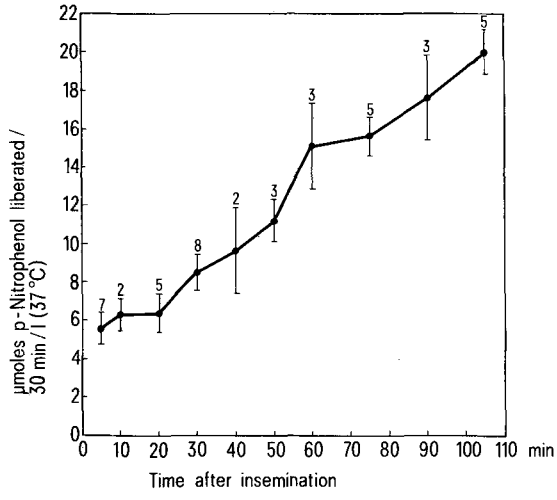

Fig. 2. Acid phosphatase activity of *Catostomus* egg homogenate during fertilization. Data presented as $\bar{X} \pm SEM$, numbers indicate the number of replicate egg groups tested.

30 min. The sections were then incubated in the following substrate[10] for up to 2 h at 37 °C: acetate buffer (pH 5) containing 1.2 g/l lead nitrate and 0.01 M Na glycerophosphate. Control solutions lacking glycerophosphate or lead nitrate were used. After incubation, the sections were rinsed briefly in distilled water, dipped in freshly made ammonium sulfide (2.5%) for 2 min, rinsed in distilled water and mounted in glycerol. Some sections were stained with Harris hematoxylin-congo red and mounted in permount.

Both gluteraldehyde and formaldehyde fixed eggs showed reaction products indicating acid phosphatase activity at the periphery of the yolk globules (figure 1). The dark brown reaction products were granular and measured approximately 0.5 µm in diameter. Localization of acid phosphatase could not be obtained in eggs stored longer than 48 h at 4 °C.

For each acid phosphatase assay, 6 fixed eggs were rinsed in distilled water, homogenized in 4 ml of distilled water and centrifuged at 2400 rpm for 5 min. 1 ml of citrate buffered (pH 4) p-nitrophenylphosphate substrate (DADE, American Hosp. Supply) was added to 1 ml of egg supernatant and incubated at 37 °C for 30 min. 5 ml of 0.1 N NaOH was added to stop the reaction. The optical density was read at 410 nm using a Beckman DB-G spectrophotometer. Serial dilutions of a p-nitrophenol solution were used to construct a standard curve. The data are expressed as µmoles pf p-nitrophenol liberated per 30 min/l.

Acid phosphatase activity of egg homogenate supernatant increased with time post insemination (figure 2). By 105 min post insemination, there was a 3-fold increase in phosphatase activity. Activity was obtained in fixed eggs stored at 4 °C for up to 1 week.

These results suggest that acid phosphatase activity, which is localized at the periphery of yolk globules, is membrane bound. This hypothesis appears to explain the loss of localization after 48 h storage, while activity remained unchanged in homogenates over this same period. One well known type of membrane bound acid phosphatase is that associated with lysosomes[11]. Lysosomes have been found and implicated in yolk utilization in eggs from a variety of animal groups[12]. However, the present study is the first to suggest the possibility that lysosomal acid phosphatase might play a role in the early events of fertilization.

Recently, a maturation promoting factor (MPF) has been found in maturing oocytes from a variety of animal groups, including fish[2], mitotically active mammalian cell cultures (HeLa)[13] and early amphibian embryos[14]. Data suggest that phosphorylation activates MPF[15,16]. However, there has been little study of the phosphatase activity which theoretically should be present to inactivate MPF prior to pronucleus formation. In the amphibian egg, MPF activity rapidly declines during fertilization and pronucleus formation, and increases at the time of the first mitotic division[14]. Since MPF is also found in the fish egg[2], it is possible that the increase in phosphatase activity observed in the present study is somehow related to MPF inactivation and subsequent pronucleus formation.

Note added in proof: A recent paper, N. Hart and P. Pontier, Experientia *35*, 999 (1979), which came to the attention of the author after submission of this manuscript, presents data supporting the general findings contained herein.

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