As this histochemical technique probably detects cyclic AMP bound to proteins and not the total cyclic AMP, as described above, we suggest that protein-bound cyclic AMP in the cytoplasm of *Tetrahymena* increases in the late stage of cell division. Although the role of cyclic AMP in this unicellular eukaryote is not well understood, our description of the intracellular localization of cyclic AMP may lead the way to eludication of the complex mechanisms involved.

1 Acknowledgments. This work was supported in part by research grants from the Scientific Research Fund of the Ministry of Education, Science and Culture, Japan. We thank Prof. M. Ui of Hokkaido University for pertinent discussion, Mr A. Sato, Research Laboratories of Yamasa Shoyu Co. Ltd, Choshi, for the generous gift of antiserum to cyclic AMP and M. Ohara for criticism of the manuscript.

2 Send reprint requests to C.T., Department of Pharmacology, Kobe University School of Medicine, Kobe 650, Japan.
3 J. Voichick, C.E. Elson, D. Granner and E. Shrago, J. Bact. *115*, 68 (1973).
4 S. Ramanathan and S.C. Chou, Experientia *29*, 814 (1973).
5 J.R. Dickinson, M.G. Graves and B.E.P. Swoboda, FEBS Lett. *65*, 152 (1976).
6 S. Kassis and E. Zeuthen, Exp. Cell Res. *124*, 73 (1979).
7 G.A. Thompson, Jr, Biochemistry *6*, 2015 (1967).
8 A.L. Steiner, S.H. Ong and H.J. Wedner, Adv. cyclic Nucleot. Res. *7*, 115 (1976).
9 A.L. Steiner, G.W. Parker and D.M. Kipnis, J. biol. Chem. *247*, 1106 (1972).
10 N. Yoshida, C. Tanaka, K. Taniyama and M. Kondo, Jap. J. Pharmac. *29*, 781 (1979).
11 M. Honma, T. Satoh, J. Takezawa and M. Ui, Biochem. Med. *18*, 257 (1977).
12 E.F. Fallon, R. Agrawal, E. Furth, A.L. Steiner and R. Cowden, Science *184*, 1089 (1974).
13 H. Murofushi, Biochim. biophys. Acta *370*, 130 (1974).
14 J. Wolfe, J. Cell Physiol. *82*, 323 (1973).

Angiogenic activity in the CSF in human malignancies

S. López-Pousa, I. Ferrier[1], J.M. Vich[2] and J. Domenech-Mateu

Cátedra de Anatomia de la Facultad de Medicina, Universidad Autonoma de Barcelona (Bellaterra), Barcelona (Spain), 17 July 1980

Summary. Angiogenic activity, tested on the chorioallantoid membrane of the chicken embryo, was present in the CSF of patients with meningioma and glioblastoma and in patients with other malignancies with no clinical signs of CNS involvement.

From the pioneer study of Algire and Chalkey[3] a tumoral angiogenic factor has been widely recognized in human and animal[4-10] tumors, including brain tumors[11]. This factor was recently detected in the ocular fluid in cases of human eye tumors[12] using the vascularization of the chorioallantoid membrane of the chicken embryo. This study reports the results of a similar approach, studying the cerebrospinal fluid (CSF) of patients with primary brain tumors or other malignancies.

Materials and methods. CSF from 52 patients (9 with primary brain tumors, 17 with other malignancies in whom cerebral involvement was clinically excluded, and 26 controls) was aseptically obtained, filtered through a 0.22 µm Millipore filter, and stored at 4 °C after lyophilization of 0.2 ml aliquots; the tubes were given numbers to minimize subjective results. 32 of the patients were male and 20 female, and their ages ranged from 24 to 81 years (mean 57 years). The samples were implanted as powder in the chorioallantoid membranes of 9-day-old chicken embryos *(Gallus domesticus)*. The eggs were examined 48 h later under a stereomicroscope and photographs were taken. The membranes were subsequently fixed with saturated formalin solution and reevaluated.

Each sample was tested on 3 occasions and examined by more than 2 observers.

Results. The results are expressed in the table. The vascular responses were considered doubtful when the results were not uniform in the 3 tests performed in each case or there

	No cases	Vascular response		
		positive	doubtful	negative
Primary CNS tumors				
Neurinoma	2		2	
Meningioma	2	2		
Hemangioblastoma	1		1	
Papilloma	1			1
Astrocytoma	1		1	
Glioblastoma	2	2		
Other malignancies				
Carcinoma of the lung	7	3	4	
larynx	2		2	
tongue	2		2	
stomach	2		2	
prostate	1	1		
unknown origin	1			1
Leukemia	1	1		
Lymphoma	1	1		
Controls	26	1		25
Total	52	11	14	27

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.

1 Depto. Anatomia Patológica (Neuropatologia), C.S. Principes de España, Hospitalet de Llobregat, Barcelona (Spain).
2 Laboratorio Inmunolaboratorio, Barcelona (Spain).
3 G.H. Algire and H.W. Chalkey, J. natl Cancer Inst. *6*, 73 (1945).
4 M. Greenblatt and P. Shubik, J. natl Cancer Inst. *41*, 111 (1968).
5 B.A. Warren and P. Shubik, Lab. Invest. *15*, 464 (1966).
6 T. Cavallo, R. Sade, J. Folkman and R.S. Cotran, J. Cell Biol. *54*, 408 (1972).
7 T. Cavallo, R. Sade, J. Folkman and R.S. Cotran, Am. J. Path. *70*, 345 (1973).
8 J. Folkman, Cancer Res. *34*, 2109 (1974).
9 M.A. Gimbrone and P.M. Gullino, J. natl Cancer Inst. *56*, 305 (1976).
10 P. Phillips, J.K. Steward and S. Kumar, Int. J. Cancer *17*, 549 (1976).
11 P.J. Kelly, R.L. Suddith, H.T. Hutchinson, K. Werbach and B. Haber, J. Neurosurg. *44*, 342 (1976).
12 D. Tapper, R. Langer, A.R. Bellows and J. Folkman, Surgery *86*, 36 (1979).

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

T. Kawaguchi, M. Endo, S. Yokoya and K. Nakamura

2nd Department of Pathology, Central Laboratory, Division of Cell Science, Fukushima Medical College, Fukushima 960 (Japan), 15 August 1980

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 ($p < 0.01$).