retardation, not only are global values of cerebral glucose utilization lower than in relatively normal children, there is a striking regional heterogeneity of functional brain anatomy. The pattern, in fact, resembles that of relatively normal neonates (Fig. 1). Congruent with this metabolic pattern is the persistence of intrinsic subcortical reflexes (such as grasping and tonic neck reflexes) and the overall neonate-like behavior of many such children. Diminished glucose utilization in psychomotor retardation is also consistent with reported dendritic spine loss and dysgenesis in children with mental retardation (*28*), since nerve endings and dendrites utilize more glucose than other neuronal constituents (*29*).

Since the extent of neurological compromise in brain-damaged children can vary, it will be important to determine whether the pattern of glucose metabolism in development and during various stimulation paradigms can predict the type and extent of neurological affliction. Furthermore, similar studies in children with specific learning disabilities may provide important insight into the basis of their deficits by identifying sites of disturbed cerebral function.

PET provides a novel approach to studying the developing human brain, both in normal children and in the brain-impaired child. The use of FDG to measure LCMRglc is but one example of many neurodevelopmental processes that can be investigated with PET. By using appropriately labeled compounds, it will be possible to quantify developmental changes in regional brain protein synthesis, blood flow, oxygen consumption, myelination, and neuroreceptors (*30*).

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L-Isoleucine and L-Leucine: Tumor Promoters of Bladder Cancer in Rats

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A 4-week assay for screening tumor promoters of bladder cancer has been developed in which increased agglutinability of isolated rat bladder cells with concanavalin A is used as an indicator. On the basis of this assay system, L-isoleucine and L-leucine were suspected of being possible tumor promoters. Results of 40- to 60-week carcinogenesis experiments in which *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine was used as an initiator to demonstrate that L-isoleucine and L-leucine promote bladder cancer in rats. This finding may be relevant to the high incidence of human bladder cancer in Western countries, where the diet is rich in protein.

A TWO-STAGE CARCINOGENESIS PROcess, consisting of initiation and promotion, has been established in mouse skin (*1*). The same two-stage process has also been demonstrated to apply in experimental bladder carcinogenesis, where substances such as sodium saccharin, DL-tryptophan, and sodium ascorbate act as tumor promoters (*2–4*). About 70 percent of human bladder cancers are of the superficial papillary type, which is characterized by frequent intravesical ectopic recurrence after transurethral resection of the tumors. This recurrence may be due to new tumor growth from epithelial lesions unrecognized on cystoscopy (*5*). Tumor promoters may play a key role in promoting the growth into recognizable tumors of epithelial lesions too small to be visible by cystoscopy. Thus, identification of promoters of human bladder carcinogenesis may be crucial for the management of superficial papillary bladder cancer.

We have developed a 4-week assay to detect promoters of bladder carcinogenesis in rats (*6–8*). Isolated bladder cells from rats that have been treated with various types of bladder carcinogens, such as *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BHBN) or *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) show increased agglutinability with concanavalin A (Con A) (*7*). This increased agglutinability with Con A disap-

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Table 1. The numbers and incidences of carcinomas and precarcinomatous lesions in the rat urinary bladder (experiment 1). Urinary bladders were inflated by intraluminal injection of 10 percent buffered formalin solution (*p*H 7.4) and fixed. The bladders were then cut sagitally into eight strips. The strips were arranged serially, embedded in paraffin, stained with hematoxylin-eosin, and examined histologically. For quantitative analysis, urinary bladder lesions were counted by light microscopy with a color video image processor (VIP-21CH; Olympus Ikegami Tsushin Company, Tokyo). The total length of the basement membrane was measured, and the numbers of lesions were expressed as the mean ± standard error of the mean per 10 cm of basement membrane (BM). Numbers in parentheses are percentages. Statistical analysis was done by the χ^2 test with regard to incidence and Bonferroni-type correction with regard to the number of lesions per 10 cm of BM.

Group	Treatment	Effective No.	Carcinoma		Papilloma		PN hyperplasia		Simple hyperplasia incidence
			Incidence	No. per 10 cm of BM	Incidence	No. per 10 cm of BM	Incidence	No. per 10 cm of BM	
A	BHBN + 2.0% L-isoleucine	27	5 (19)*	0.17 ± 0.07*	3 (11)	0.14 ± 0.07	10 (37)	0.53 ± 0.03	25 (92.6)
B	BHBN + 2.0% L-leucine	30	4 (13)	0.11 ± 0.05	7 (23)	0.29 ± 0.10	5 (17)	0.14 ± 0.04	23 (76.7)
C	BHBN + control diet	30	0 (0)	0	5 (17)	0.16 ± 0.06	16 (53)	0.70 ± 0.14	29 (96.7)
D	2.0% L-isoleucine	18	0		0		0		0
E	2.0% L-leucine	17	0		0		0		0
F	Control diet	16	0		0		0		0

*$P < 0.05$ (group A versus group C).

pears within 3 to 6 weeks after discontinuation of treatment with BHBN or FANFT. However, treatment of the rats with established promoters of urinary bladder tumors, such as sodium saccharin or DL-tryptophan, after discontinuation of carcinogen treatment maintains the increased agglutinability of their isolated bladder cells for at least 20 weeks (*8*). Using the maintenance of Con A agglutination of bladder cells as a marker, we examined the effects of 21 amino acids. The results indicated that L-isoleucine, L-leucine, and L-valine behave like sodium saccharin and DL-tryptophan in maintaining agglutination (*9*). We describe here the promoting effects of L-isoleucine and L-leucine in 40- to 60-week in vivo carcinogenesis experiments.

In experiment 1, 155 F344 rats (6 weeks old; Charles River Laboratories, Atsugi, Japan) were divided into six groups. Groups A, B, and C (31 to 32 animals each) were given CE-2 pellet diet (CLEA, Tokyo, Japan) and 0.05 percent BHBN in their drinking water for 4 weeks and then tap water. Groups A and B were then given CE-2 pellet diet supplemented with 2.0 percent L-isoleucine or 2.0 percent L-leucine, respectively, from week 4, while group C was given control CE-2 diet throughout. Groups D and E (20 to 21 animals each) were given CE-2 pellet diet supplemented with 2.0 percent L-isoleucine or 2.0 percent L-leucine, respectively, from week 4. Group F (20 animals) was control group. All surviving animals were killed in week 40. Histological abnormalities (Table 1) in the urinary bladder in week 40 were classified as (i) simple hyperplasia, (ii) papillary or nodular (PN) hyperplasia, (iii) papilloma, or (iv) carcinoma (*10*). The incidences and numbers of these foci per 10 cm of basement membrane were recorded. The incidence and number of carcinomas in group A were significantly higher than in group C and were also higher in group B than in group C. The incidence and number of PN hyperplasia were highest in control group C. Addition of 2.0 percent L-isoleucine or 2.0 percent L-leucine without BHBN to the diet did not induce any bladder lesions in groups D and E.

On the basis of the results of experiment 1, we suspected that L-isoleucine and L-leucine had tumor-promoting activity for bladder carcinogenesis. To confirm this possibility, in experiment 2 we examined the dose dependency of L-isoleucine and L-leucine in bladder carcinogenesis. A total of 217 male F344 rats (6 weeks old) were divided into eight groups (Table 2). Groups 1 through 5 (31 animals each) were given CE-2 pellet diet for the first 4 weeks and 0.05 percent BHBN in their drinking water for 4 weeks. Groups 1 through 4 were then given CE-2 pellet diet supplemented with 2.0 percent or 4.0 percent L-isoleucine or 2.0 percent or 4.0 percent L-leucine, respectively, until week 60. Group 5 was given control CE-2 diet from week 4. Groups 6 and 7 (21 animals each) were given CE-2 diet supplemented with 4.0 percent L-isoleucine or 4.0 percent L-leucine, respectively, from week 4 to week 60. Group 8 (20 animals) received no treatment as controls. All surviving animals were killed in week 60. The incidences and numbers of carcinomas were significantly higher in groups treated

Table 2. The numbers and incidences of carcinomas and precarcinomatous lesions in the rat urinary bladder (experiment 2). The procedures followed were the same as for Table 1.

Group	Treatment	Effective No.	Carcinoma		Papilloma		PN hyperplasia		Simple hyperplasia incidence
			Incidence	No. per 10 cm of BM	Incidence	No. per 10 cm of BM	Incidence	No. per 10 cm of BM	
1	BHBN + 2.0% L-isoleucine	31	14 (45)	0.65 ± 0.14*	18 (58)	0.83 ± 0.14	25 (81)	1.06 ± 0.25	31 (100)
2	BHBN + 4.0% L-isoleucine	31	24 (77)**	1.06 ± 0.15**	19 (61)	0.77 ± 0.13	22 (71)	1.01 ± 0.16	31 (100)
3	BHBN + 2.0% L-leucine	31	16 (52)*	0.86 ± 1.18*	15 (48)	0.68 ± 0.15	23 (74)	1.49 ± 0.24	31 (100)
4	BHBN + 4.0% L-leucine	31	23 (74)**	1.18 ± 0.16**	16 (52)	0.58 ± 0.11	20 (64)	0.84 ± 0.12	31 (100)
5	BHBN + control diet	31	7 (23)	0.21 ± 0.07					
					0 (0)		0 (0)		0 (0)
7	4.0% L-leucine	21	0 (0)		0 (0)		0 (0)		0 (0)
8	Control diet	20	0 (0)		0 (0)		0 (0)		0 (0)

*$P < 0.05$; **$P < 0.01$ (all comparisons were made between each group and group 5).

with BHBN and amino acids than in the group treated with BHBN alone, and the amino acids exhibited dose-dependent effects (Table 2). However, the incidences of simple hyperplasia, PN hyperplasia, and papilloma in groups 1 through 4 were not significantly different from those in group 5. Groups 6, 7, and 8 did not show any abnormalities of the bladder epithelium. These results confirm that L-isoleucine and L-leucine have tumor-promoting effects on bladder carcinogenesis in rats.

The amounts of free L-isoleucine in the urine of rats given CE-2 diet or CE-2 diet supplemented with 2.0 percent or 4.0 percent L-isoleucine were 0, 149, and 1008 μg/ml, respectively (*11*); the amount of free L-leucine in the urine of rats given CE-2 diet without or with 2.0 percent or 4.0 percent L-leucine were 0, 309, and 1141 μg/ml, respectively. The concentrations of L-isoleucine and L-leucine as protein in the CE-2 diet were 1.03 and 1.80 percent, respectively. Animals given the CE-2 diet alone excreted neither L-isoleucine nor L-leucine in their urine. When we added 2.0 or 4.0 percent free L-isoleucine or 2.0 or 4.0 percent free L-leucine to the diet, the amounts of the free forms of both amino acids detected in the urine were less than 0.5 percent of those given orally. There may be a relation between the tumor-promoting effects of these two amino acids and the high incidence of human bladder cancer in western countries, where the diet is rich in protein (*12*).

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Expression Cloning of a Lymphocyte Homing Receptor cDNA: Ubiquitin Is the Reactive Species

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The lymphocyte cell surface receptor for the high endothelial venules (HEV's) of peripheral lymph nodes is specifically recognized by the monoclonal antibody MEL-14. Three independent complementary DNA (cDNA) clones, each of which encodes the protein ubiquitin, were detected by virtue of the expression of the MEL-14 antigenic determinant on cDNA–β-galactosidase bacterial fusion proteins. The antigenic determinant defined by MEL-14 resides in the carboxyl terminal 13-amino-acid proteolytic peptide of ubiquitin, but is undetected in intact undenatured ubiquitin and other cellular ubiquitinated proteins. Antisera and monoclonal antibodies to ubiquitin determinants bind to the surface of both HEV-receptor positive and negative cell lines. The MEL-14–identified cDNA clones hydridize to RNA transcripts that encode tandemly repeated ubiquitins. Sequence analysis of these polyubiquitin cDNA's does not identify a leader sequence for export to the cell surface. The expression of the MEL-14 epitope of ubiquitin depends upon its local environment. The steady-state levels of expression of the ubiquitin messenger RNA's do not correlate with either the tissue derivation of the RNA or the expression of the lymphocyte HEV receptor. Regulation of the expression of the HEV receptor is not likely to reflect the transcriptional control of ubiquitin genes, but rather to reflect control of the expression of the HEV core polypeptide or its level or form of ubiquitination.

MATURE LYMPHOCYTES CIRCULATE throughout the body, passing through the lymphoid organs between the blood vasculature and lymphatic systems (*1*). The mobility of these cells allows them to encounter the various microenvironments necessary for maturation, to interact with the various other lymphocyte subsets, and to interact specifically with antigen. A major event in the migration pathway occurs when lymphocytes specifically recognize and adhere to specialized high endothelial cells of the postcapillary venules (called HEV's) of the peripheral lymph nodes and the gut-associated Peyer's patches. These bound lymphocytes transmigrate into the parenchyma of the lymphoid organ, and may eventually return by way of the lymphatic system to the bloodstream (*1*).

Most normal murine small lymphocytes express receptors for HEV cells in peripheral nodes and Peyer's patches (*2, 3*). The organ distribution of lymphocyte subsets may be determined in part by the HEV adherence properties of individual lymphocytes. These adherence properties may be measured both in vivo and in vitro. Lymphocytes, incubated on a tissue section of peripheral lymph nodes or Peyer's patches, adhere avidly and specifically to the exposed HEV's (*4, 5*). Some murine lymphomas express a virtually unispecific preference for one or the other type of HEV (*2, 6, 7*). These data suggest that there are at least two types of lymphocyte HEV receptors, one for the HEV of peripheral lymph nodes and one for the HEV of Peyer's patches.

A monoclonal antibody, MEL-14, appears to recognize the lymphocyte receptor for peripheral lymph node HEV (*7*). The expression of the cell surface MEL-14 epitope correlates without known exception to the peripheral lymph node adhesion phenotype of both normal cells and neoplastic lymphoid cell populations (*7, 8*). All peripheral node HEV binding lymphomas express a MEL-14 reactive antigen, while Peyer's patch HEV unispecific or nonbinding tumors lack MEL-14 antigen. In addition, clonal variants of lymphoid tumors, selected for the expression of high or low levels of the MEL-14 antigen (*9*), express the expected adhesion phenotype. Treatment of both MEL-14 antigen-positive lymphoid tumors and normal mesenteric node lymphocytes

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