the enzyme in cerebral microvessels was much greater than that measured in vitro in peripheral endothelial cells, but the implication that a difference of such large magnitude would be present in whole vessel from another source is not yet warranted.

Secretion of CSF by the choroid plexus has been shown to be directly related to the activity of Na^+,K^+-adenosinetriphosphatase in that structure (*14*), and ouabain binding sites have been shown to be abundant on the choroid plexus epithelial surface (*15*). In addition, the concentration of K^+ measured in the nascent fluid secreted by the choroid plexus is dependent on the activity of the enzyme (*7*). It was found in other tissues that the capacity to actively transport Na^+ or K^+ is directly proportional to the activity of Na^+,K^+-adenosinetriphosphatase in that tissue, and the ratio of cation transported to enzyme activity measured was nearly equal when six different tissues were studied (*16*). We have shown that Na^+,K^+-adenosinetriphosphatase activity and cation uptake in isolated microvessels are very similar to those in choroid plexus and we infer that the brain microvessels have the capacity to transport Na^+ and K^+ by a similar mechanism. We suggest that the regulation of K^+ in the nascent brain extracellular fluid and the movement of Na^+ into that fluid from microvessels is related to the activity of Na^+,K^+-adenosinetriphosphatase present in those vessels.

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10. Rats were killed by decapitation and the brain removed immediately. Cerebral hemispheres were dissected free in cold (4°C) tris-sucrose buffer (*p*H 7.4). After removal of the white matter, the cerebral cortex was minced and homogenized with a Teflon-coated pestle and a glass homogenizer (five to ten strokes). The homogenate was centrifuged (1500*g* for 15 minutes) and the pellet was resuspended in tris-sucrose buffer and rehomogenized. The process was then repeated and the mixture filtered through 300- and 110- μm nylon mesh. The material retained on the filters was layered over a 1.0 to 1.5*M* continuous sucrose gradient in tris-sucrose buffer, followed by centrifugation (25,000*g* for 1 hour).
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Differential Competition with Cytotoxic Agents: An Approach to Selectivity in Cancer Chemotherapy

Abstract. *An approach to increasing the selectivity of cancer chemotherapeutic agents is presented in which noncytotoxic competitive substrates are used to discern the differences in structural requirements for transport of cytotoxic agents between tumor cells and a sensitive host tissue, the hematopoietic precursor cells of the bone marrow. Examples are given for two such systems, one responsible for the transport of nucleosides and another for the transport of amino acids. Cytidine is twice as effective in reducing the toxicity of showdomycin for murine bone marrow cells in culture as it is for murine L1210 leukemia cells. Conversely, homoleucine is twice as effective in reducing the toxicity of melphalan for L1210 cells as it is for bone marrow cells. These observations can serve as a basis for the development of bone marrow protective agents and for the design of cytotoxic agents that may be preferentially transported into tumor cells.*

A principal concern in cancer chemotherapy is the protection of sensitive host tissue, such as the hematopoietic progenitor cells of the bone marrow, from the action of cytotoxic agents. It may be possible to do this by taking advantage of the many differences in the architecture of the plasma membrane between normal cells and tumor cells (*1*), which could alter the specificity of transport sites. Such transport systems are present in mammalian cells for the uptake of nucleosides and amino acids and are mediated by a carrier, a component of the plasma membrane that often exhibits high affinity and limited capacity for the nutrient. The structural requirement for substrates to participate in carrier uptake depends on the individual transport system, and certain cytotoxic agents of value in cancer chemotherapy have been shown to enter cells by such transport systems (*2-5*). The limited capacity of transport carriers, termed saturability, makes them susceptible to competitive interaction, and thus the activity of cytotoxic agents that gain entrance to the cell through their agency may be con-

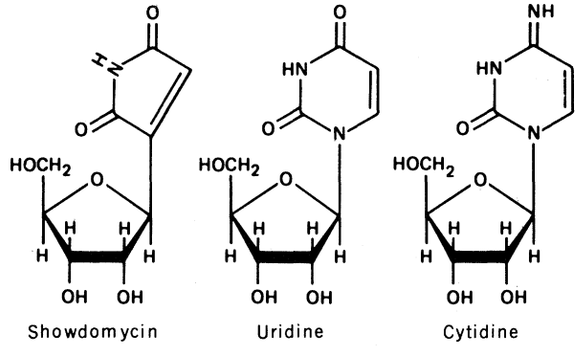

Fig. 1. Structures of showdomycin, uridine, and cytidine.

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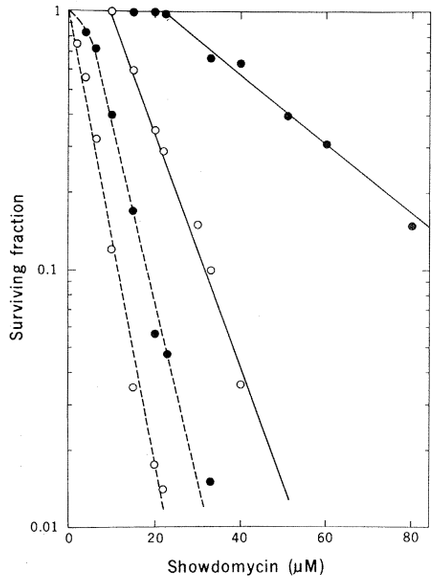

Fig. 2. Cytotoxicity of showdomycin to murine L1210 leukemia cells and bone marrow progenitor cells, and its antagonism by cytidine. The L1210 cells were grown to the logarithmic phase in RPMI 1630 medium supplemented with 20 percent heat-inactivated fetal calf serum (FCS). They were harvested, washed twice in Dulbecco's phosphate-buffered saline containing 0.1 m*M* bovine serum albumin and 0.25 percent glucose, and suspended at 1.0×10^5 cells per milliliter in the same buffer system. Bone marrow cells were obtained from male CDF_1 mice weighing 20 to 25 g. Mice were killed by cervical dislocation and femurs were removed and gently flushed with the buffer described above. The contents were washed twice and suspended at 1.0×10^5 nucleated cells per milliliter. Leukemia cells (dashed lines) or murine bone marrow cells (solid lines) were incubated for 15 minutes at 37°C in buffer (○) without or (●) with 2 m*M* cytidine. Showdomycin, prepared as a 20- to 40-m*M* stock solution in distilled water, was then added and the incubation continued for an additional 20 minutes. Cells were harvested by centrifugation, resuspended in growth medium, and washed two additional times by suspension in growth medium and centrifugation. Cytotoxicity to tumor cells was assessed by clonal growth of surviving cells for 2 weeks in soft nutrient agar according to the procedure of Chu and Fischer (*20*) with minor modifications (*18*). Cytotoxicity to murine hematopoietic precursor cells was assessed after clonal growth for 1 week in modified McCoy's 5A medium supplemented with 16 percent FCS, penicillin (20 U/ml), and streptomycin (20 μg/ml) in a humidified atmosphere of 10 percent CO_2 (*21*). Pregnant mouse uterine extract (*22*), a source of colony-stimulating factor in the bone marrow assays (*23*), was used at a concentration that resulted in maximal colony formation of 90 to 100 macrophage and granulocyte colonies per 100,000 nucleated bone marrow cells. No colony formation occurred in its absence. Cell aggregates were scored as colonies if they contained 50 or more cells.

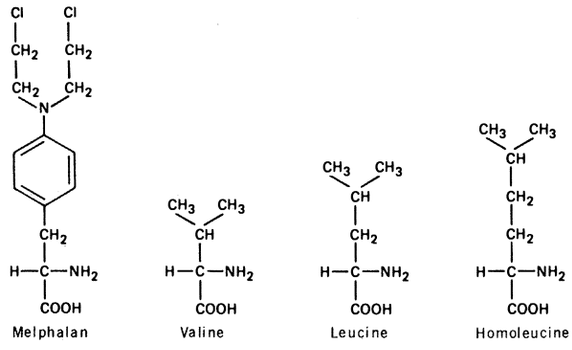

Fig. 3. Structures of melphalan, valine, leucine, and homoleucine.

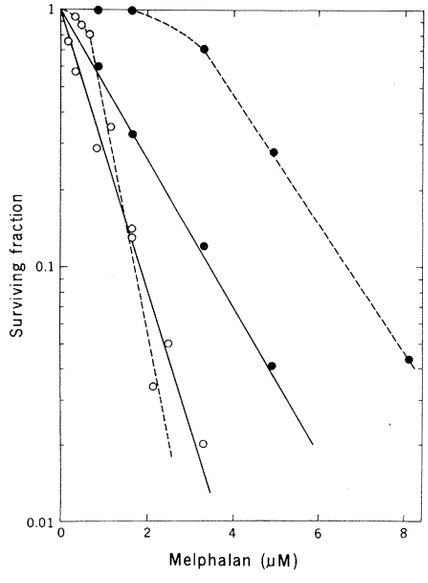

Fig. 4. Cytotoxicity of melphalan to murine L1210 leukemia cells and bone marrow progenitor cells, and its antagonism by homoleucine. Leukemia cells (dashed lines) or murine bone marrow cells (solid lines) prepared as described in the legend of Fig. 2 were incubated for 15 minutes at 37°C in buffer containing 0.1 m*M* bovine serum albumin and 0.25 percent glucose (○) without or (●) with 0.5 m*M* homoleucine. DL-Homoleucine was synthesized from isoamyl bromide by the acetamidomalonate procedure (*24*). Melphalan (L-phenylalanine mustard), prepared as a 32-m*M* stock solution in 75 percent ethyl alcohol containing equimolar hydrochloric acid, was then added and the incubation continued for an additional 35 minutes. Cell survival was estimated by clonal growth as described in the legend of Fig. 2.

trolled by competition with a noncytotoxic substrate.

We have investigated this phenomenon by means of transport and viability determinations in tissue culture (*3*) and here report on differences between the L1210 murine leukemia cells and the hematopoietic precursor cells of murine bone marrow in two such systems, one specific for nucleosides and the other for the longer-chain aliphatic amino acids. The intervention of competitive factors other than transport cannot be entirely ruled out, particularly in the case of bone marrow progenitor cells, where only one in 1000 nucleated cells forms colonies.

Showdomycin (Fig. 1) is a C-nucleoside antibiotic whose activity is due to the alkylating property of its maleimide moiety (*6, 7*). It is active against Gram-positive and Gram-negative bacteria (*7*), HeLa cells in vitro, and Ehrlich ascites carcinoma in vivo (*8*). Its uptake by *Escherichia coli* (*9*) is inhibited by a wide variety of nucleosides, but not by purine or pyrimidine bases, nucleotides, or ribose. At high concentrations showdomycin inactivates the transport site for adenosine in rabbit lung macrophages, and such inactivation can be prevented by concurrent incubation with cysteine, adenosine, or thymidine (*10*). Showdomycin cytotoxicity is determined both by its entry into the cell as a nucleoside analog and by its activity, possibly within the cell, as a maleimide sulfhydryl reagent.

The antibiotic is twice as toxic for L1210 cells as for bone marrow cells, and this differential is increased twofold by cytidine (Fig. 2). Several other nucleosides, such as uridine and deoxycytidine, as well as 5′-iodo-5′-deoxyadenosine, 5′-methylthio-5′-deoxyadenosine, and 5′-tosyladenosine protect L1210 and bone marrow cells equally (*11*). The first two nucleosides are subjects for intracellular phosphorylation, but the latter three cannot be phosphorylated and hence cannot act by metabolic competition. This suggests, but does not prove, that cytidine is active before metabolic conversion. The twofold increased protection afforded by cytidine to the bone marrow cells relative to the L1210 cells offers a potential for improving treatment in vivo by the use of showdomycin with cytidine and supports the concept of differential competition as an approach to improved selectivity.

Melphalan (Fig. 3) is an aromatic nitrogen mustard with a broad range of antitumor activity in experimental animals (*12*); single injections are curative in 60 percent of mice bearing the L1210 leukemia (*13*). It is used clinically in the

treatment of multiple myeloma (*14*), ovarian carcinoma (*15*), breast cancer (*16*), and malignant melanoma (*17*).

Cytotoxicity of melphalan in the L1210 cell system in vitro (*3, 18*) is determined by its uptake by two high-affinity amino acid transport systems of the leucine type (*3-5*). Although leucine is the most effective protector and transport competitor, its lower homolog, valine, is essentially inactive (*3, 18*). A similar pattern of protection was observed in murine bone marrow cells (*19*). However, the higher homolog of leucine, homoleucine (Fig. 3), preferentially protected the L1210 cells by a factor of 2 (Fig. 4). This suggests a higher specificity for the location of chain branching or a more limited bulk tolerance by the leucine-preferring transport system of bone marrow progenitor cells than that of L1210 leukemia cells.

No direct application of the administration of homoleucine with melphalan is apparent, since such treatment should protect the tumor and direct the cytotoxicity toward the host's bone marrow. However, the evidence for decreased specificity for the location of chain branching or higher bulk tolerance by the L1210 cell system suggests that cytotoxic compounds incorporating such features may be more selective toward the tumor cell. Differential competition for transport may therefore become a tool for the design of more selective cytotoxic agents.

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Physostigmine and Recent Memory: Effects in Young and Aged Nonhuman Primates

Abstract. *The effect of physostigmine on recent memory was evaluated in young and aged rhesus monkeys. All aged monkeys had previously shown impaired memory. The performance of the young monkeys treated with physostigmine was similar to that recently reported for young humans—no effects at low doses, some improvement at a restricted range of doses, and deficits at the highest dose. Although the aged subjects also improved at the same general doses, their overall response as a group was much more variable than that of the younger subjects. The performance of some aged monkeys was impaired by low doses that did not affect young monkeys. Continued improvement was observed in some aged monkeys at the highest dose, which typically impaired young monkeys. These variable effects across aged subjects suggest that physostigmine cannot easily or reliably be used as an agent for treating geriatric cognition. Nevertheless, the differential age-related effects suggest that appropriate manipulation of the cholinergic system may eventually be developed to alleviate some of the cognitive impairments suffered by aged subjects.*

Recent pharmacological research suggests that dysfunctions in specific cholinergic mechanisms may be partially responsible for the declines in recent memory observed with old age. For example, blocking central cholinergic mechanisms (with scopolamine) induces an amnesia in young monkeys and humans that uniquely resembles that occurring naturally in aged monkeys and humans (*1, 2*). Simultaneous administration of the anticholinesterase physostigmine reduces the scopolamine-induced amnesia in both monkeys and humans, whereas simultaneous administration of central nervous system stimulants does not (*3*). Disrupting normal cholinergic mechanisms thus impairs performance on tasks requiring recent memory, and a dysfunction in necessary cholinergic mechanisms may contribute to age-related memory impairments. This hypothesis has been supported by biochemical evaluations of aged brains showing significant reductions in choline acetyltransferase activity and muscarinic receptor binding (*4*). These evaluations suggest that age-related cognitive impairments might be reduced by treatment with certain cholinomimetic agents. Physostigmine was recently tested in young adults, with limited improvement found at a single dose and impaired performance at higher doses (*5, 6*). In aged subjects, relatively little improvement was observed, but only a single dose was tested (*7*).

Recent comparisons of young and aged monkeys have demonstrated that aged monkeys suffer behavioral impairments similar to those characteristically reported for elderly humans, the foremost of which is in memory for recent events (*8*). Initial psychopharmacological tests with aged monkeys (*9*) suggest that the aged monkey can be a valid psychopharmacological model of human aging. Therefore, several doses of physostigmine were evaluated in young and aged monkeys (*Macaca mulata*), according to the same test procedure by which age-related memory impairments were demonstrated.

The young monkeys were two feral-born males and two feral-born females, estimated to be between 5 and 7 years old. Aged monkeys were seven females and one male (all feral-born, but imported and placed in various laboratories between the ages of 3 and 7 years), and estimated on the basis of their health records to be older than 18 years. All monkeys had previously received thousands of trials in the apparatus and test procedure used in this study, and were, therefore, familiar with the requirements of the task.

The apparatus (AGED, Automated

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