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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General Experimental Device) was designed to control for many variables not related to memory per se, but known to affect performance in both drugged and aged subjects (*2*, *8*). An automated, subject-paced procedure required the monkey to remember which of nine panels had recently been illuminated (Fig. 1). Figure 1 compares the performance of aged and young rhesus monkeys under different retention conditions and illustrates the specific impairment of aged monkeys under those conditions requiring recent memory.

To evaluate the effects of physostigmine on recent memory, six different doses were given to each monkey according to a randomized block design (*10*). The effects of these doses were

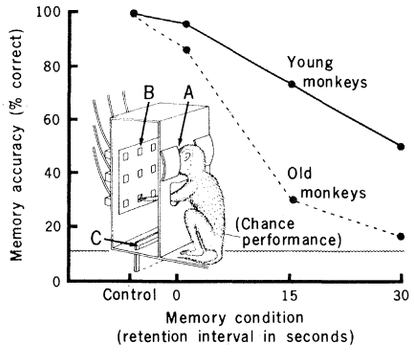

Fig. 1. Differences in performance of young and aged monkeys on automated, delayed-response procedure (used to assess recent memory), and inset drawing of the Automated General Experimental Device (AGED) used to collect these data. Features of the apparatus: *A*, stimulus observation window (through which the monkeys had been trained to look to initiate each trial); *B*, stimulus response panels (one of which is illuminated each trial, and to which the monkey must respond to obtain a food reinforcement); and *C*, a one-way viewing screen (which serves the dual functions of preventing the monkey from responding to, or seeing, the stimulus response panel during certain parts of the delayed-response trial). During the continuous-information condition, the panel remained illuminated, thus eliminating dependence on recent memory for accuracy. On the retention-interval conditions, the time between the extinction of the illuminated panel and the lowering of the one-way screen (which allowed the monkey to respond) provided an objective index of recent memory in the nonhuman primate. The control condition was presumably void of recent memory requirements, but shared all other features of the remaining retention conditions. The inability of the aged monkeys to remember the location of the illuminated stimulus declined as the retention interval was increased [$F(3,24) = 9.98$, $P < .001$] (*15*). Thus, it seems reasonable to conclude that the age-related difference on the longer retention conditions reflects genuine, age-related disabilities in functions necessary for accurate memory, and is not simply due to possible deficiencies in visual acuity, psychomotor coordination, selective attention, and so forth.

then compared with individual baseline control scores. The very steep dose-response function previously reported for young humans (*5*, *6*) was also found in young monkeys (Fig. 2A), and the same relative doses produced the greatest improvement and most reliable impairment in both species.

Although many 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 ($P < .05$) (Fig. 2B) (*11*). The performance of certain aged subjects declined significantly at the lowest doses of physostigmine (*12*), and three of the eight aged monkeys improved at the highest dose. Finally, three aged monkeys did not benefit from physostigmine at all, even though their impairment on this recent memory task was qualitatively similar to that of the other five monkeys who did improve.

The experimental design and procedure used should provide reasonable confidence that chance variations in baseline performance were not responsible for the drug effects in the aged monkeys, particularly since similar variability was not seen under saline control conditions. This variability is, in fact, reminiscent of several preliminary reports of different cholinomimetic agents in aged humans (*13*), which have often attributed the high degree of variability to methodological and measurement problems in the geriatric clinic. In the automated laboratory situation, however, in which test-sophisticated subjects served as their own controls and memory was measured objectively, problems of this sort should have been effectively controlled for or eliminated. That higher intersubject variability under physostigmine was still observed in the aged subjects implies that the differences between age groups reflect a genuine age-specific change in response to physostigmine, presumably involving some variable relevant to the mediation of recent memory. Little can yet be offered, however, in the way of a specific explanation for the age-specific changes (or their absence in certain cases). One possibility might be that the increased variability reflects day-to-day changes in individual sensitivity to pharmacological, cholinergic manipulation, perhaps related to continuously shifting imbalances in cholinergic function. Alternatively, some undefined, age-related changes in response to physostigmine may exist in certain (but not all) aged subjects. The data revealed, however, that differences in degree of impairment could not explain the differences in response to physostigmine within the aged group. More research is needed to help select between these and other hypotheses (*14*).

The lack of consistent facilitation should not be considered contradictory to the hypothesis that cholinergic mechanisms are important in age-related memory impairments. Even if one accepts that some cholinergic dysfunction is partially responsible for the deficits, it might be considered naïve to expect physostigmine to reverse the impairments in all aged subjects. Many aspects of cholinergic synthesis and transmission may become impaired during the aging process, all of which could ultimately produce a behavioral deficit in memory. Thus, simply increasing levels of acetylcholine, through the action of physostigmine, may be insufficient or ineffective in many cases of behavioral impairment resulting from an unspecified cholinergic dysfunction. It may be necessary to modify cholinergic function at more than one point in the metabolic pathway (for ex-

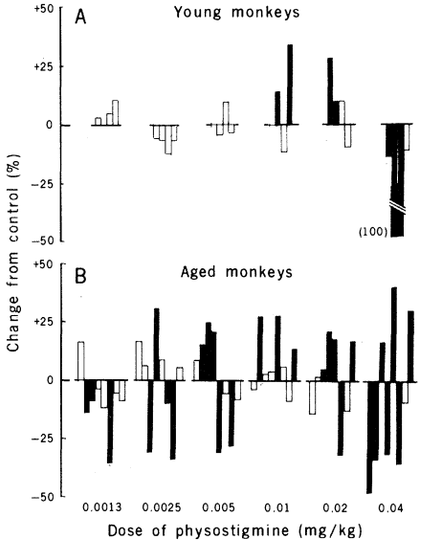

Fig. 2. Performance on memory-dependent, delayed-response trials by four young (A) and eight aged (B) rhesus monkeys injected with physostigmine. Each bar represents an individual monkey compared with that monkey's own baseline performance levels. Individual statistical confidence limits ($P = .01$) based on the control scores, were used to determine whether a change in performance under any dose of physostigmine reflected a reliable change from baseline performance for that particular monkey (solid bars). A maximum of two doses of physostigmine was given per week, with a minimum of 2 days separating each administration. Physostigmine was administered intramuscularly 15 minutes before behavioral testing. Since no differences were observed between nondrug and saline control scores, all data from these days were pooled for each monkey. Because of practical problems related to the length of this study, it was impossible to give any monkey the same dose more than once.

ample, in precursor uptake, transmitter synthesis, transmitter degradation) or, alternatively, to restore the balance between two or more age-altered neurotransmitter systems before a consistently positive effect on memory is obtained. Nevertheless, these results indicate that (i) reliable changes in performance on this memory task do occur under physostigmine, (ii) certain age-related differences in these effects also exist, and (iii) some aged monkeys perform significantly better under a number of short-term doses. Although these results do not provide strong support for the use of physostigmine as a reliable therapeutic agent for geriatric cognition, they do provide additional circumstantial evidence for an important cholinergic role in age-related memory impairments. More important, they provide an objective rationale for believing appropriate pharmacological manipulation of the cholinergic system (perhaps in conjunction with other neurotransmitter systems) may eventually be developed to alleviate some of the cognitive declines associated with advanced age.

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10. During each test session, each monkey was tested under equally distributed, 0-second (nonmemory) control intervals, as well as longer (memory-dependent) retention intervals. The duration of the longer retention intervals was adjusted so that all monkeys responded accurately between 50 and 70 percent of the time on each nondrug session. In this way, age-related differences in performance were controlled, providing an assessment of physostigmine unconfounded by differences in performance. The duration of the retention intervals for the four young monkeys (in order of appearance in Fig. 2) were 150, 68, 75, and 150 seconds. Similarly, the retention intervals for the eight aged monkeys were 40, 23, 45, 15, 23, 30, 23, and 4 seconds. These large differences in duration reflect the large difference in ability of the two age groups on this task after several months of training.
11. Individual analyses of variance revealed significantly greater variability between age groups on the three lower doses (0.0013 to 0.005 mg/kg; $F(7, 3) = 9.6$, 52.3, and 10.4, respectively). Age-related differences in variability were not observed on the three higher doses.
12. No impairment was seen at any dose in either age group on the 0-second control condition. These selective effects on the longer, memory-dependent intervals are often interpreted as evidence for disruption in centrally mediated mnemonic processes [H. J. Fletcher, in *Behavior of Nonhuman Primates*, A. M. Schrier, H. F. Harlow, F. Stollnitz, Eds. (Academic Press, New York, 1965), vol. 1, pp. 129-165].
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14. Still other alternatives include (i) Changes in the absorption and metabolism of drugs with age [J. R. Gillette, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 1901 (1979)], coupled with the relatively short half-life of physostigmine (*5*) could result in age-related differences in concentrations in the blood. These differences could result, in turn, in differences in the effective dose of physostigmine during the 30-minute test session. (ii) The two age groups might be differentially sensitive to adverse side effects of physostigmine, since no peripheral cholinergic blocking agents were administered to either group. Careful monitoring of each monkey revealed no overt effects or changes in response rates except at the highest dose. At this dose some monkeys in both age groups appeared somewhat lethargic, but three other aged monkeys actually improved. Thus, it is unlikely that differences in peripheral side effects could account for these results. The data do indicate that careful individual titration of doses of physostigmine might be necessary to enhance the chances of finding significant positive effects in aged subjects.
15. Data taken from R. T. Bartus *et al.* (*2*).
16. I thank B. Beer, D. Clody, and A. Lippa for comments on the manuscript; D. L. Fleming for helping to collect the data; and M. Concannon and C. Bartus for helping to prepare the manuscript.
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Elimination of Metabolic Cooperation in Chinese Hamster Cells by a Tumor Promoter

Abstract. *Wild-type Chinese hamster V79 cells (6-thioguanine-sensitive) reduce the recovery of 6-thioguanine-resistant cells when they are cultured together at high densities, through a form of intercellular communication (metabolic cooperation). Cooperation is inhibited by 12-O-tetradecanoyl phorbol-13-acetate, rescuing the 6-thioguanine-resistant cells. These results may be useful in the study of an aspect of the mechanism of tumor promotion and in assaying for promoters.*

Metabolic cooperation is a form of intercellular communication in which the mutant phenotype of enzyme-deficient cells is corrected by normal cells or by different mutant cells. Two types of metabolic cooperation have been observed: one requires cell-to-cell contact, the other does not. A typical example of the former is the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) system described by Subak-Sharpe *et al.* (*1*). Subsequent investigations have indicated that metabolic cooperation also occurs with the products and functions of the enzymes coded by genes for adenine phosphoribosyltransferase (*2*), thymidine kinase (*2*), Na^+, K^+-activated adenosinetriphosphatase (*3*), β-adrenergic receptor (*4*), and plasminogen activator (*5*). The second type of metabolic co-

Table 1. The effects of phorbol ester analogs on the recovery of the 6-TG^r cells. The percentage of recovery for each treatment group was obtained by averaging the results for 21 plates, each of which contained 8×10^5 6-TG^s cells and 100 6-TG^r cells. The concentration of phorbol, TPA, and all phorbol ester analogs was 1 ng/ml. Statistical significance was determined according to a modification of the Student-Newman-Keuls test. Recovery of cells treated with TPA, phorbol-12,13-didecanoate, and phorbol-12,13-dibutyrate was superior to that of the control group at the $P < .01$ level of confidence; for cells treated with 4-*O*-methylphorbol-12-myristate-13-acetate, recovery was superior to that of the control group at $P < .05$. For each treatment group there were six control plates, in which 6-TG^r cells were cultured alone. The phorbol drugs had no significant effect on their attachment and growth. Tumor-promoting potential was rated on a scale of 0 to 4, with 0 representing no significant activity and 4 the greatest activity.

Phorbol analog	Tumor-promoting activity in vivo	Percentage recovery ± standard error
Control (ethanol)	0	26.7 ± 1.3
Phorbol	0	27.5 ± 1.3
4-α-Phorbol-12,13-didecanoate	0	31.6 ± 1.5
Phorbol-12,13-diacetate	1	32.8 ± 1.1
4-*O*-Methylphorbol-12-myristate-13-acetate	1	33.5 ± 1.5
Phorbol-12,13-dibutyrate	2	51.0 ± 1.9
Phorbol-12,13-didecanoate	3	91.5 ± 2.2
Phorbol-12-myristate-13-acetate	4	100 ± 2.3

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