3. J. Mayer, *Ann. N.Y. Acad. Sci.* **63**, 15 (1955); _____ and D. Thomas, *Science* **156**, 328 (1967.
4. N. B. Marshall, R. J. Barnett, J. Mayer, *Proc. Soc. Exp. Biol. Med.* **90**, 240 (1955); A. F. Debons, I. Krimsky, A. From, R. J. Cloutier, *Am. J. Physiol.* **217**, 1114 (1969); A. F. Debons, I. Krimsky, A. From, *ibid.* **219**, 938 (1970).
5. K. Sakata, S. Hayno, H. A. Sloviter, *Am. J. Physiol.* **204**, 1127 (1963); M. J. Conway, C. J. Goodner, J. H. Werrbach, C. C. Gale, *J. Clin. Invest.* **48**, 1349 (1969); L. A. Frohman, E. E. Muller, D. Cocchi, *Horm. Metab. Res.* **5**, 21 (1973); E. E. Muller, L. A. Frohman, D. Cocchi, *Am. J. Physiol.* **224**, 1210 (1973).
6. J. P. Flatt, G. L. Blackburn, J. B. Standbury, *Metabolism* **23**, 151 (1974); E. M. Stricker and N. Rowland, *J. Comp. Physiol. Psychol.* **92**, 126 (1978).
7. E. M. Stricker, M. B. Zimmerman, M. I. Friedman, M. J. Zigmond, *Nature (London)* **267**, 174 (1977).
8. F. M. Matschinsky, J. E. Ellerman, J. Krzanowski, J. Kotler-Brajtburg, R. Landgraf, R. Fertel, *J. Biol. Chem.* **246**, 1007 (1971); G. M. Grodsky, R. Fanska, L. West, M. Manning, *Science* **186**, 536 (1974); D. M. Dean and E. K. Matthews, *J. Physiol. (London)* **210**, 258 (1970); S. Price, *Biochim. Biophys. Acta* **318**, 459 (1973); L. A. Idahl, *Diabetes* **25**, 450 (1976).
9. A. S. Pagliara, S. N. Stillings, W. S. Zawalich, A. D. Williams, F. M. Matschinsky, *Diabetes* **26**, 973 (1977); J. E. Gerlich, M. Langlois, C. Noacco, J. H. Karam, P. H. Forsham, *Science* **182**, 171 (1973); J. E. Gerlich, M. Langlois, C. Noacco, M. Lorenzi, J. H. Karam, P. F. Forsham, *J. Clin. Invest.* **58**, 320 (1976).
10. J. S. Dunn, H. L. Sheehan, N. G. B. McLetchie, *Lancet* **1943-I**, 484 (1943); F. D. W. Lukens, *Physiol. Rev.* **28**, 304 (1948); R. C. McEvoy and O. D. Hegre, *Diabetes* **26**, 1140 (1977).
11. G. Bhattacharya, *Science* **120**, 841 (1954); W. S. Zawalich and L. M. Beidler, *Am. J. Physiol.* **224**, 963 (1973); A Scheynius and I. B. Taljedal, *Diabetologia* **7**, 252 (1971); A. A. Rossini, M. Berger, J. Shadden, G. F. Cahill, Jr., *Science* **183**, 424 (1973); *Diabetes* **24**, 516 (1975).
12. M. L. McDaniel, C. E. Roth, C. J. Fink, P. E. Lacy, *Endocrinology* **99**, 535 (1976); S. J. Cooperstein and D. Watkins, *J. Pharmacol. Exp. Ther.* **204**, 230 (1978).
13. A. Niki, H. Niki, I. Miwa, B. J. Lin, *Diabetes* **25**, 574 (1976).
14. W. S. Zawalich, *Comp. Biochem. Physiol.* **44A**, 903 (1973).
15. W. Jakinovich, Jr., and I. J. Goldstein, *Brain Res.* **110**, 491 (1976).
16. P. J. Kulkosky, D. Porte, Jr., S. C. Woods, *Experientia* **31**, 123 (1975).
17. Blood samples were taken from the tip of the tail in the manner of S. C. Woods, W. Makous, and R. A. Hutton [*J. Comp. Physiol. Psychol.* **69**, 301 (1969)]. Blood glucose was determined by the microglucose oxidase method of D. O'Brien, F. A. Ibbott, and D. O. Rogerson [*Laboratory Manual of Pediatric Micro-Biochemical Techniques* (Harper & Row, New York, 1968)].
18. A. N. Epstein, S. Nicolaidis, R. Miselis, in *Neural Integration of Physiological Mechanisms and Behavior*, G. J. Mogenson and F. R. Calerasu, Eds. (Univ. of Toronto Press, Toronto, 1975), p. 148.
19. D. A. Booth, *Physiol. Behav.* **8**, 1069 (1972); G. P. Smith and A. N. Epstein, *Am. J. Physiol.* **217**, 1083 (1969). It is noteworthy that humans also have increased sensations of hunger when injected with 2-deoxyglucose [see P. Thompson and R. G. Campbell, *Science* **198**, 1065 (1977)].
20. Methylxanthenes are postulated to exert this action by inhibiting adenosine 3′,5′-monophosphate degradation [see Stricker *et al.* (*7*)].
21. Data were analyzed by a repeated measures analysis of variance. There was a significant interaction of food intake over time between the controls and the IVT-alloxan rats in each condition.
22. G. P. Smith, in *Obesity in Perspective*, G. A. Bray, Ed. (Government Printing Office, Washington, D.C., 1976); E. E. Müller, D. Cocchi, A. Forni, *Life Sci.* **10**, 1057 (1971); see also Epstein *et al.* (*18*) and Balagura and Kanner (*2*).
23. Y. Oomura, M. Ohta, H. Kita, S. Ishibashi, T. Okajima, T. Ono, Proceedings of the Seventh International Conference on the Physiology of Food and Fluid Intake (1977).
24. This research was supported by research grant AM 17844 from the National Institutes of Health (to S.C.W.). We thank N. J. Kenney, D. Porte, Jr., J. B. Simpson, and P. H. Smith for their help.

2 May 1978; revised 11 July 1978

Short-Term Memory: The "Storage" Component of Human Brain Responses Predicts Recall

Abstract. *An evoked potential component with a poststimulus peak at about 250 milliseconds is related to the storage of information in short-term memory. This storage component was found in an investigation of brain potentials in relation to a number and letter comparison task. In replications of this experiment at three different light intensities spaced 1.0 log unit apart, the component had essentially the same waveform and pattern of scores. The memory storage interpretation was confirmed in a behavioral experiment that probed short-term memory. Recall was predicted by the magnitude of the storage component.*

A critical ingredient in most human information processing is the short-term storage of incoming information so that it can be integrated with other incoming information. Various memory processes have been proposed as retainers of information for various lengths of time (*1*), for example, a sensory register, a short-term store, and a long-term store. We now present electrophysiological and behavioral evidence for a neural process related to storage in short-term memory. A latent component of electrically recorded brain responses [evoked potentials (EP's)] with a poststimulus peak about 250 msec was found to be related to the storage of stimulus information for later use in tests comparing numbers and letters.

We discovered the storage component of the EP and tentatively interpreted it as being associated with information storage in an experiment in which the latent components and component scores of the brain responses from 12 subjects were obtained by a varimaxed principal components analysis (*2*). The storage component, which is the focus of this report, was one of eight orthogonal EP components obtained from that analysis.

The generality of the storage component and its independence of the physical characteristics of the stimuli were tested in further experiments (*3*). The same stimuli and procedures were used except that the intensity of all stimuli within a run of 102 trials was ten times greater, the same, or one-tenth that of the original experiment (*4*).

Two numbers and two letters were flashed individually in random order at intervals of 3/4 second preceded and followed by a blank flash. The subject's task was to compare the two numbers on number-relevant runs, the letters being irrelevant to the task. On the other half of the runs, the numbers were irrelevant and the task was to compare the two letters (*5*).

The stimulus processing demanded by the task depended on a number of factors, including whether (i) number or letter stimuli were task-relevant, (ii) the number or letter class of stimulus could be anticipated, and (iii) the character was the first or second relevant stimulus of the pair to be compared. For the first relevant stimulus in each trial, the information had to be stored by the subject until the second relevant stimulus occurred, after which the comparison could be made. While the subject was performing the letter or number comparison tasks, electrical brain activity [electroencephalogram (EEG)] was recorded from scalp electrodes (*6*).

By averaging the brain activity evoked by stimuli for similar conditions, averaged EP's were obtained for 16 conditions: relevant and irrelevant numbers and letters at four intratrial positions. From trial to trial, the first number (or letter) occurred in intratrial positions 1, 2, or 3 and the second in intratrial positions 2, 3, or 4. To simplify interpretations, certain EEG data were discarded, so the EP's for intratrial positions 1 and 2 were based only on the first number and letter stimuli presented within each trial, whereas the EP's for intratrial positions 3 and 4 were based only on the second number and letter stimuli. For each of the three intensities, EP's were collected in the same manner, and each of the three sets of data was analyzed separately (*7*). Latent components and component scores of each of the data matrices were computed according to varimaxed principal components analysis (*2*, *8*).

In all three data sets, an EP component emerged that was strikingly similar to the storage component previously found (*2*) with regard to both waveform and relative magnitude for the 16 conditions (Fig. 1, A to C). The waveforms of these components are similar in all four sets of data, reaching their maximum about 250 msec after the stimulus. The coefficients of factorial similarity among

0036-8075/78/1215-1211$00.50/0

the waveforms from the four data sets were high, ranging between .85 and .99. For the previous experiment the maximum was at 250 msec; for the new data the maximums are at 250, 250, and 270 msec for high, middle, and low light intensities.

The storage component tends to be positive for stimuli whose information needs to be stored by the subject. Thus, the magnitude of the storage component was more positive for the first of the two relevant stimuli presented on each trial (intratrial positions 1 or 2) than for the second relevant stimulus (intratrial positions 3 or 4). The storage component was also relatively positive for the irrelevant stimuli when they occurred in intratrial position 1. Extending the storage interpretation to this result leads to the hypothesis that an irrelevant stimulus in position 1 is stored in memory, whereas irrelevant stimuli in positions 2, 3, and 4 are not. This may be related to the limited capacity of short-term memory and the interference of the storage of irrelevant information with the processing of relevant information. The difference in the storage component scores for relevant and irrelevant stimuli in intratrial position 2 is evidence that this component is not related simply to an order effect. Nor is this EP component related to the amount of processing, which is presumably greatest for the comparison operations following the second relevant stimulus, next most for the storage operations associated with the first relevant stimulus, and least for the irrelevant stimuli. Nor does this EP component reflect a general relevant-irrelevant distinction (*9*). The storage component did not consistently distinguish between number and letter processing nor between number and letter stimuli. The simplest interpretation is that this EP component is related to the storage of information in the subject's short-term memory. More specifically the component may reflect the process of reading information out of a sensory register into short-term memory. Not only were the storage component scores related to memory storage conditions, but also the timing of this EP component (waveforms in Fig. 1) is appropriate for information storage. The maximum of the storage component was at 250 msec. This is an appropriate time for storing information needed later, according to evidence that the sensory register (icon) is fading about that time (*10*).

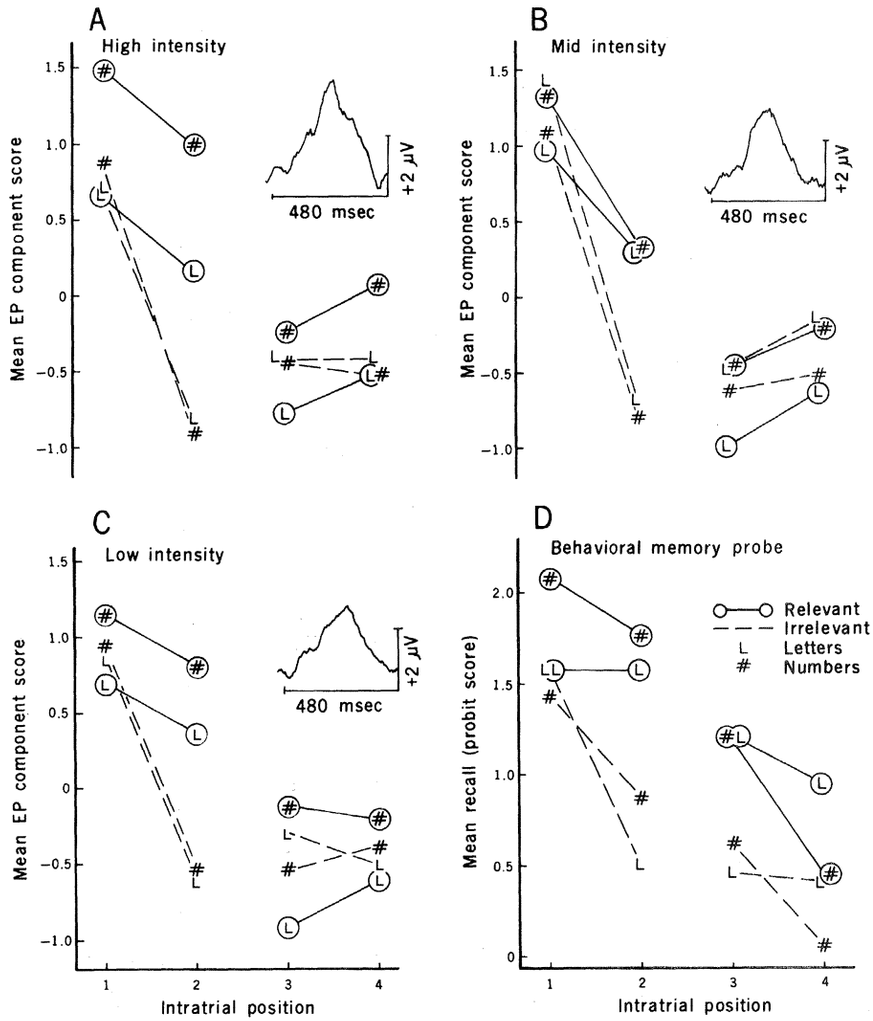

Fig. 1. Storage component of brain responses and recall for experimental conditions in which the demands of short-term memory vary. A letter (*L*) or number (*#*) was flashed in each of the intratrial positions (spaced 3/4 second apart). The task (comparision of letters or numbers) required short-term memory for relevant stimuli (circled) in positions 1 and 2. Brain response and behavioral measures are in *z*-score units. (A–C) Evoked potential (*EP*) storage components were obtained from separate varimaxed principal components analyses on evoked potentials obtained with stimuli at three light intensities spaced 1.0 log unit apart. Insets show the storage component waveforms scaled appropriately for relevant numbers in position 1; the fundamental time course of the component (rotated factor loadings multiplied at each of 102 time points by standard deviations) was multiplied by the mean storage component score for that condition; the 480-msec calibration bar begins at the stimulus flash. Storage component waveforms peaked at 250, 250, and 270 msec for high, middle, and low intensities, respectively. Evoked potentials obtained from scalp electrode at the central-parietal midline referred to linked earlobes. (D) Mean recall by 52 subjects as measured by an occasional, random memory probe ("What was the last character?") while performing the primary task of comparing numbers or letters. Percentage of correct responses was converted to equivalent *z*-scores (probits).

The results demonstrate the robustness of the storage component in the face of large differences in the physical parameters of the stimuli. That the storage component represents neural activity in the stimulus-response sequence occurring later than the simple processing of sensory input is supported by two findings. (i) It is independent of whether the stimuli are numbers or letters, and (ii) changes in stimulus intensity sufficient to markedly alter the overall EP have only a small effect on the storage component. Further, that the storage component is seen after the simple processing of sensory input, including recognition of the informational content of the stimulus, is indicated by the differences in response to identical physical stimuli when they play different roles in the information processing task. For example, in Fig. 1, compare the component scores to relevant and irrelevant stimuli in intratrial position 2 and compare the component scores to relevant stimuli in positions 1 or 2 with those in positions 3 or 4. Along the time continuum, the storage component precedes both the behavioral response and the comparison operations,

which cannot occur until intratrial positions 3 or 4. The storage component (maximum at about 250 msec) occurs before an EP component related to alphabetic comparison (maximum at about 350 msec) (*2*).

Our tentative interpretation that this EP component is related to storage was based on the differential scores for the first and second relevant stimuli within each trial. However, finding high storage component scores for irrelevant stimuli in position 1 required ad hoc interpretations in order to maintain the storage identification. Therefore, we more directly checked the storage interpretation by a behavioral experiment designed to assess storage in short-term memory.

We used a memory probe technique to test recall of individual stimuli for each of the 16 conditions in the electrophysiological experiments. Experimental sessions and data collection were conducted by experimenters not associated with the previous experiments and not aware of the hypothesis being tested. The experimental procedure was the same as for the collection of brain responses with the addition of occasional memory probes. The primary task on each trial was to compare pairs of numbers on one run of 102 trials and pairs of letters on a second run (*11*). Within each run of 102 trials, eight randomly located memory probes were selected to test the recall of a letter and a number in each of the four intratrial positions. Without prior warning of when probes would occur, blank flashes were delivered 3/4 and $1^1/_2$ seconds after the stimulus being probed was presented, and the subject was asked what the last character was. These blank flashes were used to mask the probed stimulus and to delay the recall report in order to reduce the effects of very short term sensory registers. From each subject, one such recall probe was obtained for each of the 16 conditions (eight probes each in a number-relevant and a letter-relevant run). The percentage of correct recalls from 52 subjects (29 female and 23 male college students) are given in z-score units (Fig. 1D) (*12*).

The pattern of correct recalls resembles the pattern of storage component scores (Fig. 1, A to C) with better memory for relevant stimuli in intratrial positions 1 and 2 and for irrelevant stimuli in position 1. The six correlations among the four patterns of 16 means each (Fig. 1) ranged from .71 to .97.

Three features of the data are common to both the storage component of the brain responses and the subjects' short-term memory. (i) The first relevant stimulus on a trial (intratrial position 1 or 2) gave higher scores than did the second relevant stimulus (positions 3 or 4); (ii) the scores were high for both relevant and irrelevant stimuli in intratrial position 1; and (iii) in position 2, the scores were higher for relevant than for irrelevant stimuli.

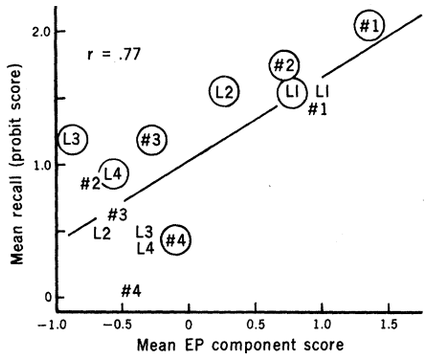

Fig. 2. Behavioral recall as a function of brain response storage component score. The linear regression line is shown. The mean EP component score is the average of storage component scores found at the three stimulus intensities (Fig. 1, A to C). Mean recall from 52 subjects was obtained with the memory probe (Fig. 1D). Experimental conditions: letters (*L*) or numbers (#); intratrial positions (1 to 4); relevant to primary task (circled) or irrelevant (not circled).

Recall performance was plotted as a function of mean storage component score (averaged over the three intensities) (Fig. 2). Thus, the storage interpretation was confirmed by predicting recall performance on the basis of the storage component of brain responses (r = .77). The accuracy of this prediction is impressive if one considers that behavioral recall is not solely a function of storage but is generally considered to be influenced by other factors, including retrieval mechanisms.

One of the reasons the storage component has not been found in other EP research is that it may be partially masked by a positive peak in the EP, which often occurs slightly before 250 msec. Hence, measurement based on peaks of the average EP may miss the latent storage component that was derived by principal components analyses that assess the relationships among all the time points and decompose EP's into independent sources of variation. Now that the storage component has been described and its waveform is known, it may be measured by computing component scores directly in other EP studies without doing a complete principal components analysis.

Regardless of the reasons stimulus information is sometimes not stored in an individual's memory, it would be useful from theoretical, experimental, educational, and clinical standpoints to be able to determine whether or not stimulus information is being stored in memory. If further research sustains the interpretation that the storage component of EP's reflects the process of storing information in short-term memory, this brain response component may be used to assess storage per se, uncontaminated by retrieval mechanisms. The storage component of EP's holds the promise of serving this practical function as well as of providing an entry to understanding the neural processes related to memory.

ROBERT M. CHAPMAN
JOHN W. MCCRARY
JOHN A. CHAPMAN

Center for Visual Science,
University of Rochester,
Rochester, New York 14627

References and Notes

1. For example, R. C. Atkinson and R. M. Shiffrin, in *The Psychology of Learning and Motivation: Advances in Research and Theory*, K. W. Spence and J. T. Spence, Eds. (Academic Press, New York, 1968), vol. 2, pp. 89–195.
2. R. M. Chapman, in *International Symposium on Cerebral Evoked Potentials in Man* (Presses Universitaires de Bruxelles, Brussels, 1974), pp. 38–42 (abstract); ———, J. W. McCrary, H. R. Bragdon, J. A. Chapman, in *Progress in Clinical Neurophysiology*, vol. 6, *Cognitive Components in Cerebral Event-Related Potentials and Selective Attention*, J. E. Desmedt, Ed. (Karger, Basel, in press). The analysis model assumes that the EP's are linear, weighted sums of a number of independent components, each with a fixed time course. The time courses of the components and the weights (component scores) are derived from the data by correlating the responses at every time point and factoring the resulting correlation matrix of 102 time points with unities retained in the diagonal. Loosely speaking, time points that vary together are identified as belonging to the same component; any time point may belong in part to more than one component. The number of components was limited by the "eigenvalue = one" criterion, and rotation was performed using normalized varimax criterion [H. Kaiser, *Psychometrika* **23**, 187 (1958)].
3. R. M. Chapman, *Electroencephalogr. Clin. Neurophysiol.* **43**, 778 (1977); in *Visual Psychophysics: Its Physiological Basis*, J. C. Armington, J. Krauskopf, B. R. Wooten, Eds. (Academic Press, New York, 1978), pp. 469–480.
4. Light intensity was controlled by interposing neutral-density filters (Wratten). The middle light intensity was approximately 0.3 cd/m² and was about 2.0 log units above threshold for character recognition.
5. By appropriately moving a two-way switch at the end of each trial, the subject indicated whether the first or second number was larger on number-relevant runs and similarly indicated the alphabetic order on letter-relevant runs. The numbers and letters were randomly selected (1 to 6, A to F) and the sequences of numbers and letters were randomized. Nearly every stimulus was processed appropriately by the subjects, with a performance accuracy of better than 99 percent. All stimuli were flashed at the same spatial location by a Bina-View display equipped with a stroboscope (Grass) [R. M. Chapman, in *Psychophysiology of Thinking*, F. J. McGuigan and R. Schoonover, Eds. (Academic Press, New York, 1973), pp. 69–108].
6. Data reported here were recorded with monopolar electrodes from the midline central-parietal area (CPZ) to linked earlobes. Frequency bandpass was 0.3 to 70 Hz; 102 samples at 5-msec intervals were obtained beginning 30 msec before each stimulus presentation.
7. Each EP consisted of amplitude measurements in microvolts at 102 time points spanning 510

msec. The data were collected from one subject over a series of ten sessions. The data set at each intensity contained 160 EP's (16 conditions by ten replications), each based on averaging the EEG to 34 to 51 stimuli (depending on the intratrial list position).

8. W. J. Dixon, Ed., *BMDP Biomedical Computer Programs* (Univ. of California Press, Berkeley, 1975), program BMDP4M.
9. The storage component was orthogonal to a number of other uncorrelated EP components, including P300 and CNV-resolution, which were also found in the analyses. Thus, this storage component is not just a variant of well-known EP components, but rather, is orthogonal to them in a formal analysis. Also, this storage component was not correlated with eye movements or with the amount of alpha in the EEG.
10. G. Sperling, *Psychol. Monogr.* **74**, No. 11 (whole No. 498) (1960); A. O. Dick, *Percept. Psychophys.* **16**, 575 (1974).
11. The order of number-relevant and letter-relevant runs was reversed for half the subjects. Practice trials on the primary task were given until ten consecutive trials were correct. The average performance on the primary task was 97.5 percent correct. The light intensity was approximately 2.8 log units above threshold for character recognition.
12. Percentage of correct responses were converted to probit scores (z-score units); 50 percent and 98 percent thus become 0.0 and +2.05 probit scores.
13. H. R. Bragdon collected the data in the EP experiment. D. Gershowitz and J. K. Martin collected the data in the behavioral memory-probe experiment. Supported in part by PHS grants EY01593 and EY01319 and contracts N00014-77-C-0037 and CNA SUB N00014-76-C-0001 from the Office of Naval Research.

22 June 1978; revised 21 August 1978

Exponential Decrease During Aging and Random Lifetime of Mouse Spermatogonial Stem Cells

Abstract. *Variation in the number of spermatogonial stem cells during the life-span of the mouse was examined by assaying the number of clonogenic cells, that is, spermatogenic stem cells, surviving known doses of radiation. The results indicated that the stem cell number decreased exponentially with age.*

In general, stem cells have been defined as undifferentiated cells with the capability of limited or unlimited self-renewal, although their real nature has not been well clarified (*1-4*). If spontaneous mutations are inevitable at every DNA replication or mitosis of mammalian cells in vitro or in vivo (*5*), and if stem cells have to be genetically stable, it seems unreasonable that they would have unrestricted infinite reproductivity. A finite lifetime of cells cultured from human embryos has been reported and has been considered to be a factor responsible for limited life-span and aging of organisms (*6*).

In the work described herein, we used a microcolony assay method for mouse spermatogonial stem cells (*7*) to determine the changes in stem cell number during the life-span of the mouse. The testes of C_3Hf/Bu male mice of various ages from our SPF (specific pathogen-free) breeding colony were irradiated from two opposing ^{137}Cs sources (1058 rad/min). Each mouse was confined in a Lucite box in air without anesthesia during irradiation. Details of the microcolony assay have been described elsewhere (*7*). Briefly, testes were removed and fixed in Bouin's solution 35 days after irradiation. Histological sections taken at the equator of each testis were stained with hematoxylin and eosin. Tubule cross sections showing regenerating seminiferous epithelium (that is, differentiated spermatogonia or cells at later stages of spermatogenesis) were scored with a light microscope (×100), while the total number of cross sections was counted with a projection microscope. On the assumption that one surviving stem cell can regenerate an island of seminiferous epithelium at any age, and since radiosensitivity did not vary significantly with age (Fig. 1), and the total number of tubules cross-sectioned remained constant, the surviving fraction after a certain dose reflects the initial number (prior to irradiation) of stem cells.

Figure 2 shows survival fractions of spermatogonial stem cells after irradiation of mice of various ages with either 1000 or 1200 rads. In both groups of mice the surviving fraction decreased exponentially with age between 7 and 122 weeks.

After Hayflick demonstrated (*6*) a lim-

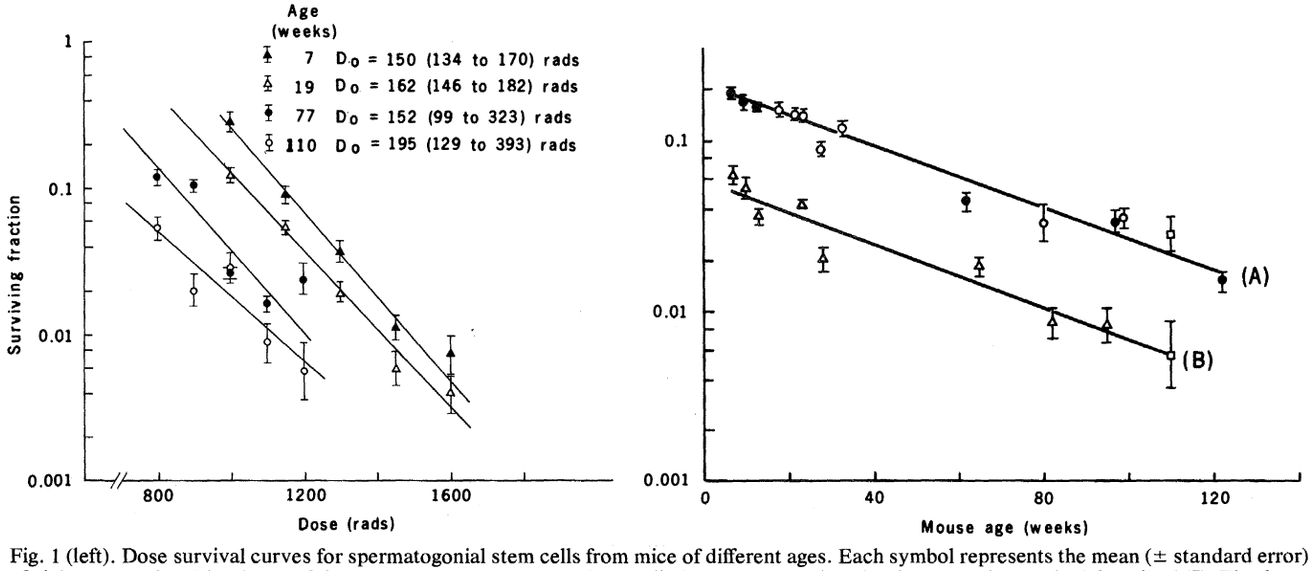

Fig. 1 (left). Dose survival curves for spermatogonial stem cells from mice of different ages. Each symbol represents the mean (± standard error) of eight to ten mice. The slopes of the curves and 95 percent confidence limits were determined by the regression method described (*7*). The doses (with 95 percent confidence limits) required to reduce survival by one natural logarithm (D_0) were 150 (134 to 170) rads at 7 weeks, 162 (146 to 182) rads at 19 weeks, 152 (99 to 323) rads at 77 weeks, and 195 (129 to 393) rads at 110 weeks. Fig. 2 (right). Fraction of tubular cross sections showing regeneration of seminiferous epithelium after irradiation with (A) 1000 rads or (B) 1200 rads at various ages. Different symbols indicate separate experiments. Each symbol represents the mean (± standard error) of ten to 15 mice. Lines were fitted by computer using a least-squares regression analysis. The times (with 95 percent confidence limits) required to reduce stem cell number by one natural logarithm were for line (A) 49 (44 to 56) weeks and for line (B) 47 (36 to 68) weeks.

0036-8075/78/1215-1214$00.50/0