was omitted in the retention trials 3 days after puromycin injection, since there was, within the design of the test, no right or wrong choice. As shown in Table 3, on testing for memory the first choice of all animals was consistent with the first learning experience, as were the large majority of subsequent choices. In view of consistent results with numerous untreated animals on various schedules of learning and reversal learning, only two control animals (Table 3) were used in this series. All these untreated animals, in sharp contrast to the experimental group, made choices consistent with their second, or reversal, learning. Because the experimental animals were able to perform the older position habit efficiently and consistently, this experiment offers strong evidence that the effect of puromycin in destroying a recent habit is not due to disorganization or incapacitation of the animal.

A beginning has been made in testing for the specificity and reversibility of the puromycin effect. Numerous control injections of saline, of subliminal concentrations of puromycin, and of puromycin hydrolyzed at the glycosidic bond were without effect on memory. Most animals treated with effective doses of puromycin were demonstrated to be capable of relearning after loss of memory, though the process of relearning, particularly with high doses of puromycin, often required considerably more trials than in the initial training experience. This aspect of the effects of puromycin will be reported more extensively at a later time.

Although the effective locus of short-term memory clearly appears different from that of longer-term memory we cannot now define the difference with precision. It does appear that the area around the caudal rhinal fissure, likely entorhinal cortex, carries the short-term memory trace, since short-term memory was retained with ventricular injections but lost with temporal injections. The part played by the hippocampus will not become evident until experiments are performed which provide for exposure of the entire temporal cortex to puromycin while the hippocampus is spared. Similarly, we cannot state whether the locus of longer-term memory is confined to the cortex or whether other parts of the brain, principally the hippocampus, are also involved. It can only be said that our observations are consistent with the evidence and conclusions of others (*4*), that the hippocampal zone is the site of recent memory and, that an extensive part of the neocortex is concerned with longer-term memory.

It must be emphasized that our results, although apparently clear-cut in important particulars, should be interpreted at this time with caution. We are in the process of obtaining more precise information, for example, on the localization of puromycin after intracerebral injection. Histological studies on the cells of the hippocampus and cortex must be completed. Determinations must be made of the degree of suppression of protein synthesis, and, particularly in view of the negative behavioral results with subcutaneous puromycin (*3*), the possibility must be kept in mind that loss of memory after intracerebral injection of puromycin may be owing to effects not related to changes in protein synthesis. Further, it remains to be shown that other learning situations, currently being investigated, and other animals are comparable to the mouse in the training experience we have used (*5*).

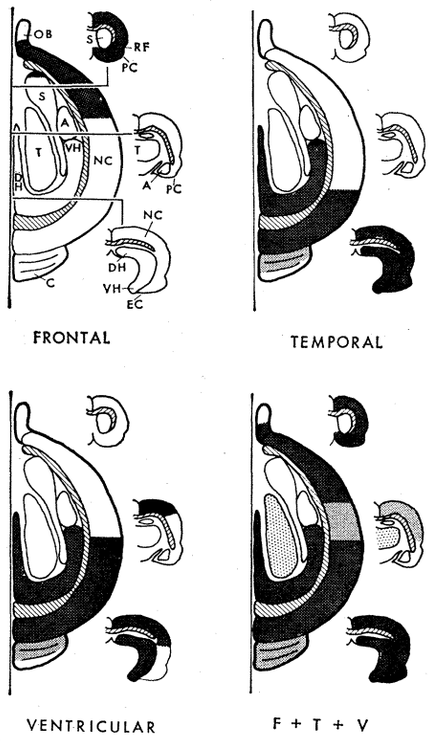

Fig. 1. Spread of fluorescein after intracerebral injection. The diagrams at the left indicate structures viewed from the top after removal of a horizontal section of the hemisphere; at the right, cross (frontal) sections of the hemispheres at the level indicated in the diagram for frontal injections. Relative intensity of staining is indicated by relative density of stippling. With all injections there was intense staining, not shown in the diagram, of the corpus callosum (cross hatched). Abbreviations: *A*, amygdaloid nucleus; *C*, cerebellum; *DH*, dorsal hippocampus; *EC*, entorhinal cortex; *NC*, neocortex; *OB*, olfactory bulb; *PC*, pyriform cortex; *RF*, rhinal fissure; *S*, corpus striatum; *T*, thalamus; *VH*, ventral hippocampus; *F* + *T* + *V*, frontal + temporal + ventricular injections.

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Fragment Sizes Produced from T5 Bacteriophage DNA Molecules by Acid Deoxyribonuclease

Abstract. *The action of acid deoxyribonuclease on T5 bacteriophage DNA results in a "random distribution" of sizes of duplex fragments as judged by electron microscopy. No preferred subunit size resulting from the "single hit" action can be detected down to lengths of 0.1 micron or less. The results are in agreement with the coexistence of a "single-hit" and a "double-hit" kinetics.*

An investigation (*1*) on the digestion of deoxyribonucleic acid (DNA) by acid deoxyribonuclease has shown that two different types of degradation take place at the same time, (i) a "single-hit" degradation causing the simultaneous breakage of both DNA strands at the same place, and (ii) a "double-hit" degradation of the type already known for pancreatic deoxyribonuclease (*2*). This second mechanism becomes effective in breaking DNA molecules only after a lag time. Results presented (*1*) suggest that in the case of DNA from chicken erythrocytes the "single-hit" degradation stops after a molecular weight of the order of 0.5×10^6 has been attained. This observation raises the possibility that the "single-hit" degradation is breaking the molecule at spe-

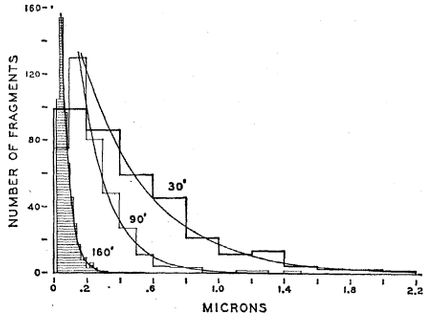

Fig. 1. Fragment size distributions in the 30-, 90-, and 160-minute digestion products of whole T5 DNA molecules. The smooth curves are the best-fit theoretical distributions calculated on the basis of random scission corresponding to 97, 231, and 832 scissions per original 40-μ molecule. For the 160-minute histogram the number scale is three times that shown.

cific sites to produce fragments which are about the size of genes. It appeared interesting to investigate this point on T5 bacteriophage DNA by studying the change in particle size during the course of digestion by enzyme.

The T5 DNA molecules are thought to be linear duplexes with a molecular weight of 77 to 82 million (*3*). Unbroken molecules were purified chromatographically and digested with a preparation of acid deoxyribonuclease purified from hog spleen (*4*). The reaction mixture of 40 μg of DNA and 0.8 μg of enzyme per milliliter in acetate buffer (ionic strength 0.15, *p*H 5.4) was held at 26°C and samples were removed at different times and mixed with equal volumes of chilled phosphate buffer (*p*H 7.5) containing 0.03*M* Mg^{++} to stop the reaction.

Samples taken at 30, 90, and 160 minutes were then examined by electron microscopy. A drop of the DNA solution, after dialysis against volatile buffer of low ionic strength (0.01*M* ammonium carbonate plus 0.02*M* ammonium acetate), was placed on each of a number of thin carbon films supported on nickel electron-microscope grids. Each drop was then drawn off horizontally with absorbent paper, and the DNA fragments were left lying oriented on the carbon film surface, where they were made visible by shadowing them broadside with Pt-Pd applied at a low angle. The grids were examined in an RCA EMU-3 electron microscope with the magnification calibrated by a standard grating replica. Local shadow angle was determined by admixed 880 Å polystyrene spheres.

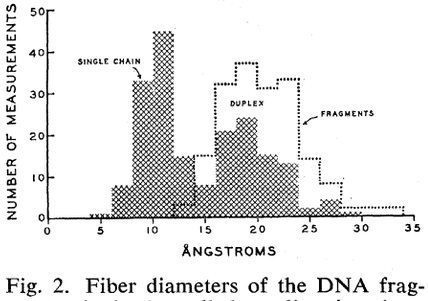

Fig. 2. Fiber diameters of the DNA fragments obtained at all three digestion times compared with control measurements of single polynucleotide chains and duplex molecules in a sample of partially renatured T4 DNA by identical procedures of microscopy and measurement. The dotted histogram corresponds to the diameters of the digestion fragments. The scatter is mostly attributable to the uncertainties in shadow-length measurement caused by background roughness in the micrographs.

Several different sets of grids were made and examined. The fragments were nonuniformly distributed over the grid surface. All fragments seen were photographed, and in every case where both ends could be clearly distinguished the lengths were measured. An exception was made in a few plates which contained more countable fragments than were needed, in which cases a strip was marked off and all particles within it were counted. In general, all fragments were well separated from one another, and no significant side-to-side aggregation (which under these conditions always leads to an apparent increase in fiber diameter) was seen. The fiber diameter on any plate appeared uniform to the eye and to measurement. In some crowded plates a fair number of fragments were rejected as a result of unclear ends and of merging with other fragments, but in the majority of plates very few had to be rejected. All measurements of length were made "blind," and no histograms were plotted until measurements had ceased. The distributions of fragment lengths in different photographs were the same for any period of digestion, but differed for the three periods of digestion. The results are shown in the histograms in Fig. 1.

The diameter as measured by shadow length was determined on plates taken from each period of digestion. In Fig. 2 the results are compared with control measurements made by the same procedure on a preparation of partially renatured T4 DNA which contained both duplex and single-chain material. The fragment-diameter histogram coincides with the duplex peak in the control, indicating that the fragments resulting from digestion are duplex.

The distributions of observed length fit what would be expected by calculations from the theory of Montroll and Simha (*5*) which assumes that scissions are being introduced at random locations. Theoretical distributions calculated for several different numbers of breaks for each original T5 molecule were tried against each observed distribution, assessing the goodness of fit by the χ^2 test. In each case χ^2 passed through a sharp minimum, indicating best-fitting values of 97, 231, and 832 breaks for each parent 40-micron molecule at 30, 90, and 160 minutes of digestion. The probabilities of fit were high, 50, 60, and 83 percent, respectively. Thus the number of breaks required to fit the data increases proportionally with the time of digestion up to 90 minutes. The last point shows somewhat more breakage than expected. Since fragments which are shorter than 0.05 to 0.10 μ cannot be reliably seen, this size was neglected in fitting the distributions.

From these experiments we conclude that (i) acid deoxyribonuclease does indeed break up the molecule, which is known to be a linear duplex, into short duplex fragments, (ii) the distribution of fragment sizes indicates that the breakage is taking place at random and generates a "random" distribution of fragment sizes; no preferred fragment size was found above the limit of visibility (about 0.1 μ), and (iii) the number of scissions per molecule is approximately linear with time, up to 90 minutes. At longer times a somewhat larger number of scissions is required to fit the data. This is compatible with the notion that a single-hit reaction is proceeding concurrently with the double-hit reaction. In view of this, it is not possible to decide about the specificity of the single-hit reaction (*6*).

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