*LOSS OF RECENT MEMORY IN MICE AS RELATED TO REGIONAL INHIBITION OF CEREBRAL PROTEIN SYNTHESIS**

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In an earlier report[1] evidence was presented that puromycin given subcutaneously to mice is without effect on memory of simple maze performance although it appeared to inhibit cerebral protein synthesis by about 80 per cent for a period lasting from the second to the eighth hour after the injection. Subsequently it was found that both recent and longer-term memory of maze performance can be destroyed by intracerebral injections of puromycin in mice.[2] Destruction of recent memory appeared to be primarily related to the effects of intracerebral puromycin on the hippocampi and temporal cortices, whereas the additional exposure of the remainder of the cortex to the antibiotic appeared necessary to produce loss of longer-term memory. The principal known effect of puromycin is to inhibit protein synthesis. If this effect is related to loss of memory, it would be expected that intracerebral injections would produce a more profound or more enduring inhibition of protein synthesis in the brain than occurs with subcutaneous injections. We have determined the degree and duration of inhibition of protein synthesis using bilateral temporal injections[2] which uniformly cause loss of recent memory and compared them to the inhibition following subcutaneously injected puromycin. In addition, we have observed the behavioral and chemical effects of another inhibitor of protein synthesis, chloramphenicol.

Materials and Methods.—We are indebted to Dr. Leon Goldman of the Lederle Laboratories Division of the American Cynamid Corporation for our supply of puromycin. The l-valine-C^{14} was uniformly labeled and had a specific radioactivity of about 50 μc/0.03 mg. Young adult albino mice weighing about 30 gm were used.

At various times after bilateral temporal or subcutaneous injections of puromycin or bilateral temporal injections of chloramphenicol, 1.3 μC of valine-C^{14} was injected subcutaneously. Forty minutes later[1] the animal was killed by asphyxiation. To establish normal rates of incorporation of valine into protein, the same amount of valine-C^{14} was injected in the same way and for the same period of time in untreated animals. As the quantity of radiovaline was not varied in proportion to the size of the animal, the observed quantity of radioactivity in pool and protein fractions was numerically adjusted to compensate for the variable dilution.

Six samples of brain weighing 18–30 mg were routinely taken; these consisted of the two hippocampi, thalami, corpora striata, and of frontal, parietal, and temporal cortices. Procedures previously used for preparation of samples[1] have been simplified with, we believe, improvements in accuracy. Tissue was dissolved in 1 ml 0.1 *N* NaOH, and aliquots were taken for determination of protein in duplicate.[3] Protein was then precipitated with 3 vol of 12 per cent TCA and the supernatant fluid saved for measurement of pool radiovaline. After thorough washing

with 6 per cent TCA, water, and acetone, the precipitate was plated. Radioactivity was measured with a liquid scintillation counter. Using methods previously described,[1] measurements were made in a few control and puromycin-treated animals of the concentrations of total free amino acids and of free valine. It will be noted that in the preparation of samples for assay of radioactivity no effort was made to remove lipids and RNA. This procedure appears preferable to that previously used[1] because inconsequential amounts of radioactivity were found in lipids and RNA and because the loss of a variable but frequently substantial amount of protein was avoided.[4]

Results.—Normal animals: Values obtained from normal mice provided a base against which to measure degree of inhibition of incorporation of radiovaline into protein produced by puromycin and chloramphenicol. The radioactivity of protein and pools of the 6 areas of the brain was determined after injection of radiovaline into 12 untreated animals. For protein, the means ± their standard deviations in counts/10 min/mg protein follow: hippocampus, 162 ± 37; thalamus, 156 ± 41; corpus striatum, 148 ± 39; temporal cortex, 186 ± 27; parietal cortex, 167 ± 31; and frontal cortex, 192 ± 44. The radioactivities of the pools of the different areas were so nearly alike that a single mean of 345 ± 63 counts/10 min/mg protein of the original solution was used.

Animals with bilateral temporal injections of puromycin: Each temporal injection contained 90 μg of puromycin in 0.012 ml water; this treatment uniformly leads to loss of recent memory.[2]

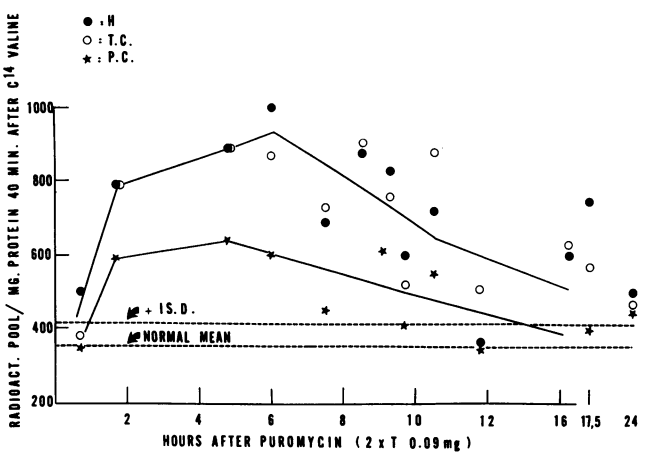

FIG. 1.—Changes with time in the radioactivity of the pools of the hippocampus (*H*), temporal cortex (*TC*), and parietal cortex (*PC*) following bilateral temporal injections each containing 90 μg of puromycin in 0.012 ml. 1 *S.D.* = 1 standard deviation.

Figure 1 gives the variation in radioactivity of the pools at various times after bilateral temporal injections of puromycin in 3 areas of the brain. The radioactivity of these areas is clearly above the normal value 2 hr after injection and is maintained at an abnormally high value for at least several hr. The magnitude of the effect is greater in the hippocampus and temporal cortex than in parietal cortex reaching a value at 6 hr in the hippocampus almost 3 times that of the normal. The duration of the effect is also greatest in the hippocampus and temporal cortex.

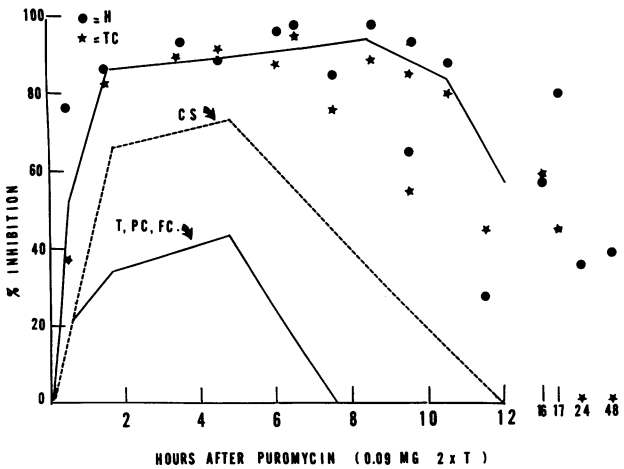

FIG. 2.—Changes with time in the inhibition of incorporation of radiovaline into protein of the hippocampus (*H*), temporal cortex (*TC*), corpus striatum (*CS*), thalamus (*T*), parietal cortex (*PC*), and frontal cortex (*FC*) following bilateral temporal injections of puromycin as in Fig. 1.

The curve for the parietal cortex is essentially the same as that for the thalamus, corpus striatum, and frontal cortex.

No change has been found in the concentration of the total valine or the total free amino acids of the pool in agreement with previously reported measurements of the total pool in the cerebral cortex and liver after subcutaneous injections of puromycin.[1] Hence, the curves of radioactivity of Figure 1 describe variations in specific radioactivity of valine after treatment with puromycin.

Furthermore, the radioactivity of the pool 10, 20, and 40 min after injection of radiovaline has been determined in normal and puromycin-treated mice. The ratio of the areas under the curves from the two sets of mice was essentially the same as the ratio of the values found at 40 min, the mean variation of the difference amounting to only 10 per cent. Thus, the specific radioactivity at 40 min provides a convenient measure of the integral of the specific radioactivity from 0 to 40 min.

The incorporation of radiovaline into protein was measured in control and treated animals. However, the rate of radiovaline incorporation is given by the product of the rate of protein synthesis and the specific radioactivity of the radiovaline pool. Hence, the ratio of radiovaline incorporated by treated and control animals has been corrected for changes in pool radioactivity in order to calculate the inhibition of protein synthesis.

Figure 2 gives the percentage of inhibition of protein synthesis in the 6 areas of the brain as a function of time after bilateral temporal injections. To avoid confusion in the figure, experimental values have been plotted only for the hippocampus and temporal cortex. The effects of puromycin on the thalamus, parietal, and frontal cortex are so nearly alike that one curve serves to illustrate the response of the 3 areas. It is apparent that inhibition is most drastic in the hippocampus and temporal cortex and that with the exception of one animal it is maintained in both these areas at a level of approximately 80 per cent or greater until 10 hr after the injection.

Animals with subcutaneously injected puromycin: As in previous experiments,[1]

12.5 mg of puromycin were injected subcutaneously. The effects on protein synthesis in the hippocampus and temporal cortex in 7 mice which became drowsy after the treatment[1] are shown in Table 1. Substantial inhibition was first observed 2–3 hr after the injection and was maintained for approximately an additional 6 hr. The mean inhibition for the hippocampus in the interval of 2–8 hr after injection of puromycin was 54 per cent and for the temporal cortex, 63 per cent. Inhibition in other parts of the brain fell between these 2 values. The mean radioactivity of the pools was increased from the normal by 60 per cent in contrast to the considerably larger elevation which occurred in the hippocampus and temporal cortex with intracerebral injections of puromycin. No increase was previously noted[1] in pool radiovaline after subcutaneous injections of puromycin using Geiger rather than scintillation counting.

TABLE 1

EFFECTS OF PUROMYCIN* AND CHLORAMPHENICOL† ON INHIBITION OF PROTEIN SYNTHESIS

	Per Cent Inhibition Hr After Treatment							
	2–3		4–5		5–7		8–9	
Treatment	H	TC	H	TC	H	TC	H	TC
Puro s.q.	36	55	48	54	58	63	35	42
			65	71	64	70	26	25
Chloramphenicol	34	35	46	32	44	29		
	66	54	16	0	0	10		

* Given subcutaneously (puro. s.q.).
† Bilateral temporal injections, each of which contained 130 or 260 (bottom line of table) μg chloramphenicol.
H, hippocampus; TC, temporal cortex.

Animals with chloramphenicol: Mice were trained to a criterion of 9 out of 10 correct responses in an avoidance-discrimination situation in a Y-maze as previously described.[1] Within 24 hr they were given bilateral temporal injections of chloramphenicol. Two of these mice received 30 μg of the drug in each of the injections; four, 130 μg; and three, 260 μg. All injections had a volume of 0.012 ml. Retention tests of learning were given 3–6 days later.[1] Injection of the antibiotic at the higher concentrations was followed by generalized convulsions which lasted as long as 90 min. None of the mice had loss or impairment of memory, the mean ± its standard deviation for savings of trials having been 91 ± 14 per cent; for savings of errors, 96 ± 6 per cent.

The degrees of inhibition of protein synthesis in the hippocampus and temporal cortex in 6 mice which received bitemporal injections are given in Table 1. When inhibition was evident, it was greater in the hippocampus and temporal cortex than in other parts of the brain. The radioactivity of the pools of the hippocampus and temporal cortex was elevated to about the same degree as with subcutaneous injection of puromycin.

Discussion.—Two of the findings given here require comment in view of those previously reported. Changes in methods of preparation and analysis of radioactive samples have led to substantially lower values for the amount of inhibition of protein synthesis produced by puromycin given subcutaneously.[1] Secondly, the spread of temporal injections of puromycin was estimated earlier[2] from the spread of fluorescein which was quite sharply limited to the hippocampus and temporal cortex. It is clear from Figure 2 that puromycin, unlike fluorescein, spreads widely from the site of the injection to other parts of the brain, but it is also clear

that its effect on protein synthesis is most marked in the hippocampus and temporal cortex. For this and other reasons[2] it appears that the results with bilateral temporal injections of puromycin are still to be regarded as consistent with the views of others that the hippocampus and temporal cortex are primarily concerned with recent memory.

From our present experience it also appears that to obtain consistent loss of recent memory of simple maze learning in mice, protein synthesis in both the hippocampus and temporal cortex must be inhibited for at least 8–10 hr at a level in excess of approximately 80 per cent. These criteria are based on the results of Figure 2 with bilateral temporal injections of puromycin in a concentration and volume which uniformly cause loss of recent memory. As the concentration of the antibiotic is decreased, it becomes progressively less effective in its behavioral and biochemical effects so that recent memory is retained in an increasing percentage of animals as the effect on protein synthesis diminishes. The failure of puromycin subcutaneously injected and of intracerebrally injected chloramphenicol to affect memory can consequently be related to their inadequate effect on the degree and duration of inhibition of protein synthesis.

The conclusion that loss of recent memory can be related to degree and duration of inhibition of protein synthesis receives support from other observations now being made. After destroying memory one or more times with intracerebral injections of puromycin, it has been found that the antibiotic is no longer effective; present evidence now indicates that the ineffectiveness of later injections is to be related to their relatively small inhibition of protein synthesis. Further, consistent destruction of longer-term memory by combined bilateral temporal plus ventricular plus frontal injections[2] seems to be related to inhibition of protein synthesis in the hippocampus, temporal cortex, and the bulk of the remaining cortex in much the same way as noted here for hippocampus and temporal cortex in recent memory.

Summary.—Evidence has been obtained that loss of recent memory of simple maze learning in mice following bilateral temporal injections of puromycin is related to the degree and duration of inhibition of protein synthesis in the hippocampi and temporal cortices. Failure of puromycin subcutaneously injected, and of intracerebrally injected chloramphenicol to affect memory is related to their inadequate effect on degree and duration of inhibition of protein synthesis.

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