to ten times the number formed (as I have mentioned) from the auto exhaust. The type of ice crystals observed and the relation between the numbers produced by various ambient temperatures were essentially the same as the exhaust experiments had shown.

The vast amount of automobile exhaust rising throughout North America could profoundly influence supercooled cloud systems if the atmosphere also contained free iodine vapor. There is a strong possibility that Grant's (*6*) "carry over" effect can be explained by this mechanism; small amounts of free iodine released by the decomposition of silver iodide could easily activate large numbers of lead particles (from auto exhausts) drifting across the mountains where this phenomenon was observed.

The massive glaciation of cumulus clouds, occurring at relatively warm temperatures (−6° to −15°C), also may be caused by this effect. Since 1 g of iodine probably suffices to activate at least 10^{18} submicroscopic particles of lead, it is obvious that such activation may be an important procedure for making large numbers of ice nuclei available in free atmosphere containing engine exhaust.

Iodine vapor may be introduced in many ways because of the high vapor pressure of crystalline iodine and the relatively easy decomposition of many of its chemical products. Although the iodine molecule adsorbs readily on atmospheric particulates, it can also transfer easily from one compound to another, especially in the presence of sunshine.

Such a new method of seeding clouds wherever auto exhaust diffuses into the atmosphere is obviously important, but other potentially important aspects need exploration. The method might be developed, in conjunction with an ice-nucleus detector, as a technique for monitoring the auto-exhaust component of polluted air. Since the automobile engine is probably the greatest source of lead particles in most urban smog, spot or continuous monitoring could indicate its contribution to the total mass of particles and show whether control methods are effective. And experiments might use this reaction to assess the effect of these hitherto-undetectable particles on respiratory ingestion.

The conversion of the particles of lead oxide to lead iodide, which then serves as a nucleation center for formation of ice crystals, is so sensitive that it is difficult to visualize its effectiveness. With the initial particle of lead iodide having an effective diameter of 0.01 μ when introduced into a supercooled cloud, it will grow to 100 μ within 30 seconds—four orders of magnitude, like a golf ball swelling to the volume of the Empire State Building.

The importance of this tremendous rate of growth is that particles that normally remain airborne for long periods, and cannot be seen or detected chemically, can be "trapped" in the centers of visible ice crystals; thus they can be collected on slides as fallout or counted by an acoustic or light signal. By replication of the crystals (*7*), the particles subsequently can be viewed under the electron microscope.

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Puromycin and Cycloheximide: Different Effects on Hippocampal Electrical Activity

Abstract. *Mice were injected in the temporal region of the brain with cycloheximide or puromycin; both agents markedly inhibit protein synthesis in the brain. Recordings of electrical activity were made in the hippocampal region 5 hours after injection of these drugs. The amplitude and frequency observed in records from mice injected with cycloheximide were indistinguishable from those injected with saline alone. Records from puromycin-injected mice were strikingly abnormal. This finding may contribute to the differences in behavioral effects of intracerebral injections of the two inhibitors of protein synthesis studied.*

Mice injected with puromycin in the temporal region of the brain learn to escape shock normally by choosing the correct limb of a maze when trained 5 hours after injection, but they have markedly impaired retention 3 hours later (*1*). Since puromycin inhibits cerebral protein synthesis (*2, 3*) this finding is consistent with a requirement for protein synthesis for the "consolidation" of memory. To test this interpretation a similar study was made with cycloheximide in doses which inhibit cerebral protein synthesis at least as extensively as puromycin does (*2*). Cycloheximide did not have an amnesic effect in this paradigm (*2*). This suggested that the puromycin effect on memory might not be due to interference with the synthesis of protein specifically required for memory storage, but, rather, to some other action. Because of the different effects of puromycin and cycloheximide on memory we studied the effects of these drugs on the electrical activity of the hippocampal region of the mouse brain. We now report the results of this study.

Male Swiss albino mice (30 to 40 g, Charles River Breeding Co.) were anesthetized with ether. Holes were made in the skulls with a stainless steel needle, and injections were administered bilaterally in the "temporal" region at a depth of 2 mm from the surface of the skull (*1, 2*). Each injection (10 μl) contained 0.04*M* NaCl either alone or in combination with 100 μg of puromycin (*4*) or 100 μg of cycloheximide. Five hours after injection, each mouse was replaced in a stereotaxic instrument after having been lightly anesthetized with ether, and a hole was made in the skull 2 mm anterior to one of the injection sites. Electrodes were then inserted at one injection site and in the hole 2 mm anterior to it. The mouse was allowed to awaken and, after a few moments of initial activity, remained docile in the apparatus.

Recordings were made of electrical activity between the two electrodes with a Grass model III electroencephalograph. In some experiments concentric electrodes were used so that activity somewhat localized to the immediately surrounding region could be recorded. Electrodes were inserted 2 mm from the outer surface of the skull and lowered by 0.05 mm every 3 to 5 minutes while continuous recordings were made. The characteristics of such records (amplitude and frequency) were similar in all mice.

After the recordings were completed, the brains were marked by direct current at each electrode site. The mice

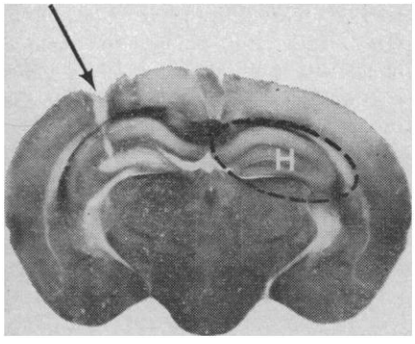

Fig. 1. Section of the brain through the region of the injection site. The arrow indicates the lesion formed in the cerebral cortex and hippocampus (*H*) after passage of a recording electrode through the injection site. The dotted line demarcates the gross limits of the hippocampus and surrounding structures which were explored with the posterior electrode in the multiple mice studied.

were killed with an overdose of ether, the brains were quickly removed and kept in formalin for 7 days, and the electrode placements were determined from frozen sections (50 to 100 μ). The typical site of injection, which served as the site for the posterior electrode, is shown in Fig. 1. The injection tract, in other studies, terminated immediately above or in the superficial portion of the dorsal hippocampus. During penetration, the posterior electrode successively passed through the dorsal hippocampus. The anterior electrode generally penetrated the anterior portion of the dorsal hippocampus and the caudate nucleus.

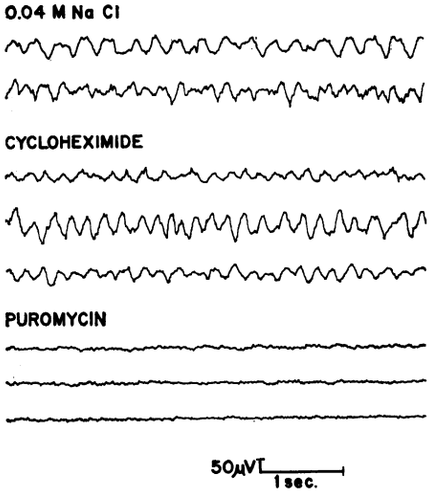

Fig. 2. Records from mice injected with cycloheximide, puromycin, or saline. Each record was taken from a different mouse.

The predominant activity in three saline-injected mice was 4 to 6 cy/sec with amplitude greater than 50 μv (Fig. 2), interpreted as normal hippocampal activity. Seven mice injected with cycloheximide and seven mice injected with puromycin were studied. Records from cycloheximide-injected mice were indistinguishable from records of saline-injected mice (Fig. 2). All seven mice in this group had predominant activity at 4 to 6 cy/sec with amplitude generally exceeding 50 μv. Records from puromycin-injected mice were consistently strikingly different (Fig. 2). Records from every mouse studied showed little activity that exceeded 10 μv and almost total absence of activity at 4 to 6 cy/sec or other rhythmic activity. When measurements were made with concentric electrodes similar marked abnormalities were observed in puromycin-injected mice at both recording sites.

Puromycin therefore differs from cycloheximide in its effect on the electrical activity of the hippocampal region of the brain. Since cycloheximide inhibits protein synthesis in the brain at least as extensively as puromycin does, it is difficult to attribute the electrophysiological effect of puromycin 5 hours after injection to interference with the synthesis of protein. The mechanism of inhibition of protein synthesis by puromycin differs from that of cycloheximide. Puromycin administration leads to the disaggregation of polysomes (*5*), and puromycin is itself incorporated into polypeptides (*6*) which are released from ribosomes. In contrast, cycloheximide does not cause the disaggregation of polysomes and does not cause the release of abnormal polypeptides (*5*). The puromycin effect on the electrical activity of the brain could therefore be related to disaggregation of polysomes or to the formation and release of peptidylpuromycin. Possibly puromycin, which does not resemble cycloheximide structurally, has a direct effect on the electrical activity of the brain.

The differences in the effects of puromycin and cycloheximide on retention of the solution of a one-choice maze 3 hours after training (*2*) may be related to their different effects on hippocampal electrical activity. However, results of this study do not indicate specifically that the hippocampus plays a part in memory storage, since the spread of puromycin after "temporal" injections is quite extensive and the drug may produce abnormalities in electrical activity in other regions of the brain. Our measurements were made in the hippocampal region because the hippocampus has been considered an important structure in the memory-storage process in rodents (*7*) as well as in other mammals. The fact that learning occurs during the altered state of cerebral organization accompanying puromycin-induced hippocampal injury (*1*) is consistent with other reports of learning after hippocampal damage (*8*).

The different electrophysiological effects of puromycin and cyloheximide may also explain the finding that puromycin (*3*), but not acetoxycycloheximide (*9*), interferes with memory in mice when administered 1 day after training. The abnormal electrical activity induced by puromycin, rather than interference with synthesis of protein which might be needed for memory storage, could be the basis of its amnesic effect when administered to mice either before (*1*) or after training (*3*). Since acetoxycycloheximide has been reported to partially impair memory in goldfish tested 3 days after injection (*10*), it would be of interest to determine the effect of this agent on brain electrical activity in this species.

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