In two additional experiments 1.25×10^5 and 5×10^5 nonprimed marrow cells of each fraction were transplanted into irradiated mice with 5×10^7 thymocytes (Table 1). Separation of precursor units for direct and indirect PFC was detected in both experiments. It was reassuring that the enrichment of indirect PFC precursors in the lower density region and of direct PFC precursors in the denser region was not missed because of a twofold dilution. Likewise, a twofold concentration did not provide enough marrow cells to compensate for the depletion of precursor units in any of the pertinent fractions.

The data can only be explained by class differentiation of marrow cells responsible for PFC production. Most likely this cell type is a precursor of PFC, dependent on cooperation with other cells and on antigen for further differentiation and maturation. However, the data do not exclude that "accessory" cells of marrow origin, with yet undefined but necessary immunological functions (*12*), are also class-differentiated, or instrumental in conferring class restriction to PFC precursors and to splenic antigen-sensitive units. The potential of transplanted marrow cells was tested for up to 9 days. It is unlikely that at later intervals precursor units would generate PFC of both classes, since in previous experiments restriction was verified at 25 and 35 days after transplantation (*2, 9*). Thus, the data are the first direct demonstration of commitment to antibody class in relatively undifferentiated and immature cells of the immune system, and of a biophysical counterpart, that is, variations of density possibly secondary to osmolality gradient (*13*). As class-differentiation of marrow cells appears to be under genetic control in mice (*14*), the combined use of genetic variants and physical separation of cells should provide a discriminating approach for elucidating one of the sources of antibody heterogeneity.

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6. A single lot of BSA (lot 119B-0250; fraction V, Sigma) was used to prepare a 35 percent stock solution in tris buffer, *p*H 7.2. The osmolarity was adjusted to 340 milliosmols by an indirect procedure (*5*). The gradient was prepared by layering 0.3 ml each of 35, 33, 31, 29, 27, 25, 23, 21, and 19 percent BSA in a glass tube (5 by 135 mm). The cells to be separated (2.5×10^8 per tube) were harvested from three donors and suspended in 0.5 ml of 17 percent BSA solution; the suspension was layered on the top of the gradient. The tubes were centrifuged (269 rotor of an International model PR-6) at 2000 rev/min for 30 minutes at 10°C. Equilibrium was presumably achieved since centrifugation at 2750 rev/min for 45 minutes did not change the yield of nucleated cells in each fraction.
7. Celloscope model 112 (Particle Data) equipped with a 100-μm aperture.
8. The concentration of each bone marrow fraction was adjusted to 5×10^5 cell/ml. Thymocytes from 9-week-old syngeneic female donors were suspended in Eagle's medium (10^8 cell/ml). Each mouse was injected with 1 ml of the cell mixture 2 to 4 hours after exposure to 900 to 950 rads of ^{137}Cs gamma radiation.
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21 October 1970

Penicillin as an Epileptogenic Agent: Effect on an Isolated Synapse

Abstract. *Penicillin enhances the excitatory postsynaptic potential of the squid stellate ganglion. This effect suggests the hypothesis that the epileptic focus created by the topical application of penicillin to the mammalian cerebral cortex is produced by the facilitation of excitatory synaptic coupling within the preexisting positive recurrent feedback system.*

Penicillin is an extremely potent epileptogenic agent widely used to create experimental foci, but its cellular mode of action is poorly understood. Recent studies with the isolated crayfish stretch receptor (*1*) revealed that penicillin increases excitability in this neuron in two ways: (i) by augmenting membrane resistance so that comparable outward current pulses produce a greater reduction of the membrane potential, and (ii) by slowly depolarizing the cell and thus bringing it nearer the firing level. Further studies of a more complex neuronal aggregate, which contains at least one excitatory synapse, seemed required to clarify the way in which this agent induces experimental cortical epileptic foci. Specifically, it was deemed essential to determine if penicillin is capable of increasing the effectiveness of excitatory synaptic action since this is a crucial postulate of recent theories of penicillin epileptogenesis (*2*).

The isolated giant axon and stellate ganglion of the squid (*Loligo vulgaris*) were chosen for these studies. The direct effect of penicillin on the membrane of the giant axon itself was also examined in order to test the generality of the earlier findings of the crayfish stretch receptor.

The giant axon and stellate ganglion were isolated in the conventional manner (*3*) and mounted in a perfusion chamber of very small volume filled with oxygenated seawater. Both pre- and postsynaptic axons were stimulated by externally applied silver electrodes, and microelectrodes filled with KCl were used for intracellular recording from the postsynaptic axon, either at the ganglion or at some distance from it. Extracellular recording electrodes were blunt micropipettes filled with seawater. Conventional cathode follower and amplifying circuits were used. Only Na^+ penicillin (Upjohn) was employed in these experiments. The high content of Na^+ in the seawater made it unnecessary to compensate for the amount of Na^+ in the penicillin salt at the concentrations used [5,000 to 50,000 international units (I.U.) per milliliter]. The technique of allowing for the measured junction potentials was previously detailed (*1*).

In general, the effects of penicillin on the membrane potential of the giant

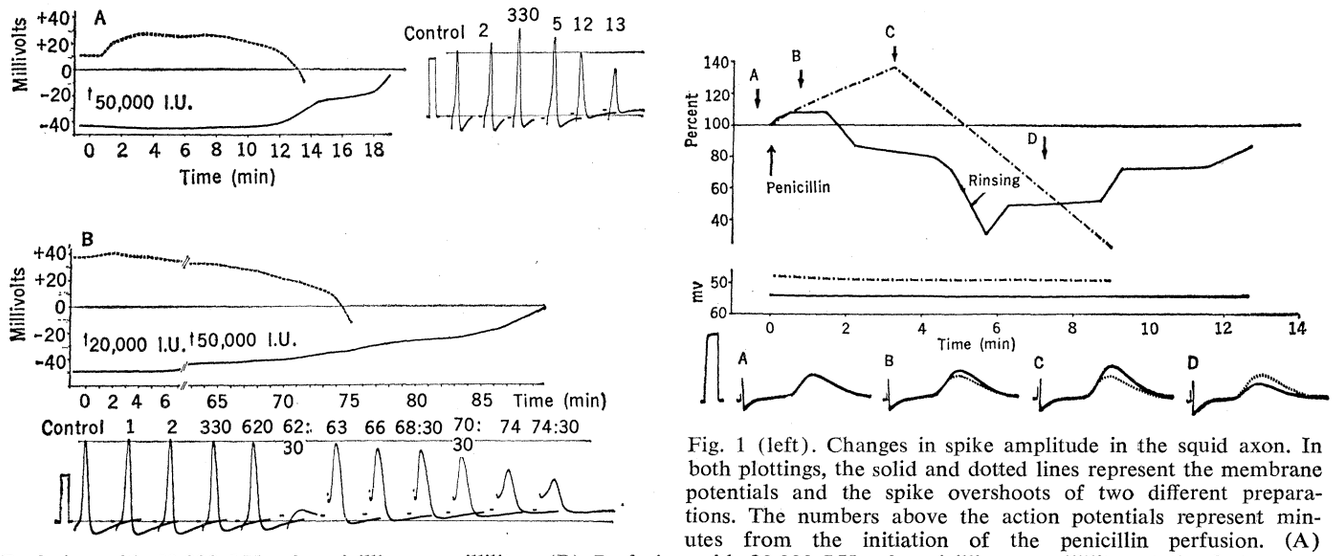

Fig. 1 (left). Changes in spike amplitude in the squid axon. In both plottings, the solid and dotted lines represent the membrane potentials and the spike overshoots of two different preparations. The numbers above the action potentials represent minutes from the initiation of the penicillin perfusion. (A) Perfusion with 50,000 I.U. of penicillin per milliliter. (B) Perfusion with 20,000 I.U. of penicillin per milliliter at the first arrow; with 50,000 I.U./ml at the second arrow, after 63 minutes from the initiation of the first perfusion. After 62 minutes, no spike could be evoked by the stimulation, but it was restored by the inversion of the stimulating electrodes. (Calibrations: 50 mv and 0.5 msec.) Fig. 2 (right). Changes in EPSP amplitude in the squid stellate ganglion. Solid lines represent membrane potential and EPSP amplitude, in percentage of the control, of a preparation perfused with 10,000 I.U. of penicillin per milliliter. The dotted lines represent membrane potential and EPSP amplitude of a preparation perfused with 5,000 I.U. of penicillin per milliliter. The EPSP's shown are taken from the preparation of 5,000 I.U./ml. Each EPSP is lettered to correspond to the lettered arrows. The dotted lines in inserts *B, C,* and *D* represent the amplitude of the control EPSP. Note the the early increase in amplitude of the EPSP and the reversal of the penicillin effect by substituting the solution of 10,000 I.U. of penicillin per milliliter with normal seawater. The other preparation was not rinsed. (Calibrations: 5 mv and 0.5 msec.)

axon were found to be similar to those on the crayfish stretch receptor (see Fig. 1). Depolarization of the membrane was established at a rate that was directly dependent upon concentration. Transient hyperpolarization preceding the onset of the slow depolarization, which was clearly demonstrated in the crayfish receptor, was equivocal in the squid axon. When evident at all, it was miniscule (0.5 to 1 mv). The action potential of the squid axon, however, was affected in a manner nearly identical to that of the stretch receptor. Initially the overshoot voltage often increased, but this increase was followed by a gradual decline and eventual failure of the action potential, which left only local responses. The decrease in the overshoot voltage preceded the onset of resting membrane depolarization but rapidly accelerated once this depolarization began. This behavior of the overshoot potential in the squid axon may be ascribed to the same factors that engender the somewhat more exaggerated changes in the action potential of the crayfish stretch receptor. The initial increase in spike amplitude probably depends upon an increase in membrane resistance. Although resistance was not measured in the present series of experiments on squid axons (*4*), perfusion with solutions having identical penicillin concentration clearly increased membrane resistance of the crayfish stretch receptor (*1*). A paralysis of the Na-K pump (the postulated action of penicillin on the crayfish stretch receptor) could produce reduction in overshoot voltage during the late phase of the squid axon response to penicillin perfusion. Accumulation of intracellular sodium produced by pump failure would eventually lead to a decrease in the diffusional electromotive force across the membrane.

The unitary synaptic potential generated in the squid giant axon by stimulation of the giant presynaptic fiber was studied in preparations fatigued or partially deteriorated so as to selectively eliminate the postsynaptic spike. Intracellular recording revealed a clear initial increase in amplitude of excitatory postsynaptic potential (EPSP) during the early period of perfusion with low penicillin concentrations (5,000 to 10,000 I.U./ml) (see Fig. 2). This was eventually followed by a rather rapid decline in EPSP amplitude; but, if penicillin was removed sufficiently early, the amplitude of the EPSP was restored.

When the extracellularly recorded complex (*5*) (which consists of presynaptic spike, focal synaptic potential, and postsynaptic axon discharge) was studied in suitable preparations, the interdependence of the different actions just described became apparent. Upon perfusion with low concentrations of penicillin (5,000 to 10,000 I.U./ml), changes occurring in the postsynaptic element closely paralleled those in the presynaptic axon. In association with the initial increase in the presynaptic spike amplitude, the previously nondischarging postsynaptic element was induced to fire an action potential to each presynaptic impulse as the result of the drug-induced augmentation of synaptic action. As would be predicted from our intracellular data, however, the postsynaptic axon eventually ceased discharging as the presynaptic spike gradually decreased in size. The ensuing decay of synaptic action could be reversed by removing penicillin sufficiently early.

These data clearly show that, at low concentrations, the cellular actions of penicillin can have, at least transiently, the overall effect of increasing the effectiveness of excitatory synaptic action. Although these data require further analysis by simultaneous intracellular study of both the pre- and postsynaptic fibers, they do suggest that solutions with low penicillin concentrations enhance excitatory synaptic action in this preparation by increasing

the overshoot voltage of the presynaptic spike (*3–5*).

The results of these studies, if also applicable to mammalian cortical neurons subjected to topical penicillin actions, lead us to restate the following hypothesis concerning the manner in which penicillin induces cortical epileptiform activity (*2*). Increased excitatory synaptic coupling in a critically large neuronal aggregate would greatly potentiate the effectiveness of preexisting recurrent excitatory pathways. This would create precisely those conditions requisite for augmented positive feedback that leads to generation of paroxysmal discharges within a large poplation of cortical neurons linked by axon collateral excitation. Indeed, a recent account of iontophoretic application of penicillin onto single cortical cells (*6*) lends further support to this proposed explanation by showing that a clear increase in test EPSP amplitude was produced by iontophoresis and, even more importantly, that full paroxysmal discharges were produced only upon surface topical application that affected much larger groups of cells.

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16 November 1970

Age-Associated Changes in the DNA of Mouse Tissue

Abstract. *The template activity of DNA with calf thymus DNA polymerase has been studied in sections of fixed brain, liver, and heart tissues from young and senescent mice. The enzyme catalyzed greater incorporation of deoxyribonucleotide monophosphates into nuclei of old mouse neurons, astrocytes, Kupffer cells, and heart muscle fibers. The results are interpreted as the accumulation of DNA strand breaks with aging.*

Questions of aging at the molecular level have been particularly difficult to pursue because of the problem of detecting subtle molecular changes. In this report we will present findings which may have some relevance to alterations in the DNA of certain cell populations of very old mice and may therefore aid those who have been addressing themselves to questions of differentiation and aging at the molecular level.

Calf thymus DNA polymerase requires partially denatured DNA with a 3′-hydroxyl group facing a single-stranded region of at least 20 nucleotides to catalyze the synthesis of complementary strands (*1*). This enzyme reaction has been carried out in fixed cell nuclei and the incorporation has been detected by an autoradiographic technique (*2–5*). Previously, nuclei of ethanol-fixed cells did not incorporate labeled deoxyribonucleotide triphosphates in the presence of the enzyme unless these cells were first treated with acid (*2–5*), alkali (*4*), or dimethyl sulfoxide (*4*) in order to denature DNA. Of particular interest are observations made on differentiating lens fibers (*3, 4*). With increasing degeneration, fiber cell nuclei, treated to denature DNA, incorporated increasing amounts of radioactivity in the presence of calf thymus DNA polymerase. This result was interpreted to indicate DNA strand scission in degenerating nuclei (*3, 4*). We now report the template activity of DNA in sections of fixed brain, heart, and liver tissues from young and senescent mice.

Tissues were collected from both young (3 to 4 months old) and aged (30 to 35 months old) (C57BL/6 Cum × C3H/Anf Cum)F_1 mice without pathological lesions; these mice have a mean life-span of approximately 30 months (Fig. 1).

In acid-denatured preparations, all types of cell nuclei were labeled (with more than three grains per nucleus) if treated with calf thymus DNA polymerase. With the exception of old brain tissue (Fig. 1A), label was localized exclusively over the nuclei. The labeling intensity, however, varied from one cell type to another (Fig. 1A). For a given cell type, labeling intensity varied between young and senescent tissue preparations. In the cerebral cortex, a gradation of labeling existed among the six different cell layers. Labeling was al-

Table 1. Mean grain counts per nucleus. Three grains per nucleus were substracted as background. At least 200 nuclei of astrocytes and neurons, 50 Kupffer cell nuclei, and 100 heart muscle cell nuclei were scored for number of grains. The similarity between the means was tested statistically by the so-called Z test (*16*) [limit of significance: probability, $P(X < -Z \text{ or } X > Z)$, $2P < .10$]. Compared to the findings for neurons and astrocytes of undenatured young brain, the labeling intensities in these cell types differed significantly in undenatured old brain ($P = .011$) and acid-denatured young brain ($P = .00013$). On the other hand, mean grain counts between undenatured old brain and acid-denatured young brain did not differ significantly ($P = .11$). A detailed analysis has been described (*6*). The intensity of labeling in nuclei of acid-denatured, old Kupffer cells was statistically greater than that of acid-denatured, young Kupffer cell nuclei ($P = .0286$); of undenatured, young Kupffer cell nuclei ($P = .0028$); and of undenatured, old Kupffer cell nuclei ($P = .0898$). The labeling intensity in undenatured, old Kupffer cell nuclei was not statistically different from that in undenatured, young Kuffer cells ($P = .1371$). Labeling in nuclei of acid-denatured cells from old heart muscle differed statistically from labeling in nuclei of acid-denatured, young ($P = .0474$) and undenatured, young ($P = .0010$) heart muscle cells. Labeling in undenatured, old heart muscle nuclei was not statistically different from that in nuclei of acid-denatured, old ($P = .1240$) and undenatured, young ($P = .3681$) heart muscle.

Cell type	Mean grain counts per nucleus			
	Young		Old	
	Undenatured	Acid-denatured	Undenatured	Acid-denatured
Neurons and astrocytes	8.92	30	22.13	*
Kupffer cells	2	4.5	6.28	13.9
Cardiac muscle	0.67	1.58	3.5	8.63

* Nuclei were too heavily labeled to obtain grain counts.