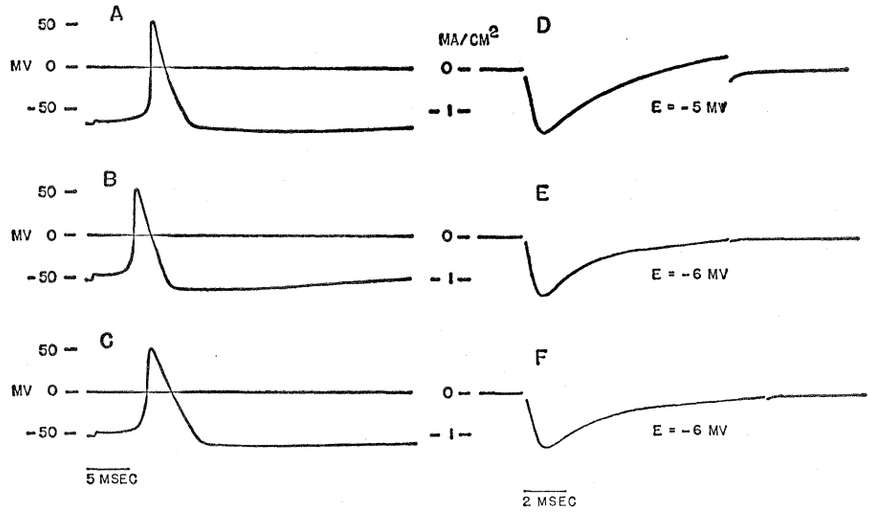

Fig. 3. Demonstration of the protection against the deleterious effects of internal chloride afforded by a low concentration of fluoride in the chloride perfusion medium. Records *A*, *B*, and *C* are membrane action potentials; *D*, *E*, and *F* are maximum sodium currents during a voltage-clamp, depolarizing, step pulse in membrane potential, from the holding potential of —65 mv to the values shown on the records. Records *A* and *D* were obtained initially upon potassium fluoride perfusion; *B* and *E*, after approximately 45-minute internal perfusion with a mixture of nine parts chloride and one part fluoride solution; *C* and *F*, 10 and 15 minutes, respectively, after reperfusion of the axon with fluoride solution. Axon 65–68; temperature, 4°C.

the decline in sodium conductance and coincides with the rise in leakage conductance. This idea suggests that the chloride effect is on some one component of the membrane complex that would alter these values oppositely and exactly in coincidence. Tasaki *et al.* (*1*) have temptingly suggested that the macromolecular structure of the membrane itself is altered by the "unfavorable" anion. The results of less-extensive studies, made with K_2SO_4 internal-perfusion solutions, clearly indicated that $SO_4^=$ was slightly more favorable than Cl^- but considerably less favorable than F^-.

The data in Fig. 3 demonstrate that 90 percent of the favorable fluoride ion may be replaced by the unfavorable chloride anion without the increase in leakage current and decrease in sodium current observed when only chloride ion is present. This fact agrees with the observation of Tasaki *et al.* (*1*) that the ratio of favorable to unfavorable ions must exceed 1 : 10 before the protection of the favorable ion is lost. Explanation of these ion interactions awaits further investigation and understanding of ion-macromolecular interactions in isolated, well-defined systems (*6*).

The initial component of membrane current in the voltage clamp, which has been shown by Hodgkin and Huxley's (*7*) mathematical empirical description of specific membrane conductances to predict the rising phase of the action potential, is greatly decreased by the action of an unfavorable internal anion such as Cl^-. This effect implies that unfavorable internal anions affect the membrane macromolecular conformations in such a way that admission of ions to membranes is altered.

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Enzyme Changes in Neurons and Glia during Barbiturate Sleep

Abstract. *During barbiturate sleep of rabbits, the succinoxidase activity in isolated neurons and glia from the caudal part of the reticular formation was lower than that during physiological sleep. No rhythmical, inverse enzyme changes were detected in barbiturate sleep in the neuron-glia unit, such as were found in physiological sleep.*

In a previous study we have found rhythmic enzyme changes in isolated neurons and glia during sleep and wakefulness (*1*). During sleep, the succinoxidase activity, expressed per nerve cell and unit glia tissue per hour, was increased in the neurons and lowered in the glia in the caudal part of the reticular formation. During wakefulness, this situation was reversed. In the oral part of the reticular formation, rhythmic changes occurred only in the neurons. We also tested the neurons and glia of the hypoglossal and trigeminal mesencephalic nuclei. No changes of the enzyme activity were found during sleep as compared to wakefulness. The observed rhythmic changes were interpreted as indicating that the caudal part of the reticular formation reflects in metabolic changes the biological clock behind the sleep rhythm.

Microchemical studies of the effect of stimulation on neurons and their surrounding glia and the kinetics of enzyme activities have shown that there exists a metabolic coupling between these two types of cells (*2*). The neuron and its glia respond as a unit to functional demands. The oscillation of the enzyme activities in the caudal part of the reticular formation during the circadian sleep rhythm focuses interest on autonomously regulating mechanisms in cells of specific brain regions.

In order to investigate whether sleep induced by drugs is accompanied by similar oscillatory changes in enzyme activities, we have studied neurons and glia in the caudal part of the reticular formation of rabbits after intravenous injection of pentobarbital [0.45 ml/kg; 6-percent solution of 5-ethyl 5(1-methylbutyl) malonylcarbamid, sodium salt, Mebumal, ACO Company, Stockholm]. The animals were killed ½ hour after the injection, by a remote-controlled guillotine. The animals were kept in a soundproof room during the experi-

ment. Sleep was monitored by an electroencephalograph and recorded with epidural electrodes. For the registration of the EEG, we drilled holes through the bone of the skull, taking great care not to damage the dura. A pair of electrodes (made by Titan) was placed symmetrically and epidurally over the hemispheres 5 mm anterior to the sutura coronaria, that is, just in front of the motor region. A ground electrode was placed in the most frontal part of the skull. The EEG was recorded by an oscillograph (Oscillomink, Siemens). The velocity of the paper was 0.5 cm/sec.

Sampling and free-hand dissection of the neurons and glia were performed as described previously (*3*).

Glia samples with the same volume as that of the neurons investigated were analyzed. Since the dry weight per unit volume has been found to be the same for neurons and the surrounding glia, the results can also be compared on a dry-weight basis. The activity of the succinate-oxidizing enzyme system was determined by the micro-diver technique, as described in the previous study (*1*).

The EEG recordings showed an initial appearance of high-amplitude, slow activity, with irregular bursts of spindles which are characteristic of sleep. Around 15 minutes after the barbiturate injection the EEG began to show the wakefulness pattern.

As can be seen from Table 1, the succinoxidase activity of the neurons from the nucleus reticularis gigantocellularis is significantly lower during barbiturate sleep than during physiological sleep.

The neuronal enzyme activity during barbiturate sleep does not differ from that during wakefulness. In contrast, the neuronal enzyme activity during physiological sleep is considerably higher than that during wakefulness.

The enzyme activity of the glia during barbiturate sleep proved, on the other hand, not to differ significantly from the values of the glia during physiological sleep. However, in both instances, the activities are lower than the glia activity during wakefulness.

When the neuron-glia enzyme activities are considered, the following can be said: In barbiturate sleep, the neuronal activity is slightly but significantly lower than the glial activity. This is also the case during wakefulness, although more accentuated. But, as was stressed above, the enzyme activity of the neurons during physiological sleep was the highest.

Table 1. Succinoxidase activity of neurons and glia from nucleus reticularis giganto-cellularis isolated from rabbits. The results are expressed as 10^{-4} μl of oxygen per sample per hour. Values are mean values ± standard errors; numbers of analyses are shown in parentheses.

State	Nerve cells	Glia
Wakefulness*	1.30 ± 0.25 (24)	3.06 ± 0.24 (28)
Barbiturate sleep	0.98 ± .15 (9)	1.96 ± .31‡ (9)
Physiological sleep*	3.41 ± .51† (29)	2.34 ± .18‡ (25)

* Data from Hydén and Lange (*1*). † Significantly higher than during wakefulness or barbiturate sleep ($P < 0.001$). ‡ Significantly lower than during wakefulness ($P < 0.02$).

Thus, the inverse enzyme activity changes which were observed in the neurons and glia during physiological sleep do not occur during sleep induced by a barbiturate. The physiological events demonstrated an oscillation with inverse enzyme activity changes between the neurons and the glia in the caudal part of the reticular formation, but barbiturate sleep seems to be correlated with another biochemical mechanism.

The question is whether the barbiturate used had a damping influence on the neuron-glia functional unit. Electrophysiological studies have invariably shown that barbiturates depress the impulse activity of the reticular formation and, to a lesser extent, of the cortex (*4*). These drugs also increase the threshold to electrical stimulation. Our enzyme activity values for neurons in barbiturate sleep are low. It might be possible that the electrical data and the biochemical observations during barbiturate sleep are both reflections of a depressing effect of the drug on the caudal part of the reticular formation.

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Immunological Competence: Alteration by Whole Body X-Irradiation and Shielding of Selected Lymphoid Tissues

Abstract. *The spleen and thymus of 6-week-old mice contain similar numbers of lymphoid cells. A lethal dose of x-irradiation given to animals whose thymus, midgut, or hindlimbs were shielded at the same time resulted in permanent acceptance of allografts, while a similar dose given to spleen-shielded mice so irradiated resulted in a normal rejection pattern. The return of immunological competence was related to the state of the lymphoid organs with different types of shielding.*

Thymectomy in the newborn mouse is thought to eliminate a source of immunologically competent cells and leads to a subsequent gross immunological deficit (*1*). In the adult it has a small effect in diminishing immune reactivity unless followed by a sublethal dose of whole body x-irradiation (*2*). It is thought that adult thymectomy removes a source of potentially competent cells but leaves the animal with a population of competent cells. Although the competent cells may stem from the thymus or from a source influenced by the thymus, they may be self-perpetuating (*3*). The thymus of the adult mouse produces lymphoid cells that appear not to be competent (*4*), but to acquire competence while in the thymus. It is a reasonable hypothesis that, before maturing, these cells respond to specific antigenic stimuli producing tolerance rather than immunization (*5*). In the newborn animal, tolerance would result. In the normal adult this tolerance would be masked by immune responses of the peripheral lymphoid tissue to the same stimuli. To test this hypothesis, the following experiments were performed.

Four groups of DBA/2 mice, 6