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

Table 1. Reconstitution of the germinal center, lymphocyte, and plasma cell components of x-irradiated shielded mice (see text for abbreviations). Results are expressed as 1 to 4+, 4+ being the usual quantity of these components, 0 to 3+ are fractions thereof.

Days after x-irradiation	X-irradiated shielded groups*			
	S	BM	T	GBM
Germinal centers				
5	+++	0	0	0
10	+++	0	0	0
20	++++**	++	+	+
Small lymphocytes				
5	+++	0	+++	++
10	+++	0	++++	+++
20	++++	+	++++	++++
Plasma cells				
5	+++	0	0	+++
10	+++	+	0	++++
20	++++	++	+	++++

* 12 lymph nodes were examined in each group.
** ++++, Usual population of cells.

weeks of age were given 1000 roentgens of x-irradiation from a 250-kv Westinghouse x-ray machine. In one group (T), an area of thorax containing thymus gland, some mediastinal lymphoid tissue, and sternal and vertebral bone marrow was shielded during x-irradiation. In a second group (S), the spleen was exteriorized on its pedicle and shielded. Effectively splenic lymphoreticular tissue and some extramedullary hematopoietic tissue were shielded. In another group (BM), both hind limbs containing primarily femoral and tibial bone marrow and also two small popliteal lymph nodes, were shielded. The last group (GBM), was irradiated with lead shielding of one hind limb together with a large segment of small bowel exteriorized on its mesentary. Here the lymphoid tissue of the submucosa and Peyer's patches (but not the mesenteric nodes) were shielded as well as the marrow of the hind limb. While all unshielded animals given 1000 r died between 6 and 14 days afterward, each of the shielded groups had a mortality of less than 10 percent. Calculations showed that the shielded tissue of each group received less than 60 r. Cell counts showed that the thymus and spleen contained approximately equal quantities of lymphoid cells. By estimation, the BM and GBM animals contained fewer shielded lymphoid cells than animals in the S and T groups.

After x-irradiation, each of the four groups was grafted with skin from Balb/C mice, which differ from DBA/2 mice by histocompatibility alleles controlling weak antigenic determinants (non-H2) (*6*). Mice in each group received grafts either 6 hours after x-irradiation or 7, 14, or 28 days later. Grafts were considered accepted when they had a luxuriant hair growth and no evidence of rejection 2 months later. Such acceptance was attributed to specific tolerance, since skin from B10/D2 strain mice was uniformly rejected if grafted 30 days after the grafting of Balb/C skin. The B10/D2 strain also only differs from the DBA/2 recipients in non-H2 alleles (*6*), but the two strains have common H2 alleles.

By skin grafting immediately after x-irradiation, a tolerant state could be induced in one-third to one-half of T, BM, and GBM animals (Fig. 1). By contrast all S animals were immediately reactive. When grafting was performed 7 days after x-irradiation, both T and GBM had largely returned to the competent state. The BM mice continued to show a significant absence of competence. When grafting was performed on day 14, about one-third of BM animals developed tolerance. They had returned to normal reactivity, however, by 28 days.

Histologic examination of the x-irradiated peripheral lymph nodes in each of these groups (Table 1) demonstrated that by 10 days small lymphocytes, germinal center cells, and plasma cells had all regenerated in S. However, in T there was a rapid reconstitution of a small lymphocyte population but no early return of either germinal centers or plasma cells. In GBM many small lymphocytes and also a large number of plasma cells were quickly reconstituted but germinal center cells were lacking. Reconstitution of all three of these cellular elements was slow in BM.

These results support the following conclusions: (i) Competent cells are present in the spleen (*7*). When the spleen is shielded, competent cells remain intact, and an allograft induces a standard immune response. (ii) Thymus and gut do not contain competent cells or cannot make them readily available to the skin graft, since, even when they were shielded, there was no immune response. They apparently affect the development of competent cells, since competence, as judged by immune responsiveness to allografts, returned rapidly (1 to 2 weeks) in T and GBM groups. (iii) Bone marrow seems to have no significant role in relation to production of competent cells, and when

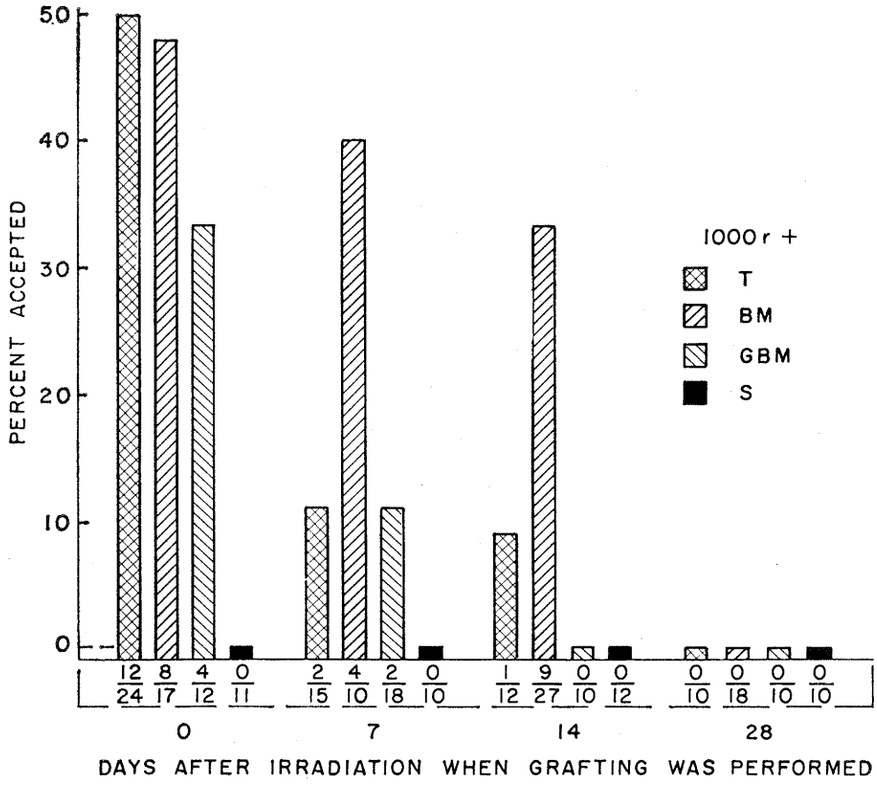

Fig. 1. Percentage of tolerant mice as a function of the time of homografting after x-irradiation when selected lymphoid tissues were shielded by lead. The fractions indicate the number of grafts accepted out of the total number of animals tested.

the marrow was shielded there was neither immune responsiveness nor rapid return of competence. Return of competence at 1 month in this group may be related to regeneration of thymus or gut, or both, implicated in item 2. (iv) The possibility that spleen may play a role in the development of competent cells cannot be evaluated since in the S group the picture was dominated by competent cells already present. (v) Tolerance will develop in one-third to one-half of adult recipient mice given allogenic skin grafts from donors with a non-H2 genetic disparity when few competent cells are present. (vi) From the morphological studies there is again evidence that after x-irradiation the thymus in the adult mouse is responsible for reconstitution of a population of mainly small lymphocytes, and that germinal center cells and plasma cells stem from other sources. Even in the absence of these other cellular elements in the peripheral lymphoid organs, if small lymphocytes were present in abundance, as at 7 and 14 days after x-irradiation in T and GBM, transplantation immunity was quite vigorous. Plasma cells may be derived from cells produced in the gut. Germinal center cells, however, only returned rapidly in animals with intact spleens. It is possible that some lymphoid tissue associated with gut, tissue that is analogous to the bursa of Fabricius in avain species, may be found as a source for both of these cell types as postulated by Good (*8*).

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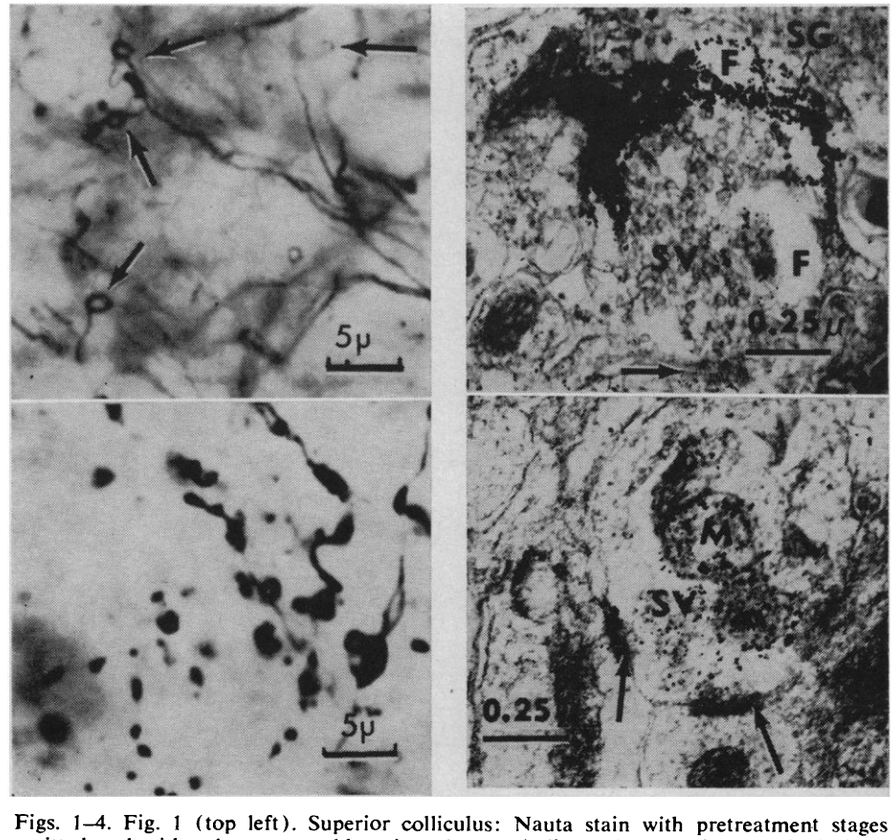
Neurofibrils and the Nauta Method

Abstract. *When the pretreatment stages are omitted, the Nauta method for degenerating axons stains neurofibrils. Under the electron microscope the stain is closely related to neurofilaments. When one uses the complete Nauta technique, the stain is specific for membrane structures.*

The differential value and merits of the Nauta (*1*) and Glees (*2*) methods have been discussed in detail by many authors (*3*). It has been suggested from chemical studies that the Nauta method stains a product of membrane breakdown (*4*). An electron-microscopic study of Nauta degeneration in the spinal cord (*5*) indicated that, under the particular experimental conditions, silver granules occurred in some terminals; but it was not clear in which component of the cytoplasm they were found or whether this was a special case. Reduced-silver techniques, comparable to the Glees method, stain neurofilaments or a component closely related to them (*6*). The filaments proliferate in degenerating nerves, giving characteristic rings or other formations in terminals. At present, the correlation between filaments and the component stained by the Glees (or other neurofibrillar) methods is largely implied, although direct correlation is strongly suggested by Gray and Guillery (*6*).

We now report on part of a larger study investigating the ultrastructural basis of silver staining of the nervous system; we wish here to emphasize similarities between the Glees and Nauta methods in an attempt to understand the mechanism of both more fully.



Figs. 1–4. Fig. 1 (top left). Superior colliculus: Nauta stain with pretreatment stages omitted and with subsequent gold toning. Arrows indicate degenerating terminal rings. Fig. 2 (bottom left). Superior colliculus: Nauta stain with subsequent gold toning. No degeneration rings; degeneration appears as swellings and granules. Fig. 3 (top right). Superior colliculus: stained as for Fig. 1 and showing stain granules (*SG*) forming part of a terminal ring in a bouton. The synaptic vesicles (*SV*) are not stained with granules. Arrows indicate the synaptic cleft. The damage caused by freezing (*F*) does not appreciably alter the distribution of stain granules. Fig. 4 (bottom right). Superior colliculus (stained as for Fig. 2): a bouton showing degeneration stain granules in mitochondria (*M*) and synaptic vesicles (*SV*). Synaptic clefts are indicated by arrows.