CHANGES IN THE PERIPHERAL NERVOUS SYSTEM OF LABORATORY ANIMALS, ASSOCIATED WITH COOLING AND WITH ARTIFICIAL HYPOTHERMIA

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The application of artificial hypothermia to clinical medicine has been steadily increasing of recent years. Insufficient study has, however, been made of the functional and morphological effects of this procedure and of cooling the organism in general. There has been particularly little work done in this connection on the peripheral nervous system.

Cajal [9] has investigated the effect of cold on regeneration of nerves. He has also described changes taking place in motor ganglion cells of lizards during hibernation. Staemmler [16] has found degenerative changes in peripheral nerves following frostbite, and associated with peri- and endoneuritis. Legouix and Thieulin [10] have examined the effects of temperature fluctuations on peripheral nerves, and found that cooling lowered conductance of stimuli.

Müller [11] examined ganglion cells of the brain of cats in a state of artificial hypothermia induced by administration of Megaphen, and found severe degenerative changes, characterized by breakdown of Nissl substance, with formation of cytoplasmic vacuoles. The changes in the brain stem were of greater severity than in the cortex.

The object of the present study was to investigate morphological changes in peripheral nerves, appearing during hypothermia. We have been unable to trace any literature references to this subject.

Our experimental material consisted of 78 rats and 10 rabbits (64 experimental animals, and 24 controls). We examined the intramural cardiac ganglia, the intramural nerve plexi of the duodenum, and the richly innervated bursae of the knee and hip joints.

We lowered the body temperature of the first group of animals to 30°, 24° and 18°, by packing ice around them. The animals were maintained at these temperatures for three to six hours, or for 10-23 hours. The group consisted of 36 animals.

We induced hypothermia in the second group by the administration of Laborit's mixture (atropine sulfate 0.0005 g, phenobarbitol 0.055 g, isocaine 0.015 g, phenergen 0.025 g, tubocurarine 0.0001 g), at this dosage per kg body weight, and introduced over a period of two hours. The animals were then cooled to 30° or 24°, for a shorter or longer period, as in the experiments with direct cooling. We used 20 animals for these experiments.

In the third group of animals we produced neuroplegia by administration of Largactil. The body temperature fell to 33-34°, and the animals were maintained at this temperature for the same periods of time as in the other groups. This group consisted of eight animals.

Rectal temperature was measured every half hour. The animals were all killed in the same way, by administration of ether either immediately after the conclusion of the experiment, or one to three days later (10 animals).

The nerve elements were made visible by impregnation with silver, using Lavrent'evs modification of the Bielchowsky-Gross method [6], and, in addition, we applied Dogiel and Shabadash's procedure [15] of staining with methylene blue. These procedures, which stain nervous system elements in different ways, complement each other very satisfactorily. Material from the control aniamls was treated in the same way as those from the experimental groups. In order to achieve greater objectivity the slides were marked with code numbers.

EXPERIMENTAL RESULTS

We examined terminal nerve fibers, pericellular structures of vegetative intramural ganglia, and also receptors. The first degree of change perceptible in the structures was their heightened stainability, the next, irregularities in the thickness of the fibers (thickening or thinning), with formation of varicosities along their axes, which is considered in the literature to be evidence of an irritated state, preceding the onset of Wallerian degeneration.

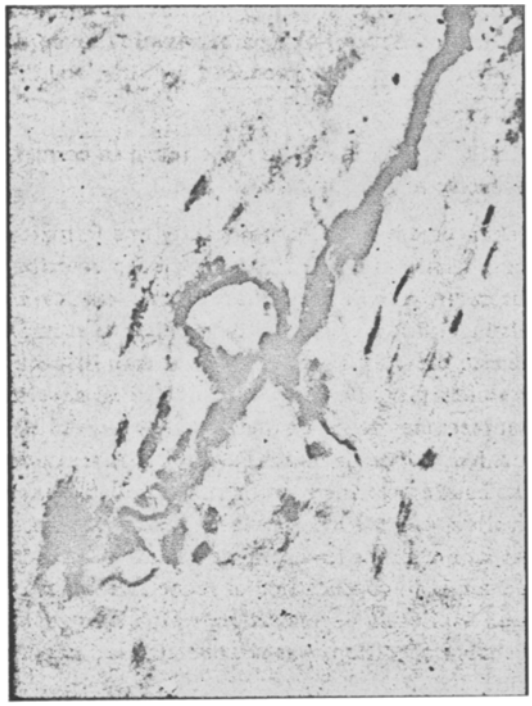

Fig. 1. Arboriform receptor from special cells. Enhanced argyrophilicity and irregular thickness of terminal fibers after cooling to 24° for 5 hours. Articular bursa of a rat. Impregnated with silver.

In the normal animal larger or smaller unmyelinated fibers are distinguished by their smooth outlines and by their uniform thickness along the whole of their length. In the experimental animals, in particular in those which had been subjected to prolonged or profound cooling, we found, in addition to heightened intensity of staining, a thickening of the fibers, with formation of large varicosities.

The ganglion cells of the intramural cardiac nodes of the normal animal are surrounded by a network of regular fine pericellular fibers with smooth contours; these fibers become thicker after administration of Largactil. This manifestation of an irritated condition is particularly pronounced in cooled animals (Figure 1).

Evidence of irritation was also seen in the fibers of the nerve plexi of the conducting system of the heart of experimental animals.

The Auerbach plexus of the duodenum of normal animals is distinguished by the uniform thickness of the nerve fibers; in artificial hypothermia some of the fibers stain more intensely, their outlines become irregular, and varicosities appear.

The commonest type of nerve endings to be found in the articular bursae are the corpuscles of Ruffini, which originate from myelinated fibers by dichotomous ramification to give unmyelinated fibers distributed between intensely staining nuclei of special cells.

These terminal arborizations are more frequently encountered in artificial hypothermia because of their increased stainability. Neurofibrillar platelets could be seen at the ends of the unmyelinated terminal fibers of the arborizations, in specimens stained with methylene blue. Stimulation of these formations in cooled animals could be perceived in the form of thickening and uneven diameter of the ramifications of the unmyelinated fibers, or of varicose swellings (Figure 1).

Another type of encapsulated receptor commonly found in the articular bursae are the modified encapsulated corpuscles of the Vater-Paccini type (Golgi-Mazzoni bodies). The increase in their staining capacity was manifested not only by the increase in the number of stained corpuscles seen, but also by the more intense staining of the inner bulb of the corpuscle; we found 245 encapsulated corpuscles in 80 animals, and the number of such encapsulated receptors in animals subjected to artificial hypothermia was 2½ times as great as in normal animals, and in cooled animals four times as many were seen. The irritated state in cooled animals was manifested by thickening of the axon and by irregularities in its thickness (Figure 2).

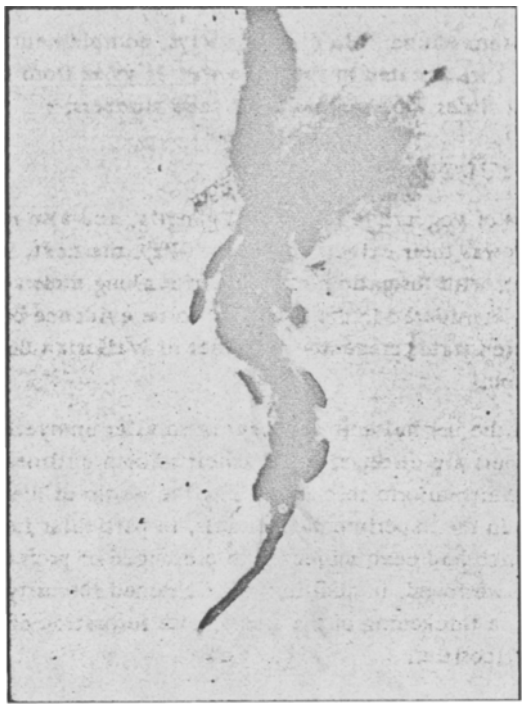

Fig. 2. Encapsulated receptor of the Golgi-Mazzoni type. Enhanced argyrophilicity and irregularity of the axon after cooling to 24° for 5 hours. Articular bursa of a rat. Silver impregnated.

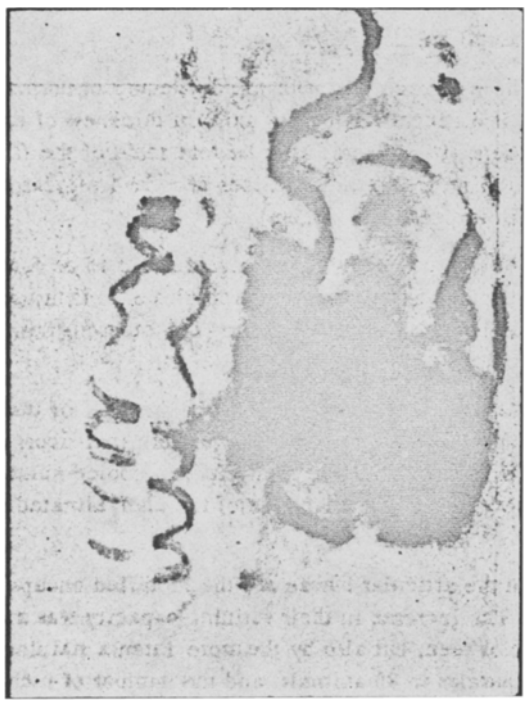

Fig. 3. Neuromuscular spindles. Enhanced argyrophilicity following cooling to 32° for 4 hours. Rat muscle. Silver impregnated.

We encountered considerable difficulties in staining the neuromuscular spindles of control animals. For this reason the greater staining capacity of the experimental animals is regarded by us as being a consequence of the cooling (Figure 3).

The enhancement of staining capacity and the signs of irritation of the nerve structures represent a nonspecific reaction of the peripheral nervous system to excessive stimulation, which may be produced by a great variety of factors. Such changes are described in the literature as being present throughout the parasympathetic system in pulmonary tuberculosis [7, 5], in hypertensive disease [4], and in oxygen hunger [2, 6]. We have also observed them under conditions of oxygen deprivation, excessive physical strain, artificially produced arthritis, and amputations [12, 13].

Similar changes are also to be found in normal animals, but to a much smaller degree.

Enhancement of staining capacity and formation of varicose swellings of the fibers have been described in the literature as being reversible phenomena. We could distinguish fewer fibers and receptors in animals killed one to three days after treatment than in those killed immediately after the conclusion of an experiment, which supports the view that the changes observed by us are reversible. It seems that metabolic changes occur in the cells, causing the increase in their capacity for staining with silver and methylene blue. It is logical to suppose that such changes in the fibers and receptors may lead to changes in conductance or receptivity of the structures. Wallerian degeneration, which is regarded as an irreversible condition, was not encountered in our experiments.

Changes in the peripheral nervous system resembling those described above have been reported by Astakhov [1], in his experiments on artificial hyperthermia. The changes due to cooling appear, however, to be more pronounced, and of longer duration.

Not all nerve fibers are equally affected. Our observations showed that thick terminal fibers proceeding from myelinated ones are the most affected, while the slender fibers accompanying the blood vessels remained unchanged. The experiments show that the sensory apparatus is less resistant than are the vegetative nerve fibers of the blood vessels, in which we could never observe any changes.

Our results show that the greatest changes in the peripheral nervous system are to be found in animals subjected to direct cooling. The effects are less pronounced (only heightening of staining capacity) in animals subjected to artificial hypothermia (by Laborit's procedure), and after administration of Largactil. These changes seem to be reversible.

The changes are greater the lower the body temperature of the experimental animals, and the longer they are maintained at low temperatures. The effects seen in the heart, the intestine, and the joint bursae are in general of the same kind.

SUMMARY

Experiments were performed on rats and rabbits. It was established that changes occur in the peripheral nervous system of the heart, intestine and the articular capsule in: 1) simple decrease of the body temperature by application of ice; 2) in artificial hypothermia induced by Laborits' method, and 3) in neuroplegia induced by largactyl. These changes are manifested by: a) increased staining; b) increased roughness of the nerve fibers and their varicosity. These changes mainly affect the thick terminal fibers and nerve endings, sensory function, while slender fibers of the vessels, vegetative by function, remain unchanged. The lower the body temperature in the animals and the longer the experiment, the more pronounced the changes in the nerve fibers. The most considerable changes appeared in the animals which were cooled by means of ice. In artificial hypothermia the changes were manifested mainly by increased staining of the nerve fibers and were reversible. Experiments show that hypothermia affects the whole organism. In the peripheral nervous system it mainly has an effect on the sensory nerves. However, these changes are not specific since they are also found in other prolonged interventions.

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