INTRAVITAL MICROSCOPY AND HISTOCHEMICAL INVESTIGATION OF THE AUTONOMIC NERVOUS SYSTEM OF THE SMALL INTESTINE OF ANIMALS WITH THERMAL BURNS

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It was shown by intravital microscopy that oxidoreductases can be demonstrated histochemically and changes in their activity studied in the intramural nervous plexuses of the small intestine in the living animal. Severe thermal injury was shown to disturb the permeability of the mitochondrial membranes of the nerve cells of Auerbach's plexus in animals, as a result of which the activity of the oxidoreductases located inside the mitochondria is modified. Lumbar procaine block, carried out immediately after burning, was shown to restore the normal state of the mitochondrial membranes of the nerve cells.

KEY WORDS: intravital microscopy; autonomic nervous system; succinate dehydrogenase; thermal burns.

Investigations have shown that after extensive thermal burns of the skin the activity of all the systems and organs of the animal are disturbed and all types of metabolism are changed. The nervous system also is involved in the pathological process [1-3, 5-7]. Notwithstanding the understanding of the role of the nervous system in the pathogenesis of burns, there have as yet been no systematic investigations of the dynamics of the morphological changes in the central and peripheral nervous systems in various types of burns, especially the nonlethal variety, nor has any attempt been made to study the histochemical changes. Accordingly the investigation described below was carried out in order to study, by the method of intravital microscopy as developed by A. A. Vishnevskii and B. I. Lavrent'ev, changes in the activity of one of the oxidoreductases, namely succinate dehydrogenase (SD), in the autonomic nervous system of the rabbit small intestine after thermal burns of various degrees of severity, and also to examine the effect of lumbar procaine block on these changes, for this procedure is widely used in burns as a method of acting upon the nervous system.

EXPERIMENTAL METHOD

To demonstrate the intramural nervous plexuses of the small intestine, which were the test object of this intravital study, and to examine the changes in SD activity taking place in them, we used a tetrazolium salt for the first time. Nitro-blue tetrazolium, a hydrogen acceptor, under the influence of dehydrogenases, forms a bluish violet diformazan which is insoluble in water; depending on the quantity and distribution of the diformazan produced the activity and localization of SD can be determined (the Leitz Ultropak was used for microscopy). The results obtained by intravital microscopy were compared with those of histochemical investigation of SD activity. The method of Seligman and Rutenberg, with nitroblue tetrazolium, was used to detect SD.

A thermal burn was produced by pouring water at a temperature of 98°C over the lower half of the back and hind limbs of a rabbit for 20, 30, or 50 sec.

Lumbar procaine block was carried out 30-40 min after burning, by injecting 4 ml/kg body weight of a 0.25% procaine solution. These experiments were carried out on 24 rabbits.

During investigation by intravital microscopy the histochemical reaction for SD activity was carried out by applying a solution of the substrate (sodium succinate with nitro-blue tetrazolium, made up in phosphate buffer, pH 7.4) to the serous surface of a segment of small intestine brought out of the peritoneal cavity [4].

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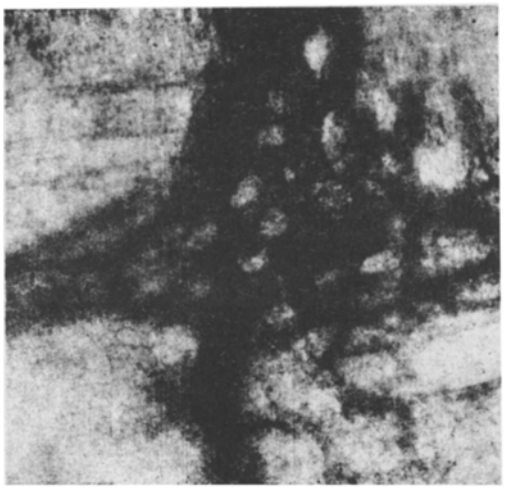

Fig. 1

Fig. 2

Fig. 1. Auerbach's plexus of rabbit small intestine 24 h after burns; nerve cell with high SD activity. Intravital microscopy; 250×.

Fig. 2. SD activity in nerve cells of Auerbach's plexus of intact rabbit 24 h after lumbar procaine block. Seligman–Rutenberg reaction for SD, 250×.

EXPERIMENTAL RESULTS

The results of these experiments showed that in intact animals 8-12 min after application of the solution to the serous surface of the small intestine, single nerve trunks of Auerback's plexus were revealed as a result of diformazan formation, and after 30-40 min all nerve trunks and ganglia of the plexus were clearly visible. The quantity of diformazan formed was small. The nerve cells were not demonstrated under these circumstances.

The nerve trunks and ganglia of Auerback's plexus 24 h after burn trauma of definite severity (98°C, 30 or 50 sec) became visible sooner (4-7 min after application of the solution), and much more diformazan was formed than in the intact animals. Nerve cells with high SD activity also were found (Fig. 1).

The demonstration of the intramural nervous plexuses of the small intestine of intact rabbits undergoing lumbar procaine block, by means of a solution of the substrate, depended on the time elapsing after injection of procaine. Between 1 h 30 min and 4 h 30 min after the block the nerve trunks and ganglia of Auerbach's plexus were revealed a little later (11-13 min after application of the solution), and less deformazan was formed than in the control animals. The intramural nervous plexuses became visible sooner (after 7-9 min) 24 h after the block and the intensity of diformazan produced was greater than normally. No nerve cells could be seen in intact animals undergoing lumbar procaine block.

The use of the lumbar procaine block after burns led to restoration of the normal state of the autonomic nervous system of the rabbit's small intestine. The nerve trunks and ganglia of Auerback's plexus were revealed later (after 11-20 min) 24 h after thermal trauma, and fewer nerve cells with SD activity were revealed than in burned animals not receiving procaine.

The results of the histochemical investigation of SD activity in the intramural nervous plexuses of the small intestine differed somewhat from the results obtained by intravital microscopy. For instance, during incubation of pieces of small intestine of intact animals in a solution of the substrate not only the subserous plexus and the nerve trunks and ganglia of Auerbach's plexus were demonstrated, but sometimes nerve cells also; nerve cells were particularly numerous in intact animals undergoing lumbar procaine block (Fig. 2). In control experiments, during incubation of pieces of small intestine of intact rabbits in a solution of the substrate from which sodium succinate was excluded, only the subserous plexus and the nerve trunks and ganglia of Auerbach's plexus took up the stain and the nerve cells remained colorless. This shows that reduction of nitro-blue tetrazolium by structures of the intramural nervous plexuses took place not through the oxidation of sodium succinate, but through its interaction with the phospholipids of nerve tissue [8, 9].

As the intravital microscopy experiments showed, SD can be detected in the nerve cells of Auerback's plexus only of animals after severe burns. This enzyme is known to be located on the inner mitochondrial membrane; diformazan formation is therefore possible only if the dye can pass through its membrane. In the control animals the intact mitochondrial membranes of the nerve cells of Auerback's plexus evidently prevented penetration of the tetrazolium salt inside the mitochondria, whereas in animals exposed to thermal trauma the mitochondrial membranes of these cells underwent definite changes. As a result of these changes molecules of nitro-blue tetrazolium were able to penetrate inside the mitochondria, and as a result the enzyme could be detected. The results of these experiments with intravital microscopy also showed that any type of procedure carried out on the animal (thermal trauma, procaine block) is reflected in the kinetics of the reactions of reduction of nitro-blue tetrazolium by structures of the intramural nervous plexuses of the small intestine.

These experiments showed that oxidoreductases can be demonstrated histochemically and changes in their activity studied in the living animal, i.e., under conditions precluding histological treatment of the nervous system.

Unlike the results obtained by intravital microscopy, the results of histochemical investigation of SD activity in the nerve cells of Auerbach's plexus of intact animals and, in particular, of animals undergoing lumbar procaine block, showed that during incubation of pieces of small intestine in a solution of the substrate post mortem changes take place in the mitochondrial membranes.

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