MORPHOLOGY AND PATHOMORPHOLOGY

EFFECT OF LEAD NITRATE ON THE SUPRAVITAL STAINING OF CELLS OF POIKILOTHERMS

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The present paper is devoted to a study of the effect of lead nitrate poisoning of poikilotherms, using a method involving supravital staining of the whole organism.

Supravital staining techniques are widely applied in cytology and physiology [9]. In most cases they are applied to isolated organs, and the resulting findings cannot always be taken as valid for the organism as a whole.

Various methods have been used in the study of lead poisoning, but we have been unable to find any references to the application of supravital staining. This method, however, has many advantages over the usual histological methods, since it permits of the study of reversible changes, does not require the use of powerfully acting fixatives, and permits the observation of changes in the physiological state of cells.

D. N. Nasonov [12] and P. V. Makarov [5] introduced neutral red into the gastrointestinal tract of animals, using a specially designed instrument for this purpose. A. A. Braun and M. F. Ivanov [1] injected the dye into the body cavity. The most useful supravital stain is neutral red [7, 8], which stains most tissues, including nervous tissue elements. E. V. Gubler [4], N. I. Grebenskaia [3], A. A. Savich [10], and E. M. Gramenitskii [2] have applied supravital staining of the whole organism to the study of the nervous system of a variety of animals.

EXPERIMENTAL METHODS

Autumn and winter frogs (males) were given injections into the body cavity of 1% aqueous neutral red solutions, at dosage levels of 0.3-0.5 mg of dye per g weight of animal. Over 300 experiments were performed in all. In most of the experiments, the organs under study were removed an hour after the injection of the dye, and were subjected to microscopic examination. Such studies of surviving tissues were conducted for the central nervous system, spinal ganglia, sympathetic nerve chains, liver, and kidneys.

We selected sensory nerve cells of the spinal ganglia for study because they represent links of reflex arcs, an analysis of the activity of which is relatively simple and readily performed. A study of the sympathetic nerve cells permits the assessment of the state of the autonomic nervous system, which is known to suffer injury in lead poisoning; cellular elements of the liver and kidneys were studied because these organs are involved in the accumulation and excretion of lead.

The techniques of isolation of spinal ganglia and sympathetic chains, and of preparation of surviving liver and kidney tissues for microscopic examination, have been described in the literature [2, 6, 11]. Observation of individual animals was conducted over several days. Injection of the stain had no perceptible effect on the condition of the animals. The distribution of the stain over all the organs of the body was assessed by means of colorimetric and microscopic examination.

EXPERIMENTAL RESULTS

Figure 1,a illustrates supravital staining of nerve cells of sympathetic chain ganglia 1 hour after injection of dye. Numerous, well defined granules of dye appear in the cytoplasm. These granules are distributed mostly

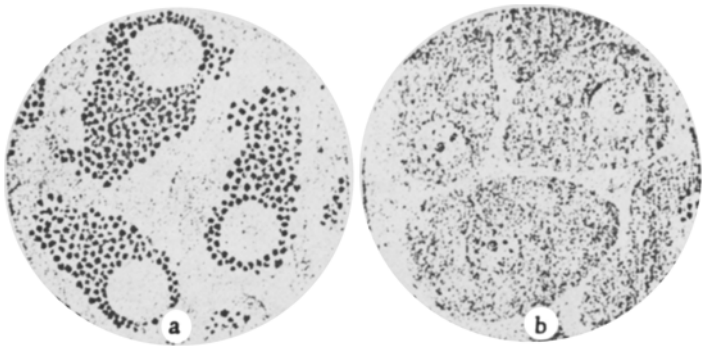

Fig. 1. Supravital staining of sympathetic nerve cells 1 hour after injection of neutral red and lead nitrate.
a) Control experiment; b) after simultaneous injection of neutral red and lead nitrate.

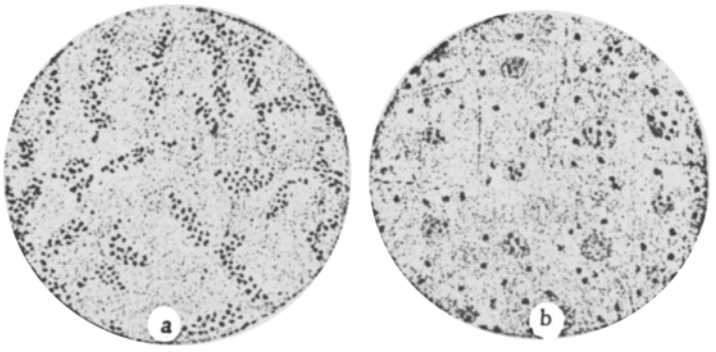

Fig. 2. Supravital staining of liver cells 1 hour after injection of neutral red and lead nitrate.
Explanation of Figure as for Fig. 1.

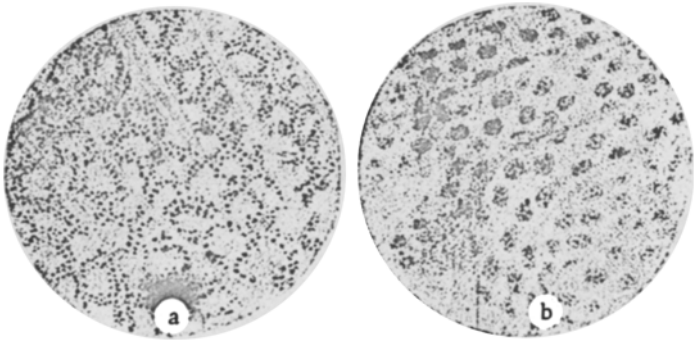

Fig. 3. Supravital staining of cells of the renal tubules 1 hour after injection of neutral red.

in the perinuclear region, and are never encountered in the neurite; they occupy a limited zone of the cytoplasm, corresponding with the intracellular apparatus. The neutral red granules have an irregular, angular shape, and are of different sizes. They are usually of a yellowish red color. The cytoplasm itself, and the nucleus, remain unstained. The distribution of the dye in the cytoplasm of uninjured spinal ganglion cells is similar,

with the sole difference that the cytoplasm takes on a light orange tinge, and the granules are smaller. No change in the reflex activity of the animals could be observed in the control experiments, showing that the supravital staining of the cells described is characteristic of normally functioning cells.

Large, deep red granules of dye were sparsely distributed in separated groups at the periphery of liver cells bordering the bile ducts. Dark granules of pigment may be encountered among the dye granules. The cell granules are not clearly defined. The nucleus and nucleolus are not stained (Fig. 2,a). The cells of the renal tubules are usually viewed from a polar aspect, for which reason the granules of neutral red are seen at the peripheries of the cytoplasm. They have a reddish tinge, and they are distributed along the cell boundary (Fig, 3,a).

It thus appears that in the intact organism, with uninjured, normally functioning cells, neutral red is deposited in the form of granules.

The frogs were poisoned by injection of aqueous solutions of lead nitrate, at dosage levels of 1-10 mg per g. The dye and the lead salt were injected simultaneously, but into different parts of the body, viz., the lead salt was injected into the dorsal lymph sac, and the dye into the body cavity.

Microscopic examination showed that administration of lead nitrate affected the nature of the supravital staining of the cells. With rising dosages of lead nitrate, diffuse staining of the cytoplasm appears, and the nucleus and nucleolus become visible, while the process of granule formation is suppressed, i.e., the complex of changes characteristic of paranecrosis supervenes [9].

Paranecrotic changes were seen in liver cells at dosages of 3 mg/g. The cell nuclei were stained, and granular structures appeared in them, while the cytoplasm assumed a uniform, diffuse, bluish-red coloration, and there was a marked fall in the number of granules (Fig. 2,b and Table).

Supravital Staining of Cells 1 Hour after Simultaneous Injection of Neutral Red and Lead Nitrate to Male Frogs (mean results of 50 experiments)

Dose of lead nitrate (mg/g)	Cell	Supravital staining		
		granules	nucleus	cytoplasm
3	Sensory nerve	+	0	0
	Sympathetic nerve	+	0	0
	Liver	0	+	+
	Renal tubules	+	0	0
7	Sensory nerve	+	0	0
	Sympathetic nerve	0	+	+
	Liver	0	+	+
	Renal tubules	+	0	0
10	Sensory nerve	+	0	0
	Sympathetic nerve	0	+	+
	Liver	0	+	+
	Renal tubules	0	+	+

Note: + presence of staining, 0 absence of staining.

The dose of lead nitrate needed in order to cause paranecrosis of the cells of the sympathetic chain was 7 mg/g. At this dosage, the cytoplasm was stained a uniform bluish-red color. Granules of dye were absent from the cytoplasm. The cell nuclei became clearly distinguishable owing to the diffuse pale pink staining of karyoplasm. The nucleoli were also stained (see Fig. 1,b and Table).

Raising the dosage level to 10 mg/g caused changes in the staining of the cells of the renal tubules. The cytoplasm of these cells was also diffusely stained a light bluish-red color, the process of granule formation was suppressed, and the nucleus, containing granular structures, became visible (Fig, 3,b). The diffuse staining of these cells, characteristic of the paranecrotic state, should be ascribed to the action of the lead salt, since the dye alone never gave rise to paranectotic changes in the given cells.

The nature of the staining of the sensory cells of spinal ganglia was not affected by even very high dosage levels of lead nitrate, in spite of the complete abolition of reflex excitability of the animals towards the end of the experiments. The absence of the paranecrotic type of staining in the nerve cells of the sensory ganglia cannot have been due to shortcomings in the methods used. The presence of granules in these cells shows that the dye penetrated into them in sufficient amount. Evidently lead nitrate, in causing irreversible loss of reflex activity, attacks synaptic links in the first place, without affecting the morphology of the individual cells of the nervous system. The work of P. V. Makarov [6] supports the possibility of such an interpretation of the mechanism of action of lead. In his studies on generalized narcosis of the nervous type, he showed that reflex excitability is abolished while the sensory nerve cells still retain the granular type of staining.

Our experimental material demonstrates the possibility of applying supravital staining of the whole organism with the basic dye neutral red, for the study of poisoning.

SUMMARY

A modified method of neutral red staining of the organs of cold-blooded animals in situ was used and the effect of lead nitrate on various cells was studied. The following threshold paranecrotic doses were established for this salt: 3 mg per g of body weight of liver cells, 7 mg per g for cells of kidney tubules and 10 mg per g for sympathetic nerve cells. Paranecrotic changes were not revealed in the sensory nerve cells of the spinal ganglia, although reflex activity of the laboratory animals was depressed. The modified method of staining in vivo is recommended for use in toxicology.

LITERATURE CITED

[1] A. A. Braun and M. F. Ivanov, Arkh. Anat. Gistol. i Embriol. 12, No. 1, 3-26 (1933).

[2] E. M. Gramenitskii, Biull. Eksptl. Biol. i Med. 34, No. 2, 76-78 (1952).

[3] N. I. Grebenskaia, "Analysis of the Effect of Carbon Monoxide on the Organism, using Supravital Staining Techniques,"* Thesis, Leningrad, 1951.

[4] E. V. Gubler, "Supravital Deposition of Neutral Red in Brain and Muscle under Conditions of Oxygen Deprivation,"* Thesis, Leningrad, 1949.

[5] P. V. Makarov, Arkh. Anat., Gistol., i Embriol. 13, No. 1, 41-60 (1934).

[6] P. V. Makarov, ibid., 19, Nos. 1-2, 5-104 (1938).

[7] M. E. Mandel'shtam, Arkh. Biol. Nauk 53, Nos. 2-3, 131-142 (1939).

[8] M. E. Mandel'shtam, ibid. 58, No. 2, 125-134 (1940).

[9] D. N. Nasonov and V. Ia. Aleksandrov, "Reaction of Living Matter to External Factors,"* Moscow, 1940.

[10] A. A. Savich, "Variations in Sensitivity to Narcotics in Philogenesis and Ontogenesis,"* Thesis, Leningrad, 1951.

[11] E. Ia. Smoilovskaia, Arkh. Anat. Gistol. i Embriol. 19, Nos. 1-2, 105-115 (1938).

[12] D. N. Nasonov, Protoplasma, 1932, Vol. 15, pp. 239-267.

* In Russian.