INTRAVITAL DISTRIBUTION OF CHELATING AGENTS IN THE TISSUES

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Injection of dithisone and quinoline compounds into animals leads to the intravital formation of granules of a zinc chelate in the tissues that can be detected in frozen sections. The intensity of the intravital histochemical reaction depends on the complex-forming capacity, dose, and mode of administration of the agent and also on the presence of other ligands reducing its use in the coordination of volume.

KEY WORDS: *zinc; chelating agents; intravital histochemical reaction.*

Intravenous injection of chelating agents is followed by the development of an intravital histochemical reaction with zinc, as described in [1-9]. However, the principles governing the development of this reaction have been inadequately studied.

The object of this investigation was to study these principles.

EXPERIMENTAL METHOD

Experiments were carried out on 530 animals (dogs, cats, rabbits, rats, golden hamsters, and mice) into which the following chelating agents were injected intraperitoneally: dithisone, 8-(p-toluenesulfonylamino)quinoline (8TSQ), and 8-(benzenesulfonylamino)quinoline (8BSQ). Dithisone was given in doses of between 10 and 100 mg/kg as a 1% solution in 0.5% ammonia solution, and 8TSQ and 8BSQ were given in doses of 10-50 mg/kg as a 0.5% solution in 0.1 N caustic soda solution.

The intravital histochemical reaction was studied in freshly frozen sections 10-30 μm thick cut from tissues taken 10 min after injection of the particular chelating agent. The dithisone reaction was observed in visible light and the reactions with 8TSQ and 8BSQ in reflected ultraviolet light (UFS-3 and ZhS-18 filters). To identify the cell types of the islets of Langerhans the sections with the intravital reaction were examined by dark-field microscopy [6].

EXPERIMENTAL RESULTS

Intravenous and intraperitoneal injection of dithisone and 8TSQ and 8BSQ led to the intravital formation of chelate granules. The dithisone granules were reddish violet in color and gave an orange–red luminescence in the dark field. After injection of 8TSQ and 8BSQ, granules giving yellowish-green luminescence were formed. The two types of granules were similar in shape, size, and location in the tissues. As a rule the granules were circular, with a more darkly stained membrane and paler, homogeneous contents.

After treatment of the sections with a 10% solution of sodium diethyldithiocarbamate (DDC) the color arising as a result of the intravital histochemical reaction disappeared after a few seconds. On subsequent staining of the sections with the corresponding chelating agents, no color appeared. The negative intravital reaction with dithisone, 8TSQ, and 8BSQ also was obtained if the animals were given a preliminary injection of DDC, which forms a colorless chelate with zinc.

The intravital histochemical reaction of the various chelating agents also was found in various parts of the body of animals into which a preliminary injection of globulin–zinc–

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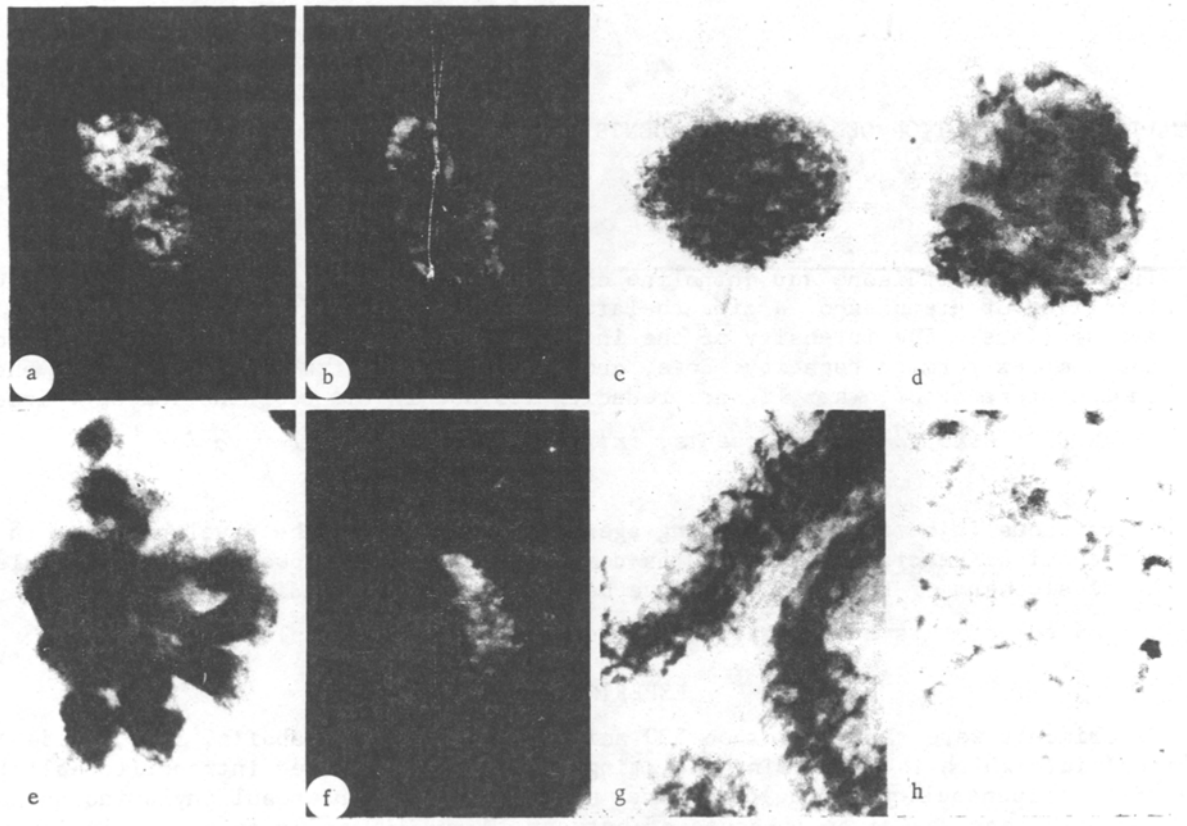

Fig. 1. Intravital histochemical reaction with zinc in various organs: a) luminescence reaction with 8-(p-toluenesulfonylamino)quinoline in the islet of Langerhans of a rabbit; b) the same islet in a dark field; c) reaction with dithisone in the islet of a hamster; d) the same but of a rat; e) the same in the small intestine of a rat; f) luminescence reaction with 8-(p-toluenesulfonylamino)-quinoline in a Paneth's cell of the rat small intestine; g) reaction with dithisone in the rat prostate; h) the same in the rabbit submandibular gland. Magnification: a-d, g, h) 200×; e, f) 900×.

insulin was given. If a mixture of the latter and DDC was injected, hardly any specific staining appeared in these areas. Finally, the presence of zinc chelate was demonstrated by spectrophotometric and fluorometric investigations of the products of the intravital reaction extracted from the homogenized tissues. Consequently, the color arising during the histochemical reaction with dithisone, 8TSQ, and 8BSQ is due to the formation of the products of their reaction with zinc atoms.

An intravital histochemical reaction was observed in the pancreas, small intestine, and prostate and submandibular glands. In the pancreas, the islets of Langerhans were stained; the reaction was strongest in rabbits, weaker in mice, hamsters, and rats, and particularly weak in cats and dogs. The staining reached a maximum of intensity after 10 min. In all species of animals except rabbits and rats, the A cells stained palely or not at all. In rats the A cells, arranged as a border at the periphery of the islets, gave a fairly strong reaction, whereas the B cells stained comparatively weakly (Fig. 1a-d).

In the small intestine a positive reaction was given by the Paneth's cells located in the basal portions of the crypts. The deepest staining was found in rats. In rabbits and mice it was much weaker. In hamsters, cats, and dogs the cells in the basal parts of the crypts did not stain (Fig. 1e, f).

Epithelial cells of the terminal portions stained in the prostate gland; the reaction was strongest in rats and dogs and much weaker in the other species of animals (Fig. 1g).

In the submandibular glands of rabbits the intravital reaction was visible at the periphery of the lobules. Even after injection of the maximal dose of dithisone it did not reach any considerable intensity, and after injection of 8TSQ and 8BSQ it was difficult to detect (Fig. 1h).

After intravenous injection of dithisone and the quinoline compounds, the histochemical reaction in the prostate gland was stronger, but in the small intestine it was weaker, than after intraperitoneal injection of the same dose of the compounds. No staining was observed in the organs after injection of the agents in a dose of 10 mg/kg.

The presence of other ligands reducing the utilization of the coordination capacity of the chelating compounds tested produced the intensity of their intravital histochemical reaction. This phenomenon was observed after preliminary injection of the ligands cysteine and glutathione.

The intensity of the intravital histochemical reaction between zinc and the various chelating agents thus depends on the complex-forming capacity, dose, and mode of administration of the agent and also on the zinc concentration and functional activity of the tissues, and on the presence of other ligands reducing the utilization of the coordination capacity of the chelating agents. The results described above and the conclusions drawn from them shed some light on the mechanisms of the selective cytotoxic action of chelating agents.

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