METHOD OF STUDYING REACTIVITY OF THE SKIN CAPILLARIES OF ALBINO MICE TO INFLAMMATORY STIMULI

Yu. N. Nuraliev and G. L. Mednik

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A modification of Monakova's method is suggested for determining reactivity of the skin capillaries to the action of inflammatory stimuli, so that tests can be carried out on albino mice.

In 1954, Monakova [3, 4] suggested a method for studying reactivity of the skin capillaries of rabbits to the action of inflammatory stimuli. She assessed the reactivity of the skin capillaries from the rate of escape of intravenously injected trypan blue in areas of skin to which the stimulus (xylene) was applied. This method has been used to study the effects of various agents on capillary reactivity [1, 2, 5].

The change from working on rabbits to working on albino mice considerably simplifies the method, makes it cheaper, and enables the action of various factors to be studied on large numbers of animals. For these reasons, albino mice weighing 18-19 g have been used. Trypan blue was injected intraperitoneally as follows. The animal was held with the left hand by the fold of skin on the dorsal surface of the neck and spine in the suspended position. The abdominal wall was punctured in layers, changing the direction of passage of the injection needle successively. The position of the needle in the abdominal cavity was verified by lifting the abdominal wall with the tip of the needle, thus forming a straight, thin ridge. Using a tuberculin syringe, 0.3 ml (50 mg/kv) of a 0.3% aqueous solution of trypan blue was injected through the needle rapidly into the animal's peritoneal cavity.

Since intravenous injection was replaced by intraperitoneal in the suggested method, and since the test objects are less sensitive animals (mice), the dose of the dye was increased by 2.5 × compared with that given during tests on rabbits.

The site of injection of the stimulus was the dorsal surface of the hind limbs, which are free from tough, thick hair and do not require any preliminary or additional treatment. The stimulating agent was m-xylene, which was applied in a dose of 0.02 ml to this area of the hind limb 10 min after injection of the dye.

The results of the test were assessed by the time of appearance of a blue color in the skin of the hind limbs at the site of application of the xylene. A conventional normal limit was established in a group of control animals (4.41 min). The earlier appearance of a blue color in the skin of the hind limbs following application of the substances studied was interpreted as the result of an increase in capillary permeability, and an increase in the time required for appearance of the blue color as the result of a decrease in capillary permeability.

The test can be repeated within 1 h on other limbs. However, the results of these tests are less demonstrative after 30-40 min, and after 1 h they are difficult to assess because of the appearance of a general blue color in the skin. The writers therefore suggest that when the antiinflammatory or pro-

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inflammatory properties of substances are investigated, and repeated tests are to be carried out, a fresh batch of animals should be used at each time interval of action of the substance. The substances to be studied must be injected at time intervals of between 15-40 min and 3-5 h before injection of the dye. If the tests are carried out in this way, their duration can be prolonged, and the dynamics of action of the substance can be judged comparatively easily and more accurately.

Using the method described above the effect of histamine and hyaluronidase, as proinflammatory agents, and of water-soluble prednisolone and mumie, as antiinflammatory substances, was studied.

The test substances were injected subcutaneously or intramuscularly in a volume of 0.2 ml, and animals of the control group received the same volume of physiological saline. The experimental results were analyzed statistically by the difference method [5].

In mice of the control group the time of appearance of the blue color of the hind limb varied from 3.5 to 5 min (mean 4.41 ± 0.1 min).

In the next series, 60 units hyaluronidase was injected intramuscularly into 10 mice 15 min before injection of the dye. A blue color at the site of application of xylene to the animals of this series appeared after 1.08-1.83 min (mean 1.57 ± 0.1 min; $P < 0.001$).

The effect of histamine was studied in 2 series of experiments. Histamine was injected subcutaneously into the animals of series I in a dose of 10 μg per animal, 15 min before injection of the dye, while in series II, twice this dose was given.

In the animals receiving 10 μg histamine, the time of appearance of the blue color varied from 2.16 to 3.91 min (mean 3.32 ± 0.07 min), while in the animals receiving 20 μg histamine, a blue color appeared in the hind limbs after 2.17-2.9 min (mean 2.52 ± 0.06 min).

Hence, as was expected, hyaluronidase and histamine sharply increased the reactivity of the skin capillaries to the action of inflammatory stimuli.

In the next series the animals received an intramuscular injection of the powerful antiinflammatory drug prednisolone (2 mg per mouse) 2.5 h before injection of the dye. In the mice of this series a blue color appeared after between 5.83 and 8.05 min (mean 7.29 ± 0.7 min), i.e., significantly later than in the control ($P < 0.001$).

Extract of mumie (12 mg) was injected subcutaneously 20 min before injection of the dye. In the mice of this series the time of appearance of the blue color varied from 4.5 to 6.5 min (mean 5.57 ± 0.07 min).

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