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A simple method for the quantitative extraction of dye extravasated into the skin

Increased vascular permeability is usually demonstrated by the leakage of certain dyes injected intravenously. Though the intensity of this reaction has been measured in various ways (Jori, Bentivoglio & Garattini, 1961; Parratt & West, 1958; Frigeni, Gazzanica & Bonanomi, 1970), the best way is to extract and determine the extravasated dye quantitatively. Of several methods for achieving this some do not give satisfactory recovery (Jancsó-Gábor, Szolcsányi & Jancsó, 1967), while others are laborious (Nitta, Hayashi & Norimatsu, 1963; Judah & Willoughby, 1962). The dye can be determined quantitatively by relatively simple procedures (Ankier & Whiteside, 1969; Udaka, Takeuchi & Movat, 1970), but this takes a long time at relatively high temperatures. We previously proposed a method in which the chopped skin is violently agitated in a mixed solvent of acetone and a commercial detergent, Emal, in a homoblendor for 15 min (Harada, Takeuchi & Katagiri, 1966). This method, however, had two demerits; (1) requirement of volume adjustment after the homoblendor process and (2) the use of a detergent that is a mixture of various compounds. Both defects have since been improved as follows. (1) Volume change due to volatilization of acetone was easily prevented by replacing the homoblendor procedure by a 24 h incubation at room temperature with occasional shaking. (2) Each ingredient of the detergent was examined individually and the component effective in the extraction was identified as sodium sulphate. The following experiment demonstrates the usefulness of this improved method.

First, the recovery of dye injected intradermally was examined. Various amounts of azovan (Evans) blue were injected into the skin of rats. After 30 min, each blue area, which was about 10–15 mm in diameter, was erased, cut into about 10 pieces with scissors and mixed with a medium composed of 14 ml of acetone and 6 ml of a 0·5% aqueous solution of sodium sulphate in a test tube. The tube was closely firmly with parafilm and left to stand for 24 h at room temperature (20°) with occasional mild shaking. Each preparation was then centrifuged for 10 min at 300 rev/min and the supernatant separated. Percentage recovery of the dye was calculated by comparing the absorbance of the supernatant at 620 nm with that of a standard sample prepared by mixing the corresponding amount of azovan blue and normal skin pieces in the same medium *in vitro*. The method gave a recovery of over

95% in all cases where the quantity of the dye injected was less than 200 μg, and a plot of the absorbance of the supernatant against the amount of the dye injected was linear. For quantitative recovery of the dye a sodium sulphate solution of 0·5% or more was necessary.

Changing the volume ratio of acetone to sodium sulphate solution greatly influenced the yield, the optimal ratio being estimated as 7:3. Raising the temperature to 37° together with continuous mild shaking had no significant effect on the extraction efficiency. Replacement of acetone by alcoholic solvents such as methanol, ethanol or butanol gave very low recovery of the dye. Pontamine sky blue injected intradermally was similarly quantitatively extracted from the rat skin. When trypan blue was used, however, the yields were lower than 90% throughout the dose range up to 200μg. The technique was also applicable to the extration of injected azovan blue and pontamine sky blue from mouse, guinea-pig, and rabbit skin.

This improved method could be applied to the extraction of dye which was exuded from the capillaries into the skin. Two blue spots were produced on the back of a rat by intradermal injection of 5-hydroxytryptamine at two sites, together with intravenous injection of azovan blue. One of the spots was extracted with the mixture of sodium sulphate and acetone, while the other was extracted with a mixture of acetone and the commercial detergent. The two extracts had almost the same absorbance.

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