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# LETTERS TO THE EDITOR

## Photometric evaluation of dye diffusion in the skin

If a local cutaneous lesion is produced by physical or chemical agents, colloidal dyes injected intravenously will diffuse in the cutaneous tissue. This phenomenon, reported by Spagnol (1929), has been used to study capillary permeability changes, after local action of inflammatory agents, or in quantitative evaluation of the protective or antagonistic action of several drugs.

Lockett & Jarman (1958) compared the intensity of the blue dye at an injection site with a series of comparator cards; Miles & Wilhelm (1955) and Jori, Bentivoglio & Garattini (1961) measured the lesion diameters; Parrat & West (1958) used simple visual grading of the response. Recently Judah & Willoughby (1962) described a quantitative method to measure trypan blue after extraction from a given cutaneous area. Young (1964), Ankier & Whiteside (1969) used a spectrophotometric method based on extraction in alkaline medium for the determination of protein-bound dye from tissues. Nevertheless, spectrophotometric methods, though sensitive, are laborious in pharmacological screening; maceration of tissue samples and centrifugation, essential for clear solutions, represent the principal difficulties.

We describe here a method for the direct measurement of the dye in rat skin with the technique used in the reading of electrophoretic strips.

Local lesions were produced by injecting inflammatory agents (like 5-hydroxytryptamine, bradykinin or histamine) by the intradermal route, in different doses for each rat and constant volume of 0·1 ml per dose, immediately after the dye. The injection sites were placed 30 mm from each other, in two rows parallel and 15 mm from the spinal cord. In different animals the injection sites of a drug were differently disposed to avoid injecting the same drug in the same area. The skin of each rat, dried and cleaned of all the subcutaneous tissue, was cut into longitudinal strips, each containing 2 or 3 blue spots. These strips were embedded for 3 h in clarifying solution [tricresylphosphate–light petroleum (40–60°)–acetone; 70:10:20] and then mounted between two glass slides. Readings were taken on a photometer for electrophoretic strips with 640 nm filter. The values obtained, expressed in $mm^2$, represent the integration of the absorbance and the area of a single blue spot.

Statistical analyses made comparatively on the results of our technique and that of Judah & Willoughby demonstrated the high sensitivity of the proposed method and its suitability for the evaluation of local capillary permeability changes.

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