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Letters to the Editor

Evaluation of changes of capillary permeability*

SIR,—The effects of drugs on capillary permeability are usually studied by means of dyes which bind to plasma proteins. Intradermal administration of agents increasing capillary permeability, such as 5-hydroxytryptamine (5-HT), histamine and bradykinin, results in the formation of coloured areas at the sites of injection when a dye is administered intravenously. The degree and extent of the colour accumulation are considered an index of the capillary lesion and are estimated by various methods. Thus, Parratt & West (1957) used the visual grading of the blueing, Ungar, Kobrin & Sezesny (1959) cut out and then weighed the pieces of skin, Judah & Willoughby (1962) determined the degree of dye leakage by extraction and photometric determination of the dye, whilst other authors presented their results in terms of the "mean lesion diameter" (Miles & Wilhelm, 1955; Sparrow & Wilhelm, 1957; Bonaccorsi & West, 1963). We have usually evaluated the degree of dye leakage into the coloured areas by measuring the two diameters (length and width) and utilising the product of these two measurements (Jori, Bentivoglio & Garattini, 1961; Bonaccorsi, Jori, & Garattini, 1963).

We have now found that the product of the two diameters of the coloured area parallels the effective movement of blue dye from the circulation into the skin, when 5-HT is used as a permeability-increasing agent.

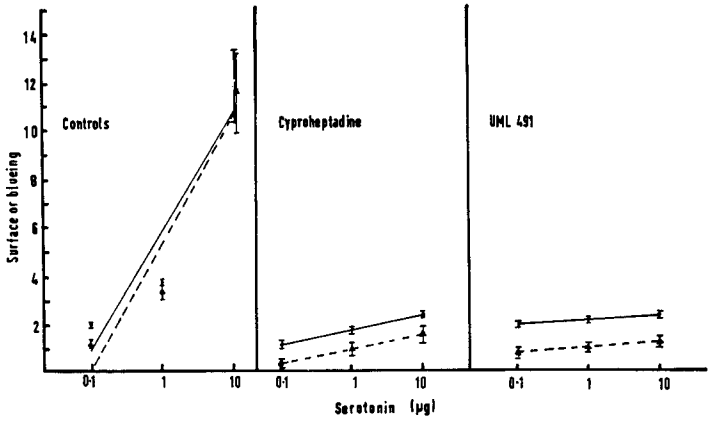

FIG. 1. Measurement of the coloured surface area (●) or of dye concentration (▲) induced by 5-HT in control animals (a) or in animals pretreated with cyproheptadine (b) or UML 491 (c). On the abscissae are shown (log scale) the concentrations of 5-HT (serotonin) injected intradermally; on the ordinates, the products of the two main diameters of the coloured spots in cm^2 or the μg ($\times$ 1/5) of azovan blue dye.
The analysis of the variance shows the following probability: regression for (a) $<0{\cdot}005$; (b) $<0{\cdot}01$; (c) $<0{\cdot}01$; deviation from linear regression for (a) 0·025; (b) 0·90; (c) 0·90; deviation from parallelism for (a) 0·50; (b) 0·70; (c) 0·40.

●—● surface (a) $y = 0{\cdot}84 + 5x$ (b) $y = 1{\cdot}10 + 0{\cdot}61x$ (c) $y = y\ 1{\cdot}74 + 0{\cdot}17x$

- - ▲ blueing $y = 0{\cdot}22 + 5{\cdot}22x$ $y = 0{\cdot}32 + 0{\cdot}57x$ $y = 0{\cdot}60 + 0{\cdot}23x$

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Sprague-Dawley rats weighing about 180 g were used. After depilation of the abdominal skin, the animals were anaesthetized with ethyl ether and injected intravenously with azovan blue (3 ml/kg of a 0·4% solution). Immediately, 0·1, 1 and 10 μg of 5-HT were injected intradermally, each in 0·1 ml of saline, into each animal. 30 min later, the rats were killed, the diameters of the coloured areas measured, and the pieces of skin removed and submitted to the azovan blue extraction and determination, according to the method of Beach & Steinetz (1961). Two antagonists of 5-HT, 1-methyl-lysergic acid butanolamide (UML 491—Sandoz) (Berde, Doepfner & Cerletti, 1960) and cyproheptadine (Periactin—Merck) (Stone, Wenger, Ludden, Stavorski & Ross, 1961), which had been shown to have a strong inhibitory activity in this test (Jori, Bentivoglio & Garrattini, 1961), were injected intravenously 30 min before azovan blue, at doses of 3 μg/kg and 100 μg/kg respectively. Fig. 1 shows that the curves of the dye concentration (blueing) and of the coloured surface area measurements are congruent. Therefore, at least in our experimental conditions, measurement of the surface of the coloured area is adequate to evaluate the degree of the dye infiltration.

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Paper chromatography and identification of *Magnolia acuminata* L. alkaloids

SIR,—In a survey of plants for steroidal sapogenins and other constituents Wall, Fenske, Garwin, Willaman, Jones, Schubert & Gentry (1959) screened some plants of the magnoliaceae family. Of the four species of the American magnolias examined, presence of alkaloids was reported in stems and leaves of *Magnolia acuminata* L. (Cucumber-tree). However, these compounds have not been isolated and characterized.

We have extracted the stems of *M. acuminata* and isolated fractions containing alkaloids. Descending paper chromatography of the quaternary chlorides was performed using three different solvent systems. Five quaternary bases (A to E) were detected (Table 1) after development with solvent system 1 and spraying with reagent I. Four bases have been identified and checked by running them with authentic specimens. A is choline chloride; B is magnoflorine chloride, D is salicifoline chloride and E is magnocurarine chloride.