which drugs injected i.c. mediate their effects on body temperature in the conscious rat. Certainly, the diversity of drugs producing a similar response pattern suggests that a single mechanism by which temperature changes occur is unlikely.

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A new method for the rapid determination of azovan blue leaked into the skin

There have been many methods for the determination of azovan blue leaked into the ventral skin after intravenous injection. These are all time consuming. Recently, an improved method was published (Harada, Takeuchi & others, 1971) but this required more than 24 h to complete the determination. We have developed a new method for the estimation of azovan blue which depends on the hydrolysis by lactic acid and hydrochloric acid of collagen composing the skin. The major advantages are that it is sensitive, reproducible with little degradation of the dye and convenient for the analysis of a large number of samples in a day.

Skin containing azovan blue is added to 3 ml of 28% lactic acid and boiled for 5 min. Then, 3 ml of 9 N HCl is added and the solution boiled for 5 min. After vigorous shaking the solution is cooled to room temperature (20°) and about 5 ml of CCl_4 is added with shaking. After centrifugation at 2000 rev/min for 5 min, the solution separates in two phases. The lower phase is removed and 5 ml of acetone is added to the tube. The supernatant obtained after shaking and then centrifugation at 3000 rev/min for 10 min is used for the colorimetric determination of the dye at 615 nm. The colour is stable when measured within 2 h.

A standard curve can be obtained by adding amounts of dye in 0·05 ml of 0·85% NaCl solution to a test tube to which a piece of the intact skin is added. The standard curve is linear over the range 25 to 125 μg of azovan blue.

Recovery was tested by injecting various amounts of dye (25–125 μg in 0·05 ml) intradermally into the abdomen of a guinea-pig after removing excess hair with clippers. After 5 min, the animal was killed and the skin containing the dye removed. The dye was measured by the method described. Recovery from treated skin was 94–106% at 25, 50, 100 and 125 μg of the dye injected.

0·05 ml of histamine in 0·85% NaCl solution was injected intradermally into the abdomen of a guinea-pig and immediately 10 mg of azovan blue in 1 ml of 0·85% NaCl solution was given intravenously. 30 min later, the animal was killed by a sharp blow on the head and bled and the skin containing the dye assayed. Table 1 shows that the amount of dye detected increased with increase in histamine dose.

Lipid should be removed from the supernatant by carbon tetrachloride and gelatin should be removed by chilling the test tube before sedimentation.

As shown in Table 1, the amount of dye leaked into the skin after histamine treatment agrees with results, using the method of Harada & others (1971). The present method had the same sensitivity as Harada's method and the advantage that lactic acid prevented the degradation of dye by hydrochloric acid.

Table 1. *The recovery of azovan blue leaked into the skin by intradermal injection of histamine.* Histamine was injected at two places of the ventral skin. Assays were performed by the method described in the text. The results are expressed as means ± s.e. (number of animals).

Administered histamine μg	Azovan blue detected (μg) proposed method	Harada's method
1·0	52 ± 10 (6)	41 (2)
2·0	74 ± 16 (6)	73 (2)
3·0	95 ± 20 (6)	93 (2)

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