Staining of experimentally-induced chorio-retinal lesions with Disulphine blue and Evans blue

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It is often difficult to assess the extent and activity of chorio-retinal lesions of inflammatory or surgical nature. Fluorescein angiography has been used with success, but this technique requires a clear media and the use of filters and other elaborate equipment. The development of an alternative simpler technique, using other dyes, to demonstrate increased vascular permeability in chorio-retinal lesions is therefore of clinical interest and forms the subject of this report.

Material and methods

Four albino rabbits weighing 2.5 to 3 kg. were anaesthetized with intravenous pentobarbital sodium.

RABBIT 1

One eye was proptosed and the sclera was exposed posterior to the equator by a semilunar incision in the conjunctiva, from 9 to 3 o'clock. Two diathermy marks, the first stronger than the second, were placed 5 to 6 mm. from one another, at a distance of 8 to 9 mm. from the limbus, thus avoiding the area of the retina containing myelinated fibres. Indirect ophthalmoscopy demonstrated a mild whitish reaction at the site of the weak diathermy application and a more marked area of coagulation in response to the stronger diathermy. Immediately after application of the diathermy, 6.2 per cent. solution of Disulphine blue* (wt./volume) was injected intravenously through the auricular vein (0.5 mL/kg.).

The fundus was examined during the course of the injection and at 2, 5, 15, 30, 60, 120, and 180 minutes, and 24 and 48 hours after the injection. Fundus photography of the diathermized areas was performed before and after the injection of the dye.

RABBIT 2

The same procedure was repeated and cryotherapy of -40°C. was applied to the sclera. A mild reaction was obtained by applying the tip of the cryostat to the sclera for a short time until there was a mild whitish reaction on the fundus. A more marked lesion was obtained by applying the cryostat for a longer time until an obvious reaction appeared at the site of application. Both reactions were controlled by indirect ophthalmoscopy.

RABBIT 3

Two photocoagulation marks of different intensity (weak and strong) were produced. The weak reaction was obtained by setting the Zeiss Photocoagulator at 20V and 40A for 1 second and the stronger by setting it at 22V and 45A for approximately 3 seconds.

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*Monosodium salt of anhydro-4:4'-bisdithyl aminotriphenyl methanol-2':2'-disulphonic acid (Sulphan blue). Manufactured by I.C.I., Wilmslow, Cheshire, U.K.
RABBIT 4

Weak and strong diathermy marks were produced as in Rabbit 1, but immediately thereafter 1.5 ml. of 2 per cent. solution of Evans blue (0.5 ml./kg. body weight) was injected intravenously.

Results

In the three rabbits injected with Disulphine blue, the whole fundus became blue within a few seconds. The intensity of the blue stain decreased rapidly and disappeared from all areas other than the injured ones within 1 minute. However, these areas retained a residual light blue colour which increased progressively in intensity during the following 15 minutes. There was no apparent difference in the colour obtained after the application of weaker and stronger stimuli, in the three different procedures used. All the lesions stained homogeneously blue, but the staining was more intense when diathermy and photocoagulation were used than after cryotherapy. Figs 1 and 2 show the appearance of a strong photocoagulation lesion before and 5 min. after the injection of Disulphine blue.

One hour after the injection of Disulphine blue, the staining in the lesions began to decrease, and about 24 hours after the injection the colour disappeared completely. A mild blue flare, which gradually disappeared after 24 hours, was observed in the anterior chamber and in the vitreous. The skin and mucosae of the rabbits stained blue immediately after injection of the dye and the colour disappeared gradually within 36 hours. The urine remained blue for the same length of time.

In Rabbit 4, injected with 2 per cent. Evans blue, the diathermized area showed initial staining 45 min. after the injection. The staining was maximal after 90 min. and was evident for the next 3 days. The skin and mucosae became bluish in colour 12 hours after the injection and remained blue for 3 days.

Discussion

Disulphine blue has been used in humans by plastic surgeons in order to assess the degree of skin burns, which were determined according to the amount of blue staining of the injured areas (Gibson and Brown, 1945; Tempest, 1958). In ophthalmology, Sorsby (1939) first used the dye to establish the extent of retinal detachment and the location of retinal holes. The dye is reported to be nontoxic in humans, and is rapidly excreted by the kidneys, although there is occasional transient nausea.

In the present study it has been shown that the dye (0.5 ml./kg.) is capable of staining chorio-retinal lesions, probably by leaking through injured vessels. The intensity of staining was somewhat stronger after diathermy and photocoagulation than after cryotherapy. This probably indicates more severe vascular damage in the two former lesions. When Disulphine blue was injected in smaller doses which did not colour the skin, it also failed to stain the chorio-retinal lesions. Evans blue was found to have the same properties, but the staining process was slower and the disappearance of the dye from the skin was more prolonged.

In the light of the above data, the use of these dyes may be of clinical importance in studying chorio-retinal lesions of surgical or inflammatory nature.

Summary

(1) Disulphine blue (0.5 ml./kg.) administered intravenously was used in vivo to stain chorio-retinal lesions produced by diathermy, photocoagulation, and cryotherapy.
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**Fig. 1** Rabbit 3. Fundus appearance of a strong photocoagulation mark

**Fig. 2** Rabbit 3. Blue staining of the same photocoagulation mark, 5 min. after Disulphine blue injection
(2) The above lesions stained homogeneously blue within a few minutes of injection of the dye and retained this colour for a period of approximately 2½ hours.

(3) The main untoward effect of the dye was a diffuse staining of the skin during approximately 36 hours.

(4) A solution of 2 per cent. Evans blue (0·5 ml./kg.) also stained chorio-retinal lesions in a similar manner but more slowly.

References