intravenous injection of the extract to the cat is followed 3–5 min later by auriculo-ventricular block, the degrees of the block increasing with time. Other rhythm disturbances including bigeminism were eventually observed (Fig. 1B). The action was over in 30 min but the maintenance of a myocardiac impregnation by the compound was evidenced by the faster and the more pronounced effect of a second injection.

Finally, the extract has a positive inotropic action on the isolated rabbit auricle preparation as described by Burn (1952) (Fig. 1C).

It is concluded that this evidence demonstrates the presence of a cardiotonic drug of bufadienolide type in *Bersama yangambiensis*.

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A simple method for measuring the amount of azovan blue exuded into the skin in response to an inflammatory stimulus

SIR,—The most characteristic sign of inflammation is the enhancement of vascular permeability. To indicate the increased permeability, the sulphonic acid dyes like azovan (Evans) blue or trypan blue, which become bound to serum proteins, are suitable. The dye-protein complex is exuded into the surrounding tissues during the enhancement of the permeability. In most reports the evaluation of the local staining effect was confined to a subjective visual estimation such as colour intensity or diameter of the coloured areas. Several methods exist for extracting the dye from minced tissues, but they are complicated, especially for routine examinations (Sachs & Lummis, 1955; Clausen & Lifson, 1956; Judah & Willoughby, 1962; Hladovec, Horáková & Mansfeld, 1961). Of these methods only the last two have been elaborated for the extraction of the dye from the skin.

We now describe a simple extraction method in which a methanolic solution of suramin (Bayer 205) is used to extract the azovan blue at room temperature from a piece of skin without maceration or mincing.

The strong linkage of suramin to protein has been known for a long time (Mayer, 1922). Jancsó (1955) showed that the suramin molecules competed with the dye molecules for the possession of the binding sites in the tissue and in this way high concentrations of suramin displaced the bound dye from the tissue structures. This competition arises because the two compounds are similar in chemical constitution, both being symmetrical naphthalene sulphonic acids.

The suramin solution slowly elutes the dye from the skin so that within 14 days all the dye passes into the clear alcoholic phase and can then be measured spectrophotometrically. An Optica-Milan spectrophotometer is suitable.

The spectrum peak of the tissue extract (620 mμ) corresponds to the spectrum peak of the pure azovan blue solution, whereas suramin alone shows no absorption over the wavelengths of the curve (475–675 mμ). Tissue extracts from rats to which dye had not been injected did not give extinction values. All the measurements were made at 620 mμ.

To establish the optimal concentration of suramin for the dye extraction, experiments were made with solutions of different suramin content. Rats were injected intravenously with 0·5 ml/100 g of 1% azovan blue solution, then the inflammatory reaction was induced by painting the skin of the hind paws with 4% mustard oil in liquid paraffin. The rats were killed by bleeding 20 min after the application of the inflammatory agent and the dorsal skin of the paws was removed, weighed and put into the suramin-methanol solution. For 100 mg of tissue 3 ml extracting solution was suitable. A solution containing 0·01% suramin did not extract any dye from the skin. The 0·1% solution extracted a large amount of dye within 2 weeks, but the skin was not completely decolourized. We then transferred the skin to 1·0% suramin solution in which it was completely decolourized in a few days and the solution turned pale blue. Taking the whole amount of azovan blue obtained by this double extraction procedure to represent 100% extraction it was established (4 experiments) that the 0·1% suramin solution extracted 88·4% of the dye contained in the skin and the 0·5 and 1% suramin solutions extracted 95·5 and 95·2%. Without changing the solution a higher extraction ratio could not be reached as tissue pieces floating in the fairly concentrated dye solution were stained by it. This was shown by the fact that this 4–5% of dye remaining could be eluted if the piece of skin was put into a few ml of pure methanol, instead of into a suramin solution.

As an example we examined the extent to which vascular permeability, i.e. the amount of exuded dye, was increased by mustard oil applications of increasing concentration. The dorsal skin of the hind paws of rats weighing 130–150 g was painted with 0·25, 1, 4 or 8% mustard oil solution after 50 mg/kg azovan blue dye had been injected intravenously. The animals were killed 20 min later and the dye was extracted from the skin of the paws with a 1% suramin solution (3 ml solution per 100 mg skin). After painting with 0·25% mustard oil a total amount of only 2 μg dye could be demonstrated, a quantity which agreed with the dye content obtained in the control paws not treated with the irritant. Hence, in such low concentrations mustard oil does not enhance the permeability, but with increasing concentrations of mustard oil there was increased recovery of azovan blue from the skin and there was a linear relation between concentration of irritant and vascular permeability as measured by the amount of azovan blue exuded. The figures were: 10 μg dye after 1% oil, 20 μg after 4% oil and 24 μg after 8% oil.

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