Mechanism of Increased Capillary Permeability Induced by *Echis carinatus* (Saw-scaled Viper) Venom: A possible new approach to the Treatment of Viperine Snake Poisoning

SIR,—Haemorrhages in various organs, in skin, and in mucous membranes, haematuria, and the accompanying shock, resulting from a widespread capillary endothelial damage, and the prolonged clotting time, determine to a large extent the ultimate outcome of the viperine snake poisoning (Ahuja and Singh, 1956). The exact mechanism by which the venom produces an increased capillary permeability is not known so far. It was, therefore, decided to determine whether or not the response is mediated through the release of 5-hydroxytryptamine (5-HT) or histamine, which have been reported to be responsible for the formation of oedema and increased capillary permeability induced by various substances (Spector, 1958; Jori, Bentivoglio and Garattini, 1961).

Capillary permeability was determined by injecting azovan blue dye, 20 mg./kg. intravenously (Parratt and West, 1957), and observing the extent and intensity of blue discoloration produced by 20 μg. each of the *E. carinatus* venom, histamine and 5-HT administered intradermally in the previously depilated abdominal skin of the rat. Male albino rats of Haffkine strain, weighing 150–200 g., were used. An interval of 15 min. was allowed for the dye to accumulate at the site of the drug administration, when the rats were killed and skin removed. The number of rats used as well as the results obtained in the control group, promethazine pretreated group, lysergic acid diethylamide pretreated group and the promethazine plus lysergic acid diethylamide pretreated group are summarised in Table I. It may be observed that the venom is almost as powerful as 5-HT, and approximately three times more potent than histamine, in increasing the capillary permeability in the rat. Promethazine pretreatment completely blocked the response to histamine, that to 5-HT remained unaltered, and that to the venom was significantly reduced. Lysergic acid diethylamide, on the other hand, completely blocked the response to 5-HT, slightly reduced that to histamine, and that to the venom was reduced to almost half. When both promethazine and lysergic acid diethylamide were given, the response to all the three drugs was completely blocked.

TABLE I

Treatment 30 min. before the test	Diameter (mm.) of area of blue discoloration 15 min. after the test drug administration in groups of 10 rats		
	5-HT (20 μg.)	Histamine (20 μg.)	*E. carinatus* venom (20 μg.)
Control	20 (18–22)	6 (5–7)	18 (16–20)
Promethazine 10 mg./kg. i.p.	19 (18–20)	nil	6 (4–8)
Lysergic acid diethylamide 1 mg./kg. i.v.	nil	5 (4–6)	10 (8–11)
Promethazine 10 mg./kg. i.p. + Lysergic acid diethylamide 1 mg./kg. i.v.	nil	nil	1–2

The results suggest that the increased capillary permeability induced by the *E. carinatus* venom in the skin of the rat is mediated through a release of both histamine and 5-HT, and the effect is partially prevented by the use of an antihistamine or an anti-5-HT drug alone, and completely blocked by the two drugs given together. Inasmuch as most of the symptoms in the viperine snake poisoning are due to an increased capillary permeability with the resultant

haemorrhages and shock, treatment with antihistamine and anti-5-HT drugs may prove to be an important advance in the clinical management of the snake poisoning.

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The Activity of Ampicillin against *Escherichia coli*

SIR,—The isolation of the penicillin "nucleus," 6-aminopenicillanic acid, has resulted in the development of new penicillins with advantages over the older existing ones. It has become clear from recent work that the penicillins show much variation in their antibacterial activity (Garrod, 1960).

Until recently, the penicillins were regarded as having only a slight inhibitory action on the growth of Gram-negative bacteria in general. Infections caused by such organisms were normally treated with either chloramphenicol or the tetracyclines; a new penicillin, ampicillin, 6[D(−)α-aminophenylacetamido]penicillanic acid, known commercially as Penbritin, has now been formulated which is more effective than either of these antibiotics in infections caused by *Escherichia coli*, *Proteus sp.*, *Shigella sp.* and *Salmonella sp.* (Rolinson and Stevens, 1961; Brown and Acred, 1961).

One of the principal mechanisms involved in the antibacterial action of benzylpenicillin is its interference with, and inhibition of, bacterial cell wall synthesis, with the resultant formation of bacterial spheroplasts. These may be regarded as bacteria which are deficient in a portion of the cell wall responsible for rigidity. At least five other penicillins are known to have a similar antibacterial action (Russell, 1962), and experiments were made to investigate whether ampicillin also possessed this property. Accordingly, 0·1 ml. of a 17-hr. broth culture of *Escherichia coli* grown at 37° was added to 10 ml. tubes of broth containing 0·33 M sucrose, 0·25 per cent w/v $MgSO_4 \cdot 7H_2O$ and varying concentrations of ampicillin. After incubation of all tubes for 5 hr. at 37°, samples were examined by phase-contrast microscopy. It was found that the minimum dose of the drug needed to induce spheroplast formation was 10 μg./ml. The minimum inhibitory concentration of ampicillin against the same organism in nutrient broth was also 10 μg./ml., this reading being taken after 24 hr. incubation at 37°. Further, by means of the method described by Rolinson and Stevens (1961) it was possible, in the space of a few days, to "train" the organism to grow in the presence of 100 μg./ml. ampicillin. Whether or not bacterial resistance to this antibiotic will present a clinical problem remains to be seen. We wish to thank Dr. G. N. Rolinson for a gift of ampicillin.

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