Association of Histamine and 5-Hydroxytryptamine with the Inflammatory Processes

SIR,—It was reported that mast cells undergo characteristic changes during aseptic inflammation in the rat (Sanyal, 1959). As histamine and 5-hydroxytryptamine (5-HT) may be associated with these cells, at least in the rat and the mouse, we have studied the tissue levels at intervals after the production of aseptic inflammation, and also the effect of previous depletion of either amine on the production of inflammatory exudate or development of fibrous tissue.

Accordingly groups of 6 rats were anaesthetised with ether, and a surgical incision was made on the back with aseptic precautions. The skin flaps were mobilised and sutured. Animals anaesthetised and then allowed to recover served as controls. Control animals, and those subjected to surgical trauma, were killed, 1, 2, 3, 4, 6, 8, 12, 16 and 26 days after the operation, for extraction and assay of histamine and 5-HT (Parratt and West, 1957a) of the skin subjected to trauma, an adjacent area, and an area away from the site of injury, namely, the legs. Skin samples from all the animals in one group were pooled for extraction and assay. The values obtained from the operated group were compared with those obtained from control animals at comparable sites. There was a 25 per cent reduction in the values for histamine obtained from the operated area, in the first 24 hr.; these values returned to control level by 4th day, and thereafter showed a progressive rise to 170 per cent by the 12th day, finally returning to the control levels by 16-26th day. The 5-HT values showed a progressive rise from the beginning, reaching a maximum of about 300 per cent of the control levels by the end of one week, thereafter returning to control levels by the 16th day. In the adjacent and distal areas, values for both histamine and 5-HT began to rise in the first 24 hr.; they reached a maximum of 200–300 per cent of the control levels in 6–8 days and then returned to control levels in 16–26 days. Results from mice were similar.

The exudative phenomenon was studied by the granuloma pouch method and the development of fibrous tissue by the cotton wool pellet method (Finney and Somers, 1958). Control animals, animals depleted of either histamine by repeated injections of polymixin B or 5-HT by injections of reserpine (Parratt and West, 1957b) were anaesthetised and either a granuloma pouch was produced by creating an air pocket in the back into which a little croton oil in arachis oil was injected or 8 weighed cotton wool dental pellets were inserted in a subcutaneous pocket. After about one week the animals were killed. The amount of exudate in the pouch was most in the control animals; in histamine depleted animals the values were about 35 per cent of control whereas values of 20 per cent of control were obtained in animals depleted of 5-HT. The latter group showed least inflammation, though in some animals the overlying skin had become parchment-like and necrotic. The groups in which cotton wool pellets were implanted were also killed after one week. The pellets were cleaned of extraneous tissues and dried in an oven to constant weight. They showed an increase in weight of about $28{\cdot}18 \pm 2{\cdot}05$ mg. in control animals, the increase in the polymixin B treated animals being $11{\cdot}89 \pm 1{\cdot}09$ mg., and in the reserpine treated animals, $7{\cdot}92 \pm 0{\cdot}60$ mg. These changes were statistically significant, all having confidence limits greater than 99 per cent. Thus the maximum reduction in the development of fibrous tissue was in the reserpine-treated animals. This effect could not be due to the depletion of catecholamines, since carbachol which also causes the excretion of catecholamines did not reduce the development of fibrous tissue. In a comparative study, the action of reserpine was only slightly inferior to prednisolone (20 mg./kg. per day for 3 days) in

preventing the development of fibrous tissues. Thus both histamine and 5-HT may be involved in both exudative and reparative stages of the inflammatory response. Recently it has been suggested that histamine liberated from mast cells may prepare many more connective tissue cells than are normally available to receive heparin or heparin containing granules, which may be used in preparing ground substances (Riley, 1962), and that 5-HT may possibly, particularly in the rat and mouse, act similarly (West, 1962). The anti-inflammatory effect of histamine and more particularly 5-HT depletion, lend support to this view.

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Demonstration of Interaction between Pairs of Antibacterial Agents

SIR,—It has been recognised that the antibacterial action of pairs of antibiotics may be that of simple addition of their separate effects, or one may enhance the activity of the other, or there may be mutual antagonism. These phenomena have been demonstrated by a number of techniques, one of which uses paper strips loaded with the compounds and laid at right angles on a seeded agar plate. Zones of inhibition are produced after incubation and the pattern of the growth between the strips gives information about the mutual effect, if any, of the pairs of compounds (Dye, 1955–56; Maccaro, 1961).

In a problem concerning the formulation of eye drops it was required to find if pairs of compounds used as bacteriostatic agents were more or less effective than each one alone or if no interaction between them occurred, and the method applied to antibiotics quoted above was investigated. In effect, the interaction of 28 combinations of antibacterial substances from the following list, phenylmercuric nitrate, 2-phenylethanol, chlorocresol, thiomersalate, chlorhexidine, benzalkonium chloride, chlorbutol and Eye-drop Solution B.P.C. were tested against *Pseudomonas aeruginosa*, NCTC 7244, *Streptococcus pyogenes*, NCTC 8708, *Staphylococcus aureus*, NCTC 4163, *Escherichia coli*, NCTC 86, *Bacillus subtilis*, NCTC 8236 and *Proteus vulgaris*, NCTC 4636.

With all six bacterial species, no antagonism was demonstrated between any pair of bacteriostats listed; there was evidence of mutual enhancement of activity between 2-phenylethanol and the organic mecurials. Antagonism between calcium thioglycollate and phenylmercuric nitrate and between chlorhexidine and lecithin can be strikingly demonstrated by this method.

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