

*Short communications*

## Role of the adrenal gland in the leucocytosis caused by bradykinin or cellulose sulphate in the rat

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Intravenous injections of bradykinin, cellulose sulphate (a kinin-releasing agent) or adrenaline cause rapid leucocytosis in the rat. The effect of the two former drugs is abolished by adrenalectomy, that of the latter is unaffected by this treatment. Bradykinin and agents capable of releasing it in plasma, may induce leucocytosis via adrenaline released from the adrenal gland.

Bradykinin causes leucocytosis in dog and man—a direct action involving the mobilization of bone marrow leucocyte reserves has been postulated (Cardoso, Amorim, Nogueira, Alencar, Corrado & Rêgo, 1967). Rothschild (1968) noted that cellulose sulphate, a kinin-releasing agent, causes leucocytosis in the rat. This work shows that in this species, leucocytosis due to bradykinin or cellulose sulphate, is indirect, mediated most probably by endogenous, released adrenaline.

**Methods.**—Female Wistar rats (200 g) were used to obtain the data presented in Fig. 1a. Solutions of cellulose sulphate (prepared according to Astrup, Galsmar & Volkert, 1944), bradykinin triacetate (Sandoz) or adrenaline (Sigma), were injected through the caudal vein. Samples of blood were withdrawn by cardiac puncture under pentobarbitone (30–40 mg/kg) anaesthesia. Leucocyte counts in peripheral blood were performed using a Neubauer counting chamber under a Zeiss microscope at 80-fold magnification. Sodium oxalate (0.2%) was used as anticoagulant. Female rats had significantly lower ( $P < 0.05$ ) leucocyte counts ( $6.540 \pm 180/\text{mm}^3$ ), than normal males ( $7.810 \pm 350/\text{mm}^3$ ). Male Wistar rats (120–150 g) were adrenalectomized under pentobarbitone anaesthesia and placed on saline as drinking fluid; 4 days after the operation they were given 400  $\mu\text{g}$  of depot methyl-prednisolone (Depo-Medrol, Up-

john), intraperitoneally; this treatment was repeated 48 h afterwards. The animals were used after another 48 hours. Sham-operated controls were kept under the same conditions. No difference between the leucocyte counts of rats submitted to adrenalectomy ( $8.240 \pm 30/\text{mm}^3$ ) or sham-operation ( $8.200 \pm 40/\text{mm}^3$ ), were noted.

**Results.**—Panel (a) of Fig. 1 shows that the leucocytosis induced by 3 mg/kg of cellulose sulphate could be reproduced by the injection of bradykinin (100  $\mu\text{g}/\text{kg}$ ). Although high when compared with that required for most of its other pharmacodynamic effects, this amount of bradykinin is released into the circulation of rats given maximally effective doses of cellulose sulphate (Rothschild, 1968). Lower doses of bradykinin, given either as a single intravenous injection of 30  $\mu\text{g}/\text{kg}$ , or as an intravenous infusion of 60  $\mu\text{g}/\text{kg}$  over a 10 min period, failed to induce leucocytosis. Figure 1 also shows that adrenaline (10  $\mu\text{g}/\text{kg}$ ) induced significant leucocytosis, apparent within the first 5 min; adrenaline (1  $\mu\text{g}/\text{kg}$ ) was also slightly effective: it succeeded in overcoming the small leucopenia caused by saline in control animals.

It has long been known that adrenaline-releasing stimuli cause leucocytosis in many species including the rat (Cress, Clare & Gellhorn, 1943). Bradykinin causes the release of adrenaline (Feldberg & Lewis, 1964). In view of the large amounts of bradykinin released by cellulose sulphate in the rat (Rothschild, 1968), the possibility that this polysaccharide as well as bradykinin cause leucocytosis by an indirect action mediated through the release of adrenaline, was investigated after adrenalectomy. Panel (b) of Fig. 1 shows that adrenalectomized rats, kept on a saline diet and glucocorticoid maintenance therapy, were insensitive to the leucocytosis-inducing effect of bradykinin. Cellulose sulphate was still able to cause leucocytosis; its efficacy was, however, 6 times smaller than in control, sham operated animals. Adrenaline was active in both control and adrenalectomized rats indicating that adrenalectomy *per se* did not impair the capacity for leucocytosis in the animal.

**Discussion.**—The results presented above provide support for the assumption that endogenous adrenaline plays a role in the leucocytosis observed in bradykinin or cellulose sulphate treated animals. Cardoso

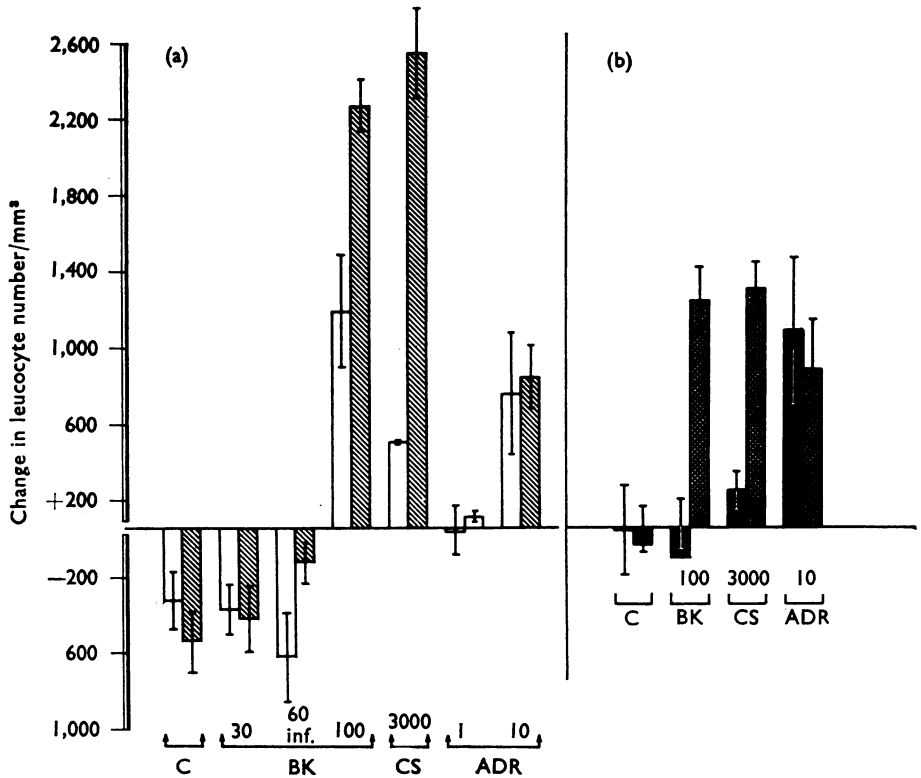


FIG. 1. Effect of cellulose sulphate (CS), bradykinin (BK), adrenaline (ADR) and saline (C) on the changes in leucocyte numbers in the blood of normal (a) and adrenalectomized and sham operated rats (b). Numbers refer to dose ( $\mu\text{g}/\text{kg}$ ) of drug intravenously injected or infused (inf) over a 10 min period. Panel (a): blood samples were withdrawn 5 min,  $\square$  and 60 min,  $\text{▨}$  after treatment. Panel (b): blood samples were withdrawn 5 min after treatment.  $\blacksquare$ , Adrenalectomized;  $\text{▨}$ , sham operated. Panel (a): means of three animals  $\pm$  S.E.M. Panel (b): means of five to seven animals  $\pm$  S.E.M.

*et al.* (1967) arrived at an opposite conclusion; it should be noted, however, that their results were obtained in dogs and in man given, respectively, doses of bradykinin 6 and 100 times smaller than those required to induce leucocytosis in the rat. Further work will decide whether these discrepancies are due to species linked differences in sensitivity, different mechanisms of production of leucocytosis or other causes.

Dougherty & White (1943), have shown that adrenocortical extracts or ACTH can cause polymorphonuclear leucocytosis, lymphopenia and a slight change in total leucocyte counts in the blood of mice and rats. My experiments do not rule out the possibility that a release of adrenocortical hormones is involved in the leucocytosis caused by bradykinin or cellulose sulphate in the rat. Nevertheless, impairment of the adrenaline-releasing ability of the adrenal gland seems to be the major cause for the decreased responsiveness to bradykinin or cellulose sulphate of the adrenal-

ectomized rat: maintenance on saline plus methyl-prednisolone, a long-acting glucocorticoid, would tend to counteract most of the symptoms of adrenocortical insufficiency in such animals (Sarett, 1959).

The leucocytosis induced by cellulose sulphate is most probably due to the rather large amounts of bradykinin which this polysaccharide releases in rat blood (Rothschild & Gascon, 1966). Rats depleted of bradykininogen by cellulose sulphate are less sensitive to passive heterologous cutaneous anaphylaxis (PCA) than normal animals (Rothschild, 1967). A role for PMN leucocytes in the aetiology of this phenomenon is suggested by the results of Takeuchi, Udaka & Movat (1969). The observation that cellulose sulphate does not cause leucopenia yet decreases sensitivity to heterologous PCA, could indicate a role for bradykinin in the aetiology of this phenomenon. Recent results by Eisen & Loveday (1970), however, demonstrate anticomplementary activity of cellulose sul-

phate in the rat. Since the complement system is a requisite for heterologous PCA in the rat (Osler, Hawrasiak, Ovary, Siqueira & Bier, 1957), it could well be an alternative site of the inhibitory action of cellulose sulphate in this form of anaphylaxis.

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