reacted with neurophysin in cryostat sections of sheep posterior pituitary. The use of ethanol-fixed tissues embedded in paraffin (Holborow & Johnson, 1967) enabled immunofluorescence staining to be followed, after postfixation in Bouin's solution, by conventional histochemical reactions for NSM.

In normal sheep, fluorescence was confined to the supraoptic and paraventricular nuclei, and to the neural portion of the HNS. In the median eminence and lower infundibular stem, the fluorescence appeared as fine fibres. In the pars infundibularis, the proximal portion showed intense fluorescence of globular appearance; in the central portion the fluorescence was more diffuse and its intensity low, but the intensity was high in the most distal portion.

In sheep with natural scrapie there was a reduction in specific fluorescence in the hypothalamic nuclei, but at the level of the median eminence the fluorescence was dramatically increased. In the distal tract there was a considerable reduction, particularly in the central portion of the neural lobe. This pattern is similar to that found for NSM by Beck, Daniel & Parry (1964).

Intense foci, 0·5–5 μm in diameter, of neurophysin-specific immunofluorescence were observed outside the HNS in the granular layer of the cerebellum and in the region of the III cranial nerve. Subsequent staining of the same sections by the Gomori CAH method revealed NSLM in precisely the same sites. Similar NSLM was found by Bignami *et al.*, in degenerating cerebellar bouton terminaux, which showed at the ultrastructural level electron dense bodies within Herring body-like structures.

These observations suggest that degenerating bouton terminaux in the cerebellum contain neurophysin or a protein sufficiently similar to cross-react with this cross-species reactive neurophysin antiserum.

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Definition of the histaminic component of the bronchoconstrictor and cardiovascular effects of anaphylatoxin in the guinea-pig

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Anaphylatoxin (AT) is a potent histamine liberator in the guinea-pig. Heparin, histaminase and catecholamines are also involved in AT-induced effects *in vivo*. How-

ever, the liberation of these known endogenous substances does not adequately account for all the observable effects, and a directly acting component has been postulated. In view of this apparent complexity of the mode of action of AT it is desirable to define the extent of the influence of the various components. To this end a quantitative evaluation of the histaminic component has been performed.

Anaphylatoxin was prepared according to the method of Rothschild & Rocha e Silva (1954), by incubation of rat serum with inulin. When administered intravenously to guinea-pigs (400–600 g) anaesthetized with pentobarbitone (60 mg/kg, i.p.) AT produced resistance to positive pressure inflation (bronchoconstriction) and a biphasic blood-pressure response. The latter comprised a brief initial depressor phase followed by a pressor component. These responses were dose dependent but to avoid any distortion due to tachyphylaxis each dose level was investigated separately in different animals, a submaximal dose (0·5 ml/kg) being selected and used in all subsequent experiments.

Upon repeated administration of AT the bronchoconstrictor and pressor responses were subject to tachyphylaxis, decreasing to a level of 20% of the respective initial values. In contrast, the depressor response showed no tachyphylaxis. Furthermore, graded doses of mepyramine (0·5–4·0 mg/kg i.v.) progressively inhibited the bronchoconstrictor and pressor responses to a residue of approximately 30% of the control values, without affecting the depressor response. This correlation between the tachyphylactic effect and the inhibition with mepyramine suggested a histamine-depleting mechanism as the basis of the tachyphylaxis. This would be in agreement with the observation by Hahn, Maack, Müller, Mitze & Ebner (1970) that the tachyphylactogenic effect of AT is associated with a decrease in histamine depots. It is, however, to be noted that the residual effect after mepyramine blockade was approximately 10% higher than the residue after complete tachyphylaxis, this difference being statistically significant (bronchoconstriction at $P<0{\cdot}001$, and pressor response at $P<0{\cdot}01$). The involvement of some other mechanism additional to histamine depletion in the observed tachyphylaxis phenomenon was thus implied.

Aminoguanidine sulphate (2 and 10 mg/kg) did not alter the responses to AT or exogenous histamine (10 μg/kg i.v.). However, it reversed the inhibitory effects of heparin (500 and 10,000 U/kg) on the bronchoconstrictor and pressor responses to both AT and intravenous histamine. The depressor response to AT in contrast to the depressor response to intravenous histamine was resistant to heparin inhibition.

It is concluded that the histamine component of AT activity in the guinea-pig with respect to the bronchoconstrictor and pressor responses represents approximately 70% of the total response but does not involve the depressor response.

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