estimations were carried out on the blood and tissues of six animals at intervals while the scorbutic diet was being administered, and changes in tissue and body weight were recorded. On the 27th day animals were spontaneously dying and showed morphological evidence of scurvy. The body weight increased until the twelfth day on the scorbutic diet in the same way as in control and hypervitaminotic animals (Odumosu & Wilson, 1970). Thereafter it decreased, and was 8·5% less than the initial weight on the 27th day ($P<0.05$). The adrenal weights increased consistently from the sixth day and finally were 65% above the initial weights. The adrenal cortisol concentration remained constant until the sixth day. It had fallen to 75% of its initial level by the eighteenth day, but the decrease then stopped until the end of the experiment. From the first day of vitamin C deprivation the plasma cortisol increased, and on the 27th day it was 27 times its initial value. Values for the biliary cortisol increased in a comparable fashion. Like the adrenal cortisol, the adrenal ascorbic acid concentration did not alter during the first 6 days of the scorbutic diet. Thereafter adrenal ascorbic acid diminished rapidly. 87% of the ascorbic acid had disappeared by the eighteenth day, and ultimately 99·5% had been lost. Plasma ascorbic acid increased during the first 6 days and then fell consistently to almost zero on the 27th day. Leucocyte ascorbic acid did not decrease until after the sixth day. By the eighteenth day 40% had been lost, but thereafter leucocyte ascorbic acid fell significantly more slowly than plasma ascorbic acid as shown by the change in angle of the regression lines relating plasma and leucocyte values. During the development of scurvy the corticosteroid secretion increases on the sixth day at the same time as a change appears in the metabolic use of ascorbic acid. On the eighteenth day adrenal production of cortisol levelled out at the same time as the leucocytes began to conserve their stores of ascorbic acid. Throughout the experiment plasma ascorbic acid showed a more extensive range of variation than leucocyte ascorbic acid. We suggest that this indicates that plasma values give a measure of metabolic demands, and leucocyte values indicate storage capacity for ascorbic acid.

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Isolation of dense-cored granules from the neurosecretory system of the vena cava of the small octopod, *Eledone cirrosa*

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A system of nerves associated with the vena cava of the cephalopod, *Eledone cirrosa*, has been proposed as having a neurosecretory function (Alexandrowicz, 1964). An extensive system of nerve terminations, containing numerous electron-dense granules, is closely associated with the inner wall of the blood vessel. A potent cardio-excitatory substance is associated with these nerve terminations (Berry & Cottrell, 1970). It is possible to scrape off this inner layer of nerve terminations. Such material provides a very suitable preparation for the isolation of the electron-dense granules.

The tissue was isolated in 1·1 M sucrose (a solution approximately isotonic with sea-water), homogenized, and prepared for centrifugation on a discontinuous sucrose

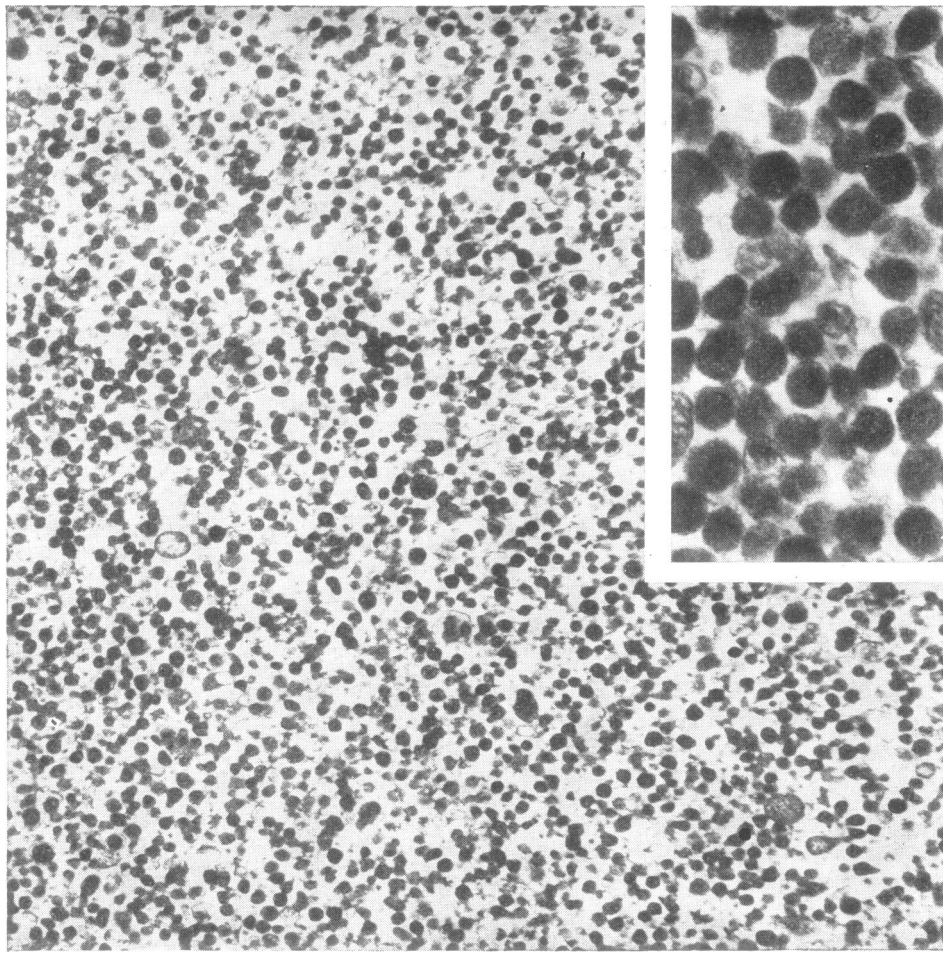

FIG. 1. Electron micrograph of the fraction equilibrating between 2·0 and 2·4 M sucrose. This fraction consists of electron-dense granules 100–125 nm in diameter. (×20,000; inset ×60,000.)

gradient after the method of Cottrell (1966). Fractions were separated on a gradient ranging from 1·3 to 2·4 M sucrose (pH 6·8–7·2). The gradient was centrifuged at 97,000 *g* for 90 min, and fractions were collected using a syringe. All processes were carried out at 0°–4° C. The volume and molarity of each fraction was adjusted to give 1 ml of 1·1 M sucrose solution. Each fraction was then spun at 97,000 *g* for 60 min and the sediments prepared for electron microscopy. The pellets were fixed in 2·5% buffered glutaraldehyde at 0° C (method from Berry & Cottrell, 1970).

The fraction equilibrating between 2·0 and 2·4 M sucrose consisted entirely of granules 100–125 nm in diameter (Fig. 1). Cell membranes forming "nerve-ending ghosts" and isolated granules separated between 1·7 and 2·0 M sucrose, while "nerve-ending bodies" containing dense-cored granules separated between 1·3 and 1·7 M sucrose.

The preparation of a pure granule fraction will facilitate the identification of the structure and function of the substance(s) associated with this neurosecretory system.

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Relative susceptibility of peripheral sympathetic nerves to adrenergic neurone blockade by bethanidine

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The intravenous doses of bethanidine sulphate necessary in cats for complete abolition of responses of the heart to sympathetic postganglionic nerve stimulation (0·4–0·8 mg/kg) were much lower than those required for inhibiting contractions of the nictitating membrane caused by indirect stimulation (3·2 mg/kg) (Boura & Green, 1963; Armstrong & Boura, 1970). The ability of bethanidine to block the responses of the heart and nictitating membranes to stimulation of their respective sympathetic postganglionic nerves has now been studied further and related to any concomitant change in effector tissue sensitivity.

Cats were anaesthetized with chloralose, and autonomic reflexes were blocked by bilateral vagotomy, sympathetic cardiac nerve section or ganglion blockade (pentacynium 2 mg/kg intravenously). Low intravenous doses of bethanidine (0·4–0·8 mg/kg) slightly reduced the magnitude of the nictitating membrane contractions caused by nerve stimulation (0·3–30·0 Hz), and potentiated responses to intra-arterial injection of 0·25–16·0 μg (−)-noradrenaline bitartrate (2·6 fold), 0·1–4·0 μg (−)-adrenaline bitartrate (2·1 fold) and 2·5–40·0 μg acetylcholine bromide (approximately 1·2 fold). Higher dose levels (1·6 mg/kg) caused a 5·5, 3·3 and a 2·8 fold increase in sensitivity to noradrenaline, adrenaline and acetylcholine respectively.

The most likely explanation for the hypersensitivity of the nictitating membranes is that bethanidine resembles other adrenergic neurone blocking agents by blocking uptake of catecholamines into tissue stores (Boura & Green, 1965), thereby permitting increased concentrations to reach effector sites. This conclusion is supported by the finding that noradrenaline was potentiated more than adrenaline, the former amine being taken up more readily from the circulation than the latter (Iversen, 1967). The potentiation of the effects of acetylcholine could be due to elevated background levels of noradrenaline as caused by cocaine (Trendelenburg, 1962).

Little potentiation of the positive chronotropic effects on the heart of noradrenaline (0·5–8 μg) and adrenaline (0·5–8 μg) given intravenously could be detected after the above doses of bethanidine. This contrast between the magnitude of the myocardial and nictitating membrane sensitivity changes to catecholamines is again analogous to that which occurs after cocaine (Innes & Kosterlitz, 1950; Fleming & Trendelenburg, 1961).

It is concluded that the apparently high resistance of the postganglionic cervical sympathetic nerve to the blocking action of bethanidine can be accounted for by the relatively greater hypersensitivity developed by the nictitating membrane to catecholamines. Any reduction in the output of transmitter, resulting from administration