The acute effect of 6-OHDA in the present study was either an increase or a fall in T_b. The hypothermic effect of 6-OHDA might result from the release of endogenous NA[10], which in turn could activate pathways driving inhibition of heat production. The lowering effect of 6-OHDA on T_b has been widely demonstrated in mammals [11-13]. The hypothermic effect of intrahypothalamic injection of dopamine (DA) on T_b of the pigeon seems to be rather slight[3]. Since the highest concentration of DA is found outside the hypothalamus[14,15] interaction between dopaminergic and cholinergic system seems unlikely. Furthermore the effects obtained with 6-OHDA are not necessarily a result from its effect on dopaminergic neurons.

The slight increase in T_b, when the injection site was close to the tractus septomesencephalicus, points to a transient, probably unspecific activation of pathways driving thermogenesis. This indicates that 6-OHDA did not reach the catecholamine terminals, hence NA was not released and no hypothermia ensued.

In discussing the possibility of the interaction between the noradrenergic and cholinergic systems, an assumption is made that stimulation of either of these systems simultaneously with the other adds to hypothermia induced by one system alone. Since near the tractus opticus the hypothermic response to CCh was markedly attenuated in birds in which 6-OHDA induced a decline of T_b, we might reason that due to the degeneration of NA terminals this system could not contribute to CCh hypothermia. This also indicates that the noradrenergic system is involved in cholinergic hypothermia via the release of NA.

In conclusion, the results of the present work suggest that in a circumscribed area in the pigeon hypothalamus the noradrenergic neurons may be involved in hypothermia induced by injection of CCh.

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Ultrastructural preservation of human atrial intrinsic innervation after the cold ischemic anoxic asystole during cardiac surgery[1]

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Summary. The method of induced cold ischemic anoxic asystole used in the course of cardiac surgery for protection of the myocardium also preserves well the inbuilt intrinsic nervous apparatus of the heart, including the small dense-cored vesicles of the adrenergic nerve terminals. However, if the protective procedure fails, the resulting damage to the myocardial cells is accompanied by severe destruction of the neural elements.

Protection of the myocardium from ischemic anoxic injury during open-heart surgery is of crucial importance in order to ensure adequate cardiac function after the corrective procedure. Various techniques have been widely used for myocardial preservation. The efficacy of these techniques has been studied also with the electron microscope[2-7]. Attempts have been made to define criteria of irreversible damage[6], and the ultrastructural picture of the 'stone heart' (extreme irreversible ischemic contracture of the left ventricle following extended normothermic ischemic arrest) has been described also in humans[8]. Until now, the main interest has been focused on the myocardial cell, while very little is known of possible injury to the inbuilt intrinsic nervous apparatus of the heart. The present study was undertaken partly to fill this gap in our knowledge.

Patients and methods. Right atrial myocardial biopsies were excised in the course of prosthetic aortic valve replacement operation from 8 consecutive patients a) before starting the extra-corporeal circulation, b) after the cold ischemic anoxic asystole (aortic cross-clamping (50–70 min) during general hypothermia (30 °C) associated with local cardiac cooling with +4 °C saline solution) and subsequent coronary reprefusion (20–30 min, until decannulation). 2 specimens were excised from each patient. The 1st specimen (which served as the control) was excised from the right auricular appendage at the insertion of the venous perfusion cannula. The 2nd specimen was excised from the root of the right auricular appendage, below the purse-string suture, at decannulation. Thin myocardial tissue strips were immediately fixed by immersion in 5% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.2) at 0 °C for 5 h, postfixed in 2% osmium tetroxide for 1 h, dehydrated in graded series of aethyl alcohol, and finally embedded, through propylene oxide and a mixture of equal amounts of propylene oxide and epoxy resin, in an epon-araldite mixture. Ultrathin sections exhibiting silver to gold interference colours were stained with saturated uranyl acetate and 2.5% lead citrate. The sections were viewed and photographed with a Philips electron microscope EM 300, operated at 60 kV.

Observations. In most cases, the ultrastructure of the nerve

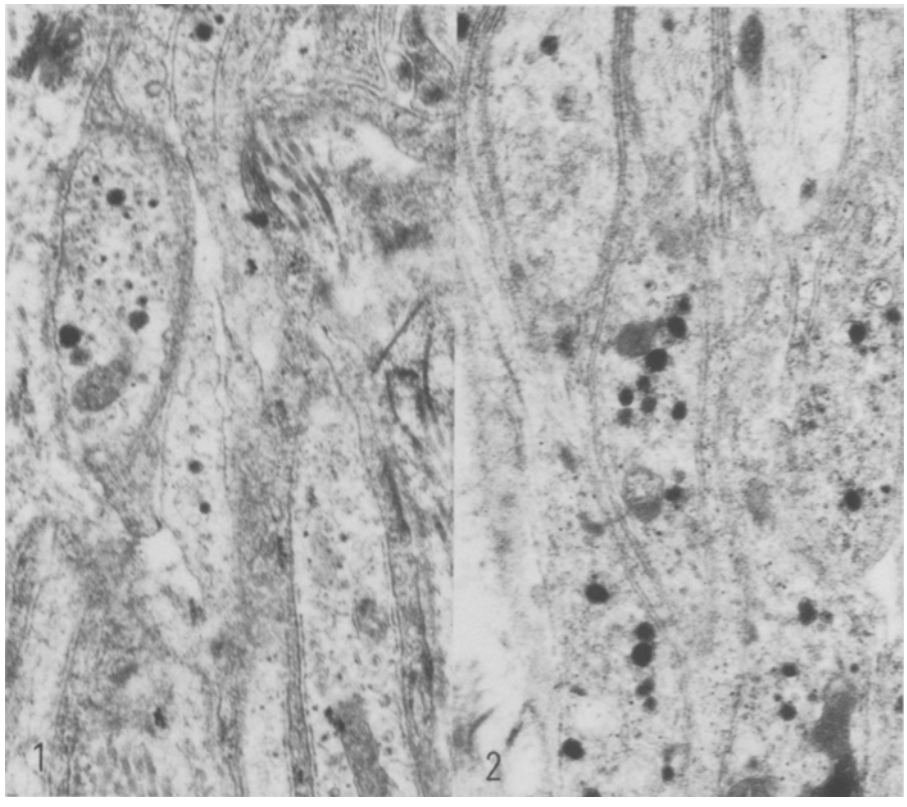

Fig. 1. Group of nerve terminals, most of them adrenergic, i.e. containing also small dense-cored vesicles. Control specimen biopsed from the right atrial myocardium at the beginning of the prosthetic aortic valve replacement operation. × 9200.

Fig. 2. Normal ultrastructure of the nerve terminals in the specimen biopsed from the same patient after the induced cold ischemic anoxic asystole and subsequent coronary reperfusion. Also the small dense-cored vesicles of the adrenergic nerve terminals can be clearly identified, although the amine granules are not quite as prominent as in the respective control specimen (figure 1). × 11500.

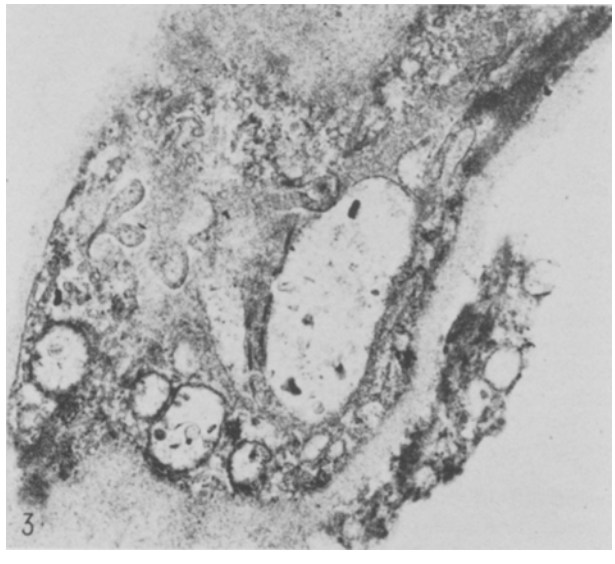

Fig. 3. Severe destruction of the nerve terminals in the specimen biopsed from another patient after the induced cold ischemic anoxic asystole and subsequent coronary reperfusion (severe damage ot the myocardial cells also occurred, and the recovery of the patient was complicated by long-lasting low output syndrome). × 11500.

terminals was well preserved after the cold ischemic anoxic asystole (and subsequent coronary reperfusion), including the small densecored vesicles of the adrenergic nerve terminals (figures 1 and 2). In these cases, the ultrastructure of the myocardial cells was also well preserved, and the patients made a smooth recovery. In one case, severe destruction of the myocardial cells and nerve terminals occurred. These nerve terminals contained degenerated mitochondria, irregular lamellar myelin figures, lysosomal vacuolization products (autophagic vacuoles) and occasional normal-looking mitochondria. Many terminals were filled with vacuolized, quite homogeneous, finely electron dense material, in which no vesicles or mitochondria could be identified. No small dense-cored vesicles could be identified, and even the large dense-cored vesicles were degenerated to ghosts. Some nerve terminals were degenerated to pale, empty, deformed vacuoles (figure 3). This patient, too, ultimately recovered but only after a long-lasting severe low output syndrome (cardiogenic shock). In the respective control specimen, biopsed at the beginning of the operation, the nerve terminals were completely normal.

Discussion. The results of the present study clearly indicate that the method of induced cold ischemic anoxic asystole used in the course of cardiac surgery for protection of the myocardium also preserves well the inbuilt intrinsic nervous apparatus of the heart, including the small densecored vesicles of the adrenergic nerve terminals, in principle. However, if the protective procedure fails, the resulting damage to the myocardial cells is accompanied by destruction of the neural elements. This may be, per se, of considerable clinical importance: it is obvious that even 'spot-like' destruction of the nerve terminals is potentially harmful, because it may interfere with the peripheral neuro-neuronal interaction despite near-normal total amounts of neurotransmitter substances. Loss of integrity of the nerve net can obviously lead to non-homogenous spread of the excitation wave front. Such disparity has been shown by marked intra-cardiac variations in inotropic response, in tissue refractoriness, and in patterns of ventricular repolarization during controlled stimulation of cardiac sympathetic nerves[9]. Preservation of the noradrenaline-containing adrenergic nerve terminals is probably not of crucial importance to maintain the basic myocardial con-

tractility, but their destruction or amine-depletion would remove a potentially important compensatory mechanism for augmentation of the myocardial force development and velocity of contraction in the failing heart[10,11]. In conclusion, preservation of the inbuilt intrinsic nervous apparatus of the heart should be taken into consideration in association with the problem of myocardial preservation.

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Intravenous injections of cholecystokinin and caerulein suppress food intake in domestic fowls

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Summary. As with various mammals, cholecystokinin (CCK) and caerulein have short-term, dose-related, inhibitory effects on feeding when injected i.v. in domestic fowls. It is estimated that in meals lasting more than about 6 min there could be time for ingested food to reach the duodenum and for the release of CCK to act as a satiety signal.

Cholecystokinin (CCK) is a polypeptide hormone which is released in the duodenum and jejunum upon entry of ingesta[2,3]. It causes contraction of the gall bladder, inhibits gastric emptying, stimulates pancreatic enzyme secretion and is also found in the brain[3,4]. It has a dose-related inhibitory effect on feeding when injected in various mammals[5-9] and has been proposed as a short-term physiological satiety signal[4]. There is evidence that it occurs in the brain and gut of birds[10,11], and porcine CCK stimulates pancreas activity in turkeys[12], but Snapir and Glick[13] were unable to demonstrate a significant reduction in intake in domestic fowls following i.p. injections of CCK and caerulein, a decapeptide chemically similar and with similar properties to CCK. However, they tested only 1 dose of CCK (20 Ivy dog units/kg b.wt) and 2 of caerulein (1 and 2 µg/kg), their samples were small and it is possible that i.p. injections are less effective with birds than mammals. We now report that CCK and caerulein suppress feeding in a dose-related manner when injected i.v. in domestic fowls.

Methods. For the CCK experiment 10 immature medium-hybrid hens (Rhode Island Red × Light Sussex) were housed and tested in individual cages on a 14-h photoperiod (07.00–21.00 h) and were given access to a commercial pelleted diet for 6 h/day (10.00–16.00 h), having been trained to this schedule for a week before testing started. They were tested for 5 weeks from 12 to 17 weeks of age, when their mean body weights increased from 1.46 ± 0.02 (SE) to 1.89 ± 0.04 kg. CCK has a half-life of only 2 or 3 min[14] and its effects are short-lived[5,7], so food consumption was measured in the periods 10.00–10.15 h and 10.15–16.00 h every day from Monday to Friday. At 10.00 h on Tuesdays and Thursdays each bird was injected by wing vein, immediately before receiving its food, with either 1, 5, 10, 20 or 40 Ivy dog units (IDU)/kg of the synthetic C-terminal octapeptide of CCK (Squibb Institute for Medical Research, New Jersey; assuming that 1 mg CCK = 3000 IDU[9]) dissolved in 0.9% NaCl solution (10 IDU/ml), or an equivalent volume of the 0.9% saline. Injected volumes varied from 0.15 to 8 ml. Every bird received each of the 10 injections once, in random order, according to a Latin square arrangement. Half the birds received CCK on Tuesday and the corresponding dose of saline on Thursday of the same week, and the other half were injected in the reverse order. With each bird and dose, food consumption from 10.00–10.15 h and 10.15–16.00 h on CCK and saline injection days was compared with the corresponding control intake, taken as the mean from Monday, Wednesday and Friday of that week. To test the significance of effects, error variances were estimated by performing analyses of variance between and within weeks.

For the caerulein experiment the above procedure was repeated with 10 immature hens of a different, light-hybrid (White Leghorn), strain. They were tested from 15 to 20 weeks of age and weighed from 1.25 ± 0.02 to 1.52 ± 0.03 kg. The 5 doses of caerulein (Sigma, London) injected were 0.1, 0.5, 1,2 and 4 µg/kg, dissolved in 0.9% saline (1 µg/ml), as before. Injected volumes of caerulein and saline varied from 0.13 to 6 ml.

Results and discussion. I.v. injections of CCK, caerulein and saline caused significant reductions in food intake in the period 10.00–10.15 h compared to that on control (non-injection) days (figure, table). The reductions due to CCK and caerulein were greater than those due to saline, and increased significantly with dose (table), whereas those due to the isotonic saline were not related to dose, and may have been a consequence of the actual handling and injection of the birds. The suppression of food intake due to injection of the peptides disappeared in the period 10.15–16.00 h, when there was a compensatory increase in feeding on CCK and caerulein injection days which was not related to dose. Intake from 10.15 to 16.00 h on saline injection days decreased significantly with dose in the case of the light-hybrids (caerulein), but not the medium-hybrids (CCK), and with both strains did not differ signifi-