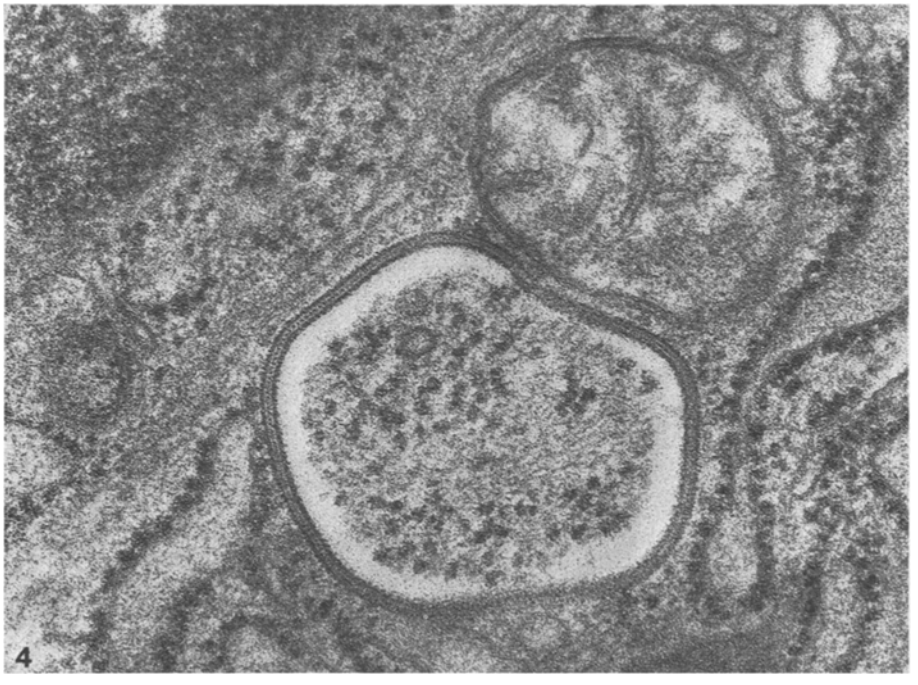

Fig. 4. An intracytoplasmic inclusion body is found in close contact with a mitochondrion and in the vicinity of the nucleus, a part of which is seen in the upper left corner of the picture. The granular core of the body is separated from the pentalaminar limiting body membrane by an electron-lucent halo (×120,000).

structure was found located near the outermost layer of the body membrane (figure 2). The intermediate dotted layer of the body membrane appeared to be discontinuous at sites of saccule location (figures 2 and 3).

The spherical membrane-bound bodies found in the metaphyseal trabecular bone tissue of the tibia of mice occurred mainly in osteoblasts and occasionally in preosteoblasts. They were not observed in capillary endothelial cells, osteoclasts and osteocytes. All bodies were located freely in the cytoplasmic matrix of cells. Continuities of the limiting body membrane with the limiting membrane of the cell within which a body was located were never detected.

Discussion. The bodies described in the present study are similar in their ultrastructure to those which several authors have found in cells from various normal or neoplastic tissues of hamsters[6], rats[8], rabbits[3,7], sheep[5] and men[2,4]. The structures have been observed in luteal cells of the ovary[2], cells of the membrana granulosa of the ovarian follicle[3], cultured cells from a small-cell carcinoma of the adrenal cortex[4], keratinizing cells of the wool follicle[5], cells of estrogen-induced renal adenocarcinomas[6], cells of vitamin A acid-treated keratoacanthomas[7] and periosteal cells of normal and lathyritic femurs[8]. Such inclusion bodies have not, to our knowledge, yet been reported as occurring in bone cells from the proximal tibial metaphysis of normal mice.

It has been presumed that the spherical structures might originate from intercellular gap junctions by bulging of such junctions into the cytoplasm of cells and by pinching off of the invaginations from the cell membranes[2,3,5,6]. According to this postulated development, a so-called annular gap junction must contain a portion of the cytoplasm from an adjacent cell[2,3,5]. The functional significance of such a cell-to-cell transfer of cytoplasmic components remains obscure at present.

Since the greatest number of intercellular gap junctions are found between osteoblasts in active bone formation[11], it is speculated that the so-called annular gap junctions, which we found predominantly in apparently protein-synthesizing osteoblasts, may also be somewhat associated with osteogenesis. In this respect, it should be emphasized that the so-called annular gap junctions present in metaphyseal bone cells contained mainly granules resembling ribosomes on which protein synthesis normally takes place.

Turnover of 5-hydroxydopamine in adrenergic nerves

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Summary. In adrenergic nerve endings of the guinea-pig vas deferens the population of small granular vesicles increases from 19% in control animals to 80–90% 1–3 h after the administration of 5-hydroxydopamine, and gradually declines to control values in 10 days. Large granular vesicles were also loaded but the loss of enhanced granulation was more rapid than in the small granular vesicles.

The osmiophilic false-transmitter 5-hydroxydopamine (5-OHDA) has been used as a specific electronmicroscopical marker for adrenergic nerves[2–6], and to investigate the storage of transmitter within the adrenergic neurone[7–8]. In the present experiments the time-course of the uptake and loss of 5-OHDA into synaptic vesicles of adrenergic nerves innervating the vas deferens has been studied.

Adult male guinea-pigs weighing 400–460 g were given 5-OHDA HCl (Sigma, 170 mg/kg in 1 ml isotonic saline

containing 17 mg L-ascorbate, i.p. on 3 occasions separated by 4 and 16 h). Control animals were given L-ascorbate only. The animals were killed by cervical dislocation and exsanguinated at various times after the final drug administration. The vas deferens was fixed with 5% glutaraldehyde buffered to pH 7.4 with 0.1 M Na cacodylate and postfixed in 2% OsO_4 in cacodylate buffer. Tissue blocks were stained with saturated aqueous uranyl solutions, dehydrated and embedded in Araldite. Thin sections cut on glass knives were stained with lead citrate and viewed in a Philips 300 or 301 electron microscope.

4 tissue blocks from the middle third of both vasa deferentia were prepared from each animal. From each block thin sections were cut and 25 varicosities within the outer longitudinal muscle layer were photographed at $\times 12{,}000$ onto 35-mm-film and printed to a final magnification of $\times 84{,}000$. Varicosities were defined as axonal profiles (1–2 µm in diameter) containing 20 or more synaptic vesicles of the small type. For each varicosity photographed (100 per experimental animal) the ratio of small granular vesicles to total small synaptic vesicles was determined.

The vas deferens is known to receive a dual cholinergic and adrenergic innervation; the longitudinal muscle layer and the circular layer are both densely innervated by adrenergic nerves while the major cholinergic input is to the circular layer[4]. Bundles of adrenergic nerve fibres penetrate the muscle coat and divide repeatedly to form individual junctions on smooth muscle cells with a gap of 20 nm. Adrenergic varicosities were identified by the presence of small (50 nm) synaptic vesicles containing a granule or dense 'core' (figure 1)[9–10].

In control vas deferens adrenergic varicosities in the longitudinal muscle layer contained small granular vesicles (SGV) which, with glutaraldehyde/OsO_4 fixation, accounted for only $19 \pm 6.8\%$ (Mean ± S.D., $n = 100$) of the total small vesicle population. In the circular muscle layer $36.5 \pm 3.0\%$ (Mean ± S.D., $n = 100$) of the small vesicles of adrenergic varicosities were granular. Small vesicles entirely filled with electron-dense material were occasionally present in adrenergic endings within the circular layer in control animals but were rare in the

1 M. R. C. Scholar. I am greatly indebted to Prof. G. Burnstock, Prof. G. Gray and Dr G. Gabella for helpful advice and criticism. The technical assistance of J. Barron and G. Barrett is gratefully acknowledged.

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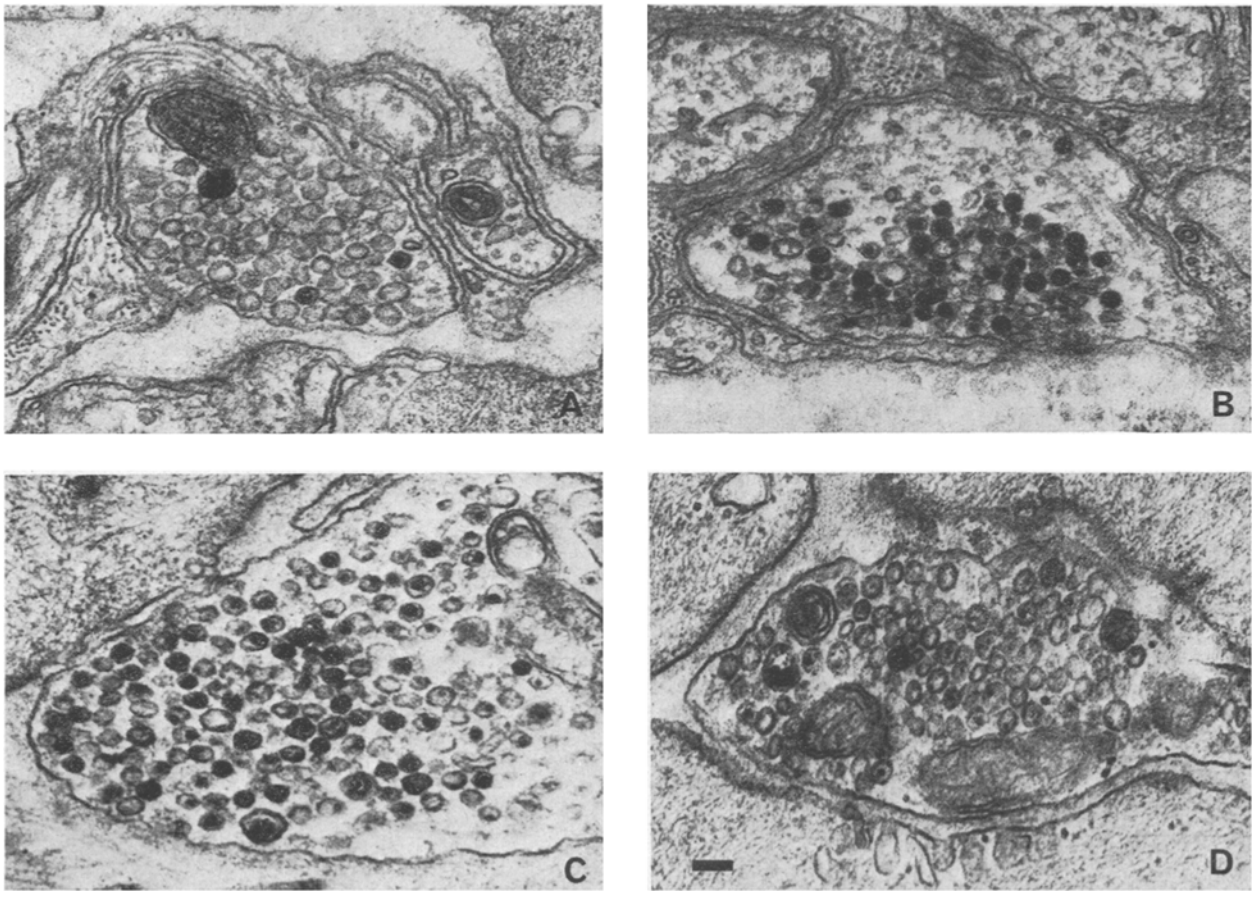

Fig. 1. Adrenergic variocosities in the outer longitudinal muscle layer of the guinea-pig vas deferens. *A* Control. *B* 1 h after 5-OHDA. *C* 24 h. *D* 48 h. × 54,000, bar line (*D*) 0.1 µm.

longitudinal layer. Following 5-OHDA both large and small vesicles contained highly electron-dense material which filled the entire vesicle (figure 1)[8]. The diameters of these vesicles were greater than those of controls (control: 50 ± 6 nm, 5-OHDA: 52 ± 5 nm, mean diameter ± S.D., n = 60) confirming previous observations[8]. Figure 2 shows the percentage of SGV's in adrenergic nerves in the longitudinal muscle layer of the vas deferens at various times after the final administration of 5-OHDA. At 1–3 h 80–90% of the small synaptic vesicles

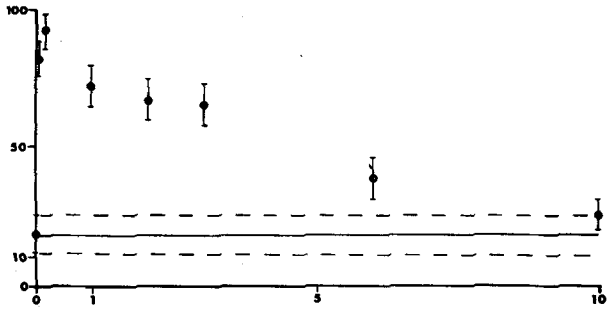

Fig. 2. Graph showing the change in the SGV percentage in adrenergic nerve terminals in the outer longitudinal muscle layer of the guinea-pig vas deferens after administration of 5-OHDA. Ordinate: SGV percentage, abscissa: time (days). Vertical bars represent 1 SD (variability among different terminals). The value at time zero represents the control (continuous line) and its SD (broken line).

were of the granular type as compared to 19% in controls. At these times the large granular vesicles were also loaded. In time the percentage of SGV's steadily declined until at 10 days the percentage of SGV's was similar to that of controls (figure 2). Parallel to their decline in number the SGV's showed less prominent granules and completely filled vesicles became rare. The percentage of large granular vesicles showing enhanced granulation decreased at 24 h and were rarely encountered at 48 h whereas the percentage of SGV's with completely filled matrixes began to decrease at 72 h and few were encountered at 144 h.

Following 5-OHDA the qualitative changes in the granulation of the synaptic vesicles in adrenergic nerves in the circular layer were similar to those in the longitudinal layer, although in the former the decline in the SGV numbers took place more slowly; this is true for both the large and small synaptic vesicles. At 72 h SGV's having completely filled matrixes were frequently encountered in the circular layer but rare at this time in the longitudinal layer.

Treatment of animals with reserpine (Sigma, 2.5 mg/kg in 1 ml isotonic saline containing 20% L-ascorbate, i.p.) 24 and 4 h before 5-OHDA resulted in a much lower (20–30%) percentage of SGV's than after 5-OHDA alone. Paradoxically, however, in animals given reserpine alone (2.5 mg/kg 24 and 4 h before death) although the majority of small vesicles had no core, 5–10% had electron-dense matrixes which entirely filled the vesicle.

Effect of early bursectomy on allocrafts survival in chicken

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Summary. Surgical bursectomy in chicken at 62 h of incubation produces a delay in skin allograft rejection, whereas later bursectomized chickens show normal rejection time. It is proposed that the Bursa of Fabricius in early stages of development influences the development of cellular immunity.

The primary lymphoid organs in the chicken are the thymus and the bursa of Fabricius. Both are lymphoepithelial organs where development of lymphoid stem cells into T and B lymphocytes respectively occur. The thymus is responsible for the cell-mediated immunity mechanisms such as graft rejection, and in addition thymus derived cells can act as suppressor or helper cells in antibody production. Late surgical bursectomy, chemical or hormonal procedures have been applied to determine the role of the bursa. Chickens in which the bursa has never functioned are unable to produce antibody. The results of surgical bursectomy vary with the age of the animals when bursectomy is performed. It has been shown that bursectomy, if not combined with sublethal irradiation, must be performed before the 18th day of incubation to result in complete agammaglobulinemia and lack of plasma cells and germinal centers[2].

Surgical bursectomy at hatching does not measurably influence cellular immunological functions[3,4]. Considering that the bursa exports cells to the thymus earlier in embryonic life, it is possible that thymus function is affected by the bursa.

The aim of the present paper was to establish whether very early surgical bursectomy by impeding cell migration or other early humoral interactions between bursa and the thymus can influence the development of cell mediated immunity mechanisms.

Hy-line fertile chicken eggs were maintained at 38.5 °C in a forced air incubator. All embryos were surgically bursectomized or sham-operated at 62 h of incubation (stage 17 Hamburger-Hamilton)[5]. Surgical bursectomy was performed under aseptic conditions by ablation of the tail bud caudally to the leg buds, according to the technique described by Fitzsimmons et al.[6]. Sham-operations were done following the same surgical steps with the exception of bursa ablation. In all, a total of 17 successfully bursectomized (Bx) and 21 sham-operated (Sh) chicken embryos were used in these experiments.

The chickens were skin-grafted within 24 h of hatching. Full-thickness grafts, 10 × 10 mm in size, were removed from middorsal region of chicken donors and placed in similar sites on each Bx and Sh chicken. Histoacryl was applied to hold the grafts in place. The transplants were

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