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

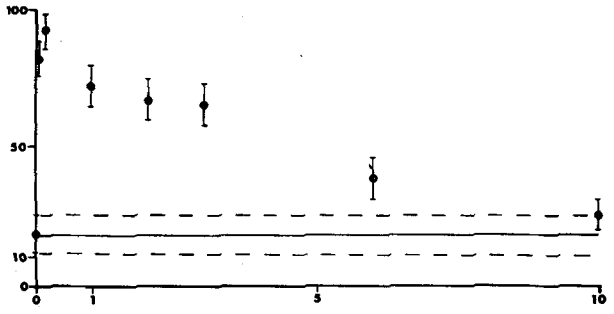

Fig. 2. Graph showing the change in the SGV percentage in adrenergic nerve terminals in the outer longitudinal muscle layer of the guinea-pig vas deferens after administration of 5-OHDA. Ordinate: SGV percentage, abscissa: time (days). Vertical bars represent 1 SD (variability among different terminals). 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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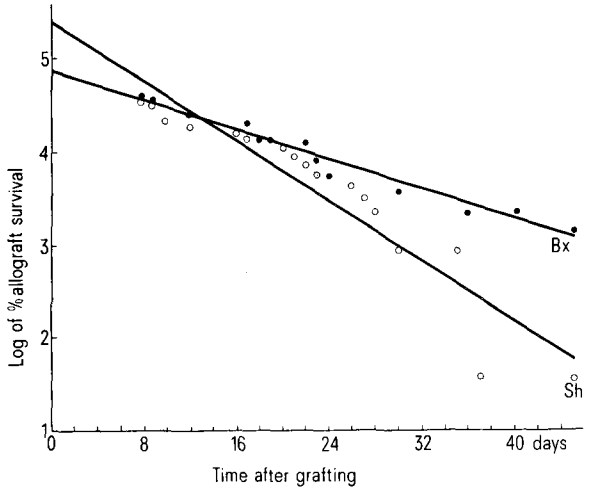
turned 180° so that the feathers grew anteriorly, making identification of the grafts easier. As anaesthetic, sodium pentobarbital (i.p. 30 mg/kg) was used during grafting. Examination of the grafts was not attempted until 1 week after grafting and then they were evaluated every 2 days during the following 7 weeks. A graft was considered as rejected when necrotic shrunken and/or crusty areas appeared. Autopsies were performed on several Bx and Sh chickens when 12 weeks old. The spleen and thymus were removed and stored in 10% buffered formalin until prepared for histological examination. The specimens were embedded in paraffin, sectioned and stained with haematoxyline and eosin. Careful necropsy was performed in search of bursal tissue remmants in bursectomized animals; none were found.



Per cent skin allograft survival in sham-operated (Sh) and bursectomized (Bx)/chicken. The difference between the 2 linear regressions is highly significant ($p<0.001$).

Effect of bursectomy on skin allograft survival in chickens

Group	Graft survival 10 days	20 days	30 days	40 days
Control sham-operated	16/21	12/21	4/21	1/21
Bursectomized	16/17	10/16	5/14	4/14

The data from all Bx and Sh birds are grouped together in the table. Percentage skin allografts survival in Sh and Bx birds showed 2 curviline functions that are plotted semilogarithmically. The differences between these 2 linear regressions are highly significant ($p < 0.001$; figure).

The outstanding feature of splenic morphology was the absence of germinal centers in the Bx birds in contrast to the Sh chickens. Cross sections of the thymus showed no clear-cut morphological differences between the 2 groups.

Our results clearly show that early bursectomy produces a delay of skin allograft rejection. This observation may imply an alteration in thymus function. The bursa of Fabricius in the chicken exports lymphoid cells to the thymus early in embryonic life. The very early export of cells from the bursa can explain the first appearance of immunoglobulin-containing cells in other lymphoid organs, on the 17th to 19th day of incubation[8]. Late surgical bursectomy, at 17 days of incubation[3] or later, leaves open the consideration of possible cellular[6] or humoral interactions preceding the operation that may affect thymus functions.

In contrast with the results obtained after late bursectomy, our experiments in early bursectomized chicken suggest some interaction between the bursa of Fabricius and the thymus or other elements of the immune system, which find their expression in a delayed rejection of skin allografts.

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Erythrocyte 2,3-diphosphoglycerate in anaemic sheep[1]

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Summary. Experimental anaemia resulted in an increase of red cell 2,3-DPG from 0.11 μM/g Hb to 0.99 μM in haemoglobin A sheep and from 0.21 μM to 1.5 μM in haemoglobin B sheep. Production of haemoglobin C as a result of anaemia was confined to haemoglobin A only. The results, therefore, appear to suggest that the rise in 2,3-DPG in the red blood cells of different haemoglobin types is independent of haemoglobin C.

A considerable amount of evidence now available[2–5] indicates that 2 organic phosphate compounds, 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) moderate haemoglobin function in man. 2,3-DPG causes a concentration dependent decrease in the oxygen affinity of haemoglobin by its binding to specific sites on the β-chain. ATP also reduces the affinity of haemoglobin for oxygen. However, 2,3-DPG is present in the erythrocyte in a much higher concentration than ATP and is, therefore, considered to be more important in moderating haemoglobin oxygen affinity.

The relationship between red cell 2,3-DPG and haemoglobin function has been investigated in several mam-

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