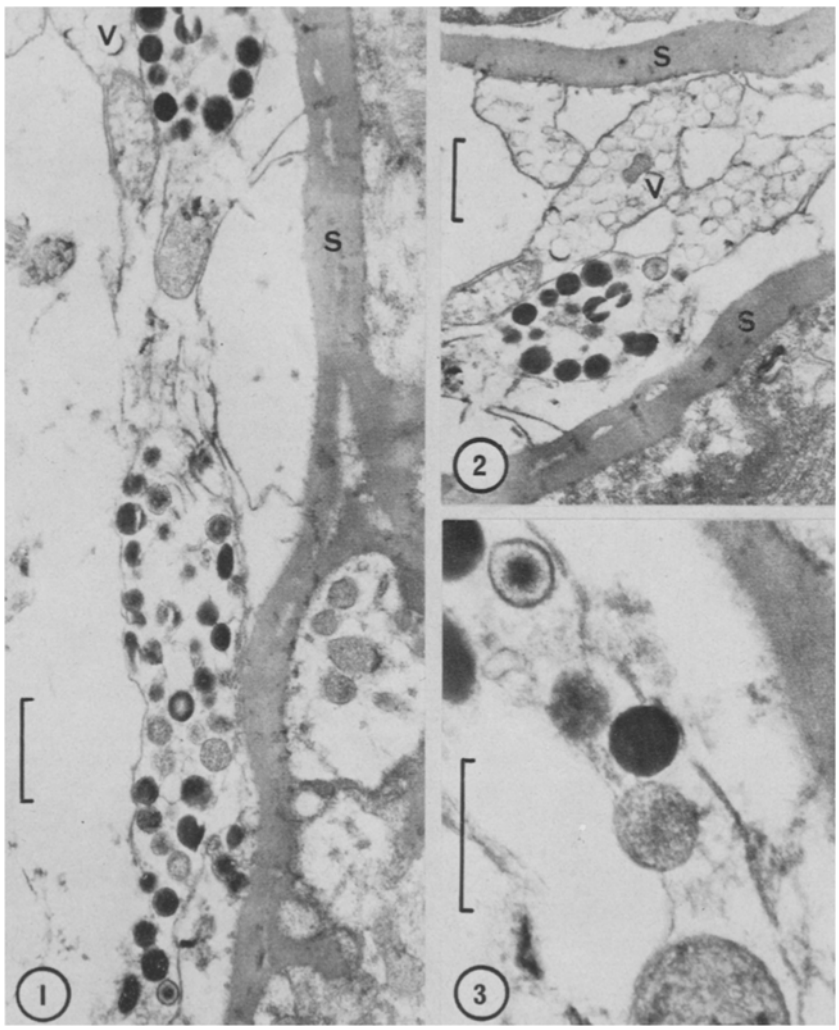


Fig. 1. Longitudinal section through part of a strap-like neurosecretory axon terminal. The axon, somewhat convoluted along its length, makes loose contact at several points with the extracellular connective tissue sheath(S) surrounding the muscle fibre (not shown). The axon, at this point, is packed with large granular or dense membrane-bounded vesicles. A few small clear vesicles(V) are evident in the upper left-hand corner. ×14,000; scale 1 μm.

Fig. 2. Portion of a neurosecretory axon running in a cleft between 2 fibres completely bounded by extracellular sheath material(S). Note the large aggregations of medium-sized electron-lucent vesicles(V). ×11,100; scale 1 μm.

Fig. 3. High-power view of some neurosecretory vesicles showing the variations in size and appearance. ×42,000; scale 0.5 μm.

Several reports[12–14] have now identified substances such as noradrenaline, dopamine and 5-hydroxytryptamine in neurosecretory vesicles and ATWOOD et al.[5] have tentatively suggested that the contents of neurosecretory vesicles may mediate the well established trophic effect of nerve on muscle[15]. If this is true, then the only difference between the endings described here and those of ATWOOD et al.[5] is that in *Carcinus* the trophic vesicular element has become separated from the synaptic vesicular element at the peripheral sites.

*Résumé.* Un axone neuro-sécréteur à extrémités localisées dans le muscle squelettique du crane *Carcinus* est décrit. Ces extrémités contiennent 6 sortes de vésicules ayant les diamètres de 1300 a 3700 Å. Il est suggéré que les petites vésicules correspondent à la phase de rétablissement de la membrane, pendant la décharge des granules neuro-sécréteurs. On suppose aussi que cet axone peut-être l'intermédiaire d'une influence trophique du nerf sur le muscle.

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*17 January 1972.*

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## Immunological Activity of Transplanted Spleens in *Xenopus laevis*

Adults of *Xenopus laevis*, like other amphibians[1], are known to show immunological responses when maintained at a temperature of 20°C or above. These responses include the production of circulating antibody to soluble or to particulate antigen[2,3]. Allograft rejection occurs[2] and its immunological basis is shown by the specific accelerated rejection of second set grafts[4].

In our experience with skin transplants, *Xenopus* maintained at 24°C behaves in a manner broadly comparable with mammals and birds. We have found that autografts,

if surgically successful, always take. First set allografts are normally rejected in between 14 and 20 days although in 15% of cases they are accepted. Since accepted grafts are commoner between siblings than between other members of our colony (24% as against 9%) we believe that chance genetic and antigenic compatibility is responsible for failure to reject. Second grafts from the donor of a rejected graft succumb in between 8 and 12 days. In this species, however, it is difficult to define a precise end-point of the rejection process.

Specific immunological tolerance can be induced in embryos which receive tissue grafts at stage 22 (NIEUWKOOP and FABER[5]). In our experiments massive grafts are used which include presumptive skin, body wall and gut and, probably, blood forming cells. The tolerance inducing graft is not distinguishable in later development, but the tolerant state can be confirmed by test grafts of skin. Tolerance has been successfully induced in 93% of our attempts, the few failures may be due to unobserved extrusion of embryonic graft cells in the post-operative period. The alternative explanation, that the graft is made too late or is too small to induce tolerance, should be borne in mind in the light of evidence[6,7] that even younger embryos can be immunized by neural crest grafts in anurans. This possibility is strengthened by the observation that the test skin grafts were, in these animals, rejected in the fashion of second set grafts. The reactivity of hosts bearing tolerated grafts to grafts from other animals is not impaired.

As a prelude to experiments which required the adoptive transfer of immunity, we have tested the ability of whole grafted spleens to undertake immunological reactions in their hosts. Spleens were used since we have found no lymph nodes in post-metamorphic *Xenopus* (but see[8] for development of lymphoid tissue in this animal). Three experiments will be reported here. In the first, to provide experimental controls, pairs of embryos were rendered mutually tolerant by exchange of embryonic tissues (Figure 1). In post-metamorphic life tolerance was confirmed by exchange of skin grafts, and later the spleen from 1 animal was removed and transplanted whole beneath the skin of the ventral surface of the thigh of the other. Some crude observations on such spleens are possible through the skin which is pigment free in this region. They are normally pale pink on transplantation but within 2–3 days have become vascularized by the host and have flushed red. One of 19 spleens atrophied soon after grafting, the others remained visible withouth change for several (2–8) months. Histological examination of such spleens showed them to be normal in structure.

In the second experiment (Figure 2) a graft versus host (gvh) reaction was sought. Tolerance was induced unilaterally between pairs of embryos. In post-metamorphic life the tolerant animal offered 2 successive skin grafts to its partner. After the rejection of the second the immunized host's spleen was transferred to the tolerant animal. 11 pairs were so treated. In 7 the grafted spleen was the focus of visible evidence of a gvh reaction. The skin lying above it became inflamed, and a progressively larger area of skin suffered from vasodilation and heamostasis. Two of these animals died, one 17 and the other 65 days after receiving the spleen. The other 5 showed recovery followed by the disappearance of the grafted spleen.

In none of the 7 animals showing a gvh reaction was the tolerated test graft of skin from the spleen donor unequivocally rejected. In 4 of the 7 the test grafts showed some reaction in vasodilation and haemostasis after the spleen graft had been made, but in each case the skin

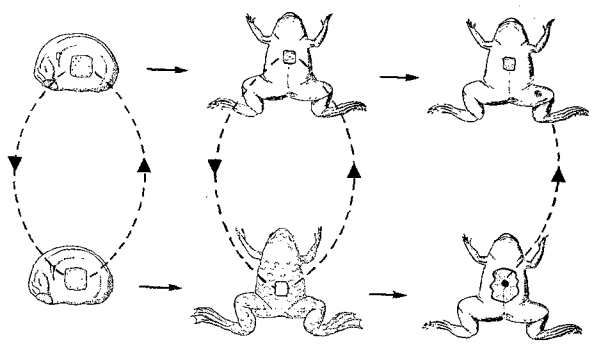

Fig. 1. After the induction of mutual tolerance, confirmed by reciprocal test grafts of skin in post-metamorphic life, spleen grafts normally thrive.


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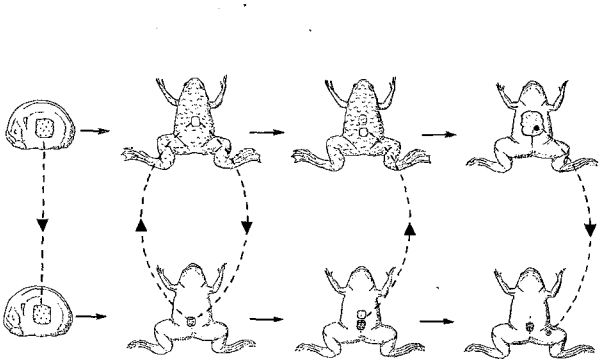

Fig. 2. After unilateral induction of tolerance and the immunization of the donor against recipient grafts, the grafted immunized spleen may mount a gvh reaction.

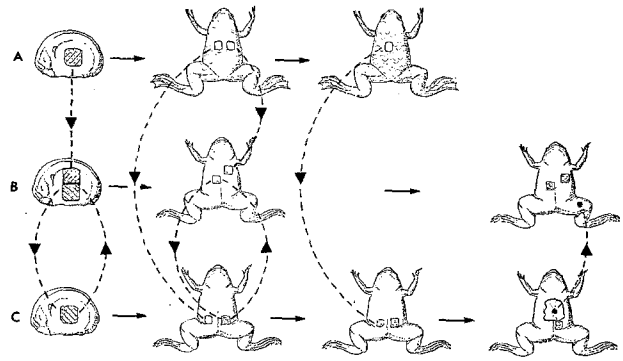


Fig. 3. A grafted spleen tolerant of its host, and tolerated by it, may react against a skin graft from a third animal previously tolerated by its host.

appeared to recover. The skin graft ultimately survived in all 5 animals in which the spleen itself disappeared.

Of the 4 remaining hosts the grafted spleens were lost in 2 and the earlier tolerated test grafts in these animals were simultaneously rejected. The other 2 retained both grafted spleens and test grafts.

It thus appears that whole grafted immunized spleens can evoke gvh reactions in adult *Xenopus*, but that the severity of the reaction varies as indeed it does in adult mammals[9]. Two observations remain difficult to explain. The loss of immunologically reacting but presumably tolerated spleens (9 out of 11) when compared with tolerant ones has perhaps a parallel in the postulated 'allergic death' of transferred lymphocytes in radiation chimaeras[10,11]. However, the transient discomfort or rejection of hitherto tolerated skin test grafts (4 out of 7) suggests that they may be under attack. It should, of course, be borne in mind that the atrophy of the grafted spleen does not preclude the survival elsewhere in the host of cells of graft origin.

In the third experiment (Figure 3) 2 animals B) and C) were made mutually tolerant and one of them B) unilaterally tolerant to a third A). In post-metamorphic life A) gave a tolerated test skin graft to B) and 2 successive immunizing grafts to C). The mutual tolerance of B) and C) was also tested by skin grafts. The spleen of C) was then transplanted to B). Of 12 sets of triplets so treated only one spleen host provided a clear rejection of the skin graft from A). 10 further animals showed some reaction in the A) skin but this later recovered. In 4 of these animals a very slow process of creeping replacement of the A) graft occurred. One animal showed no reaction in the A) graft. Three of the hosts died at 19, 62 and 158 days after the spleen graft was inserted. The latter 2 animals died 6 and 26 days after receiving a second challenge with A) skin.

These results are complicated by one consideration. The grafted spleen, if immunologically active against A) tissue, would attack both the test skin graft from A) and the cells descended from the tolerance inducing embryonic graft. This could explain the death of 3 of the hosts. It also makes it reasonable to consider the possibility of partial tolerance or paralysis in some of the grafted spleens induced by the massive quantity of antigenic material present.

Our results suggest that transplanted immunized spleens in *Xenopus* can effect immunological reactions in tolerant hosts but that in the gvh situation they are themselves subject to attack which may also affect, though less strongly, long-standing skin grafts of the same genotype.

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*Resumé.* Des rates immunisées de *Xenopus laevis* qu'on greffe dans des hôtes qui les acceptent, peuvent y produire des réactions immunologiques. Ces réactions se dirigent ou contre l'hôte ou contre des greffes épidermiques, prises à un troisième animal que l'hôte tolère. Au cours de ces réactions de greffe-contre-hôte, la rate se trouve parfois attaquée.

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## Adjuvanticity and Delayed-Type Hypersensitivity by Bacillus Calmette-Guérin (BCG)

Complete Freund's adjuvant, which contains mycobacteria, is widely used as a potent adjuvant in experimental immunology. It has been reported, however, that too large amounts of mycobacteria do not act as adjuvant[1,2]. Mycobacteria are an antigen eliciting a high degree of delayed-type hypersensitivity (DTH), the antigenic determinants seem to be located in the glycopeptide of the bacterial cell wall[3]. The adjuvant masking effect of too large amounts of mycobacteria seems to be attributed to the competing antigen of mycobacteria[4]. We report here that the adjuvant activity of BCG after acetic-anhydride treatment seems to be related to a low degree of DTH induced by the acetylation treated bacillus.

*Materials and methods.* 1. Bacteria. The bacteria used in the present investigation was *Mycobacterium* of the BCG strain originating from Pasteur Institute, Paris, France. The organisms were grown in Santon's medium for 17 days at 37 °C. 6.7 g of viable BCG (VB) were harvested, from culture medium, by filtration through a sintered glass filter and then washed successively with: a) a large quantity of distilled water, b) a mixture of ether-ethanol (1:1, v/v), and c) pure chloroform in the manner described by Anderson[5]. 5 g of bacillary residue (BR) thus obtained from 6.7 g VB (yield: 74.6%), were treated with 100 ml of a mixture of pyridine-acetic anhydride (30:25, v/v) at 37 °C for 2 h, then filtered and washed with diluted hydrochloric acid (0.1 *N*) and with distilled water until all traces of pyridine and acid were removed. The bacillary mass was then washed with acetone and exhaustively extracted with pure chloroform. The acetylation treated bacillus (ATB) thus obtained was dried in vacuum at room temperature and stored over a desiccant until used.

2. DTH tests. Female albino guinea-pigs of 400 to 500 g were sensitized by injection into each hind foot-pad of 0.1 ml of a water-in-oil emulsion consisting of one part paraffin oil containing 0.5 mg VB or 0.5 mg ATB, 2 parts Tween 80 and 7 parts saline. On day 21 after sensitization, the animals were skin-tested by intradermal injection of various amounts of test antigen [glycopeptide from wax D of human mycobacteria strain peurois[6]] in 0.1 ml saline into the clipped flank of guinea-pigs; the reactions were read 24 h later.

3. Antibody production and determination. F1 (DBA/2 × $C_{57}$Bl/6) mice weighing approximately 20 g were injected i.p. with $10^9$ sheep red blood cells (SRBC) per animal; at 1 min intervals 0.1 ml water-in-oil emulsion containing 0.25 mg VB or 0.25 mg ATB was injected i.p. into the same site. 4 days later, the animals were killed and the

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