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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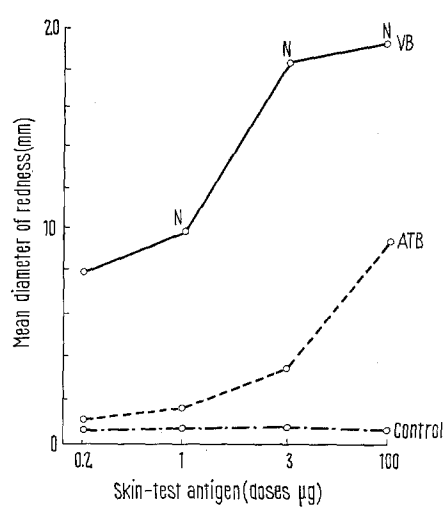
Influence of viable BCG and acetylation treated bacillus on the average number of plaque forming cells in F1 (DBA/2×C_{57}B1/6) mice 4 days after immunization with SRBC

	PFC/Spleen[a]	PFC/Spleen average number
SRBC (10^9)	24,000 22,000 26,000 20,000	23,000
SRBC (10^9)[a] WOE^{++} (0.1 ml)	29,100 33,600 30,200 35,200	32,000
SRBC (10^9)[a] VB (0.25 mg) in 0.1 ml WOE	56,200 68,000 52,000 64,100	60,000
SRBC (10^9)[a] ATB (0.25 mg) in 0.1 ml WOE	153,600 141,200 161,700 143,500	150,000

[a] Number of 19S plaque forming cells/spleen. 4 animals/group.
++ Water-in-oil emulsion which consisted of 1 part paraffin oil, 2 parts Tween 80 and 7 parts saline.



Graphic representations of the results of skin tests in guinea-pigs sensitized with 0.5 mg viable BCG (VB) and with 0.5 mg of its derivative, acetylation treated bacillus (ATB). The mean diameter of redness in guinea-pigs skin at sites of injection is indicated on the ordinate and the skin-test antigen doses are shown on the abscissa. N = central area necrosis.

number of spleen cells forming or releasing antibody to SRBC was determined by the JERNE technique[7].

Results. The Figure illustrates various degrees of DTH induced by 0.5 mg VB and 0.5 mg ATB. All animals sensitized with either 0.5 mg VB or 0.5 mg ATB showed positive skin reactions when 100 µg of skin test antigen were used. However, large diameters of redness and central areas of necrosis at injection sites were observed only in the group receiving 0.5 mg VB. When the skin test antigen was reduced to 0.2 µg, animals sensitized with 0.5 mg VB still showed positive skin reaction, while no visible skin reaction was observed in the group receiving the same dose of ATB (0.5 mg). The absence of skin reaction when small amounts of test antigen were used indicated that ATB was a weaker sensitizer.

The results shown in the Table indicate that animals treated with 0.25 mg ATB produced a significant increase in the number of plaque-forming cells 4 days after immunization with 10^9 SRBC (an average of 150,000 in contrast to 23,000 and 32,000 in controls receiving, respectively, antigen alone and antigen plus oily emulsion). Injection of 0.25 mg VB raised slightly the number of plaque forming cells.

Discussion. It has been reported that the composition of the glycopeptide of the cell wall of mycobacteria is similar to that of the glycopeptide obtained from wax D of various human strains of mycobacteria[8]. The glycopeptide of the wax D was shown to contain free hydroxyl groups which can be converted readily to ester groups by acetic-anhydride under conditions described above[9]. From these data, the ability of ATB to increase significantly antibody forming cells and to induce only a low degree of DTH appears to be related to the chemical changes of cell surfaces by acetylation of the hydroxyl groups of the cell wall glycopeptide.

Résumé. Le BCG, lavé à l'eau distillée, puis à l'ether-ethanol, puis au chloroforme, est traité par l'anhydride acétique. Le bacille ainsi traité par acétylation perd, à poids égal, par rapport au bacille vivant, de son pouvoir d'induire une hypersensibilité retardée spécifique, mais accroit son pouvoir de stimulation non spécifique des réactions immunitaires.

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Effect of Irradiation and Alkoxyglycerol Treatment on the Formation of Antibodies After *Salmonella* Vaccination

The alkoxyglycerols have proved to be of medical interest[1-3]. To some extent they prevent leucopenia and thrombocytopenia. The administration of alkoxyglycerols to patients with cancer of the uterine cervix results in higher survival rates than if radiation treatment alone is given[1,3]. Furthermore the alkoxyglycerols promote the growth of *Lactobacillus lactis*[1].

The body's ability to react against cancer cells that are not eliminated by radiation treatment could possibly depend upon an immunological process. In this context it would be relevant to know whether the capacity for forming antibodies after a vaccination can be influenced by treatment with alkoxyglycerols. Consequently 54 patients with cancer of the uterine cervix were vaccinated