Epidemiol. Infect. (2005), **133** (Suppl. 1), S25–S27. © 2005 Cambridge University Press
doi:10.1017/S0950268805004280

8. Development of a stable smallpox vaccine

Collier L. J Hyg 1955; **53**: 76–101

AN APPRECIATION BY DERRICK BAXBY

35 Galston Avenue, Rainhill, Merseyside, UK

Introduction

During its first century the journal attracted well over 100 papers on poxviruses. During the last 50 years this was probably due in part to the fact that Allan Downie, the world's leading poxvirus authority helped to edit the journal from 1951 to 1982. This expertise and association was acknowledged by the publication of a special academic tribute of 12 papers, including 5 on poxviruses, in 1982 (**89**: 353–478).

Significant papers on poxviruses published by the journal include: what became standard methods for the titration of virus [1] and neutralizing antibody [2]; analysis of the immune response to smallpox and vaccination [3]; details of the simplest biological method for differentiating orthopoxvirus species, by determining their maximum temperature of growth [4]; a series of four papers in 1969 (**67**: 603–630) in which the results of laboratory investigations contributed to our understanding of the clinical features and epidemiology of smallpox; proof that bankvoles and woodmice are the reservoir hosts of cowpox virus [5]. Rather less auspicious was publication of probably the last claim to have transformed variola virus into vaccinia virus [6].

Although the papers published covered the whole range of poxvirus genera, 46 were concerned with myxomatosis. Thirty-two of them, including a linked series of eight by Frank Fenner, Ian Marshall and their colleagues, describe attempts to control wild rabbit populations in Australia by introducing myxoma virus. Although the exercise was only partially successful, these papers document the fascinating interaction between the originally highly susceptible rabbit and the originally highly virulent virus. This resulted in the simultaneous evolution of rabbits more resistant to the virus [7] and also of less virulent virus strains [8]. This situation is still monitored and remains an interesting and important exploration of the host–pathogen relationship [9]. The appearance of the Australian papers in the journal no doubt attracted papers describing similar surveys conducted in Britain (see e.g. [10]).

Heat-resistant smallpox vaccine

Any of the above papers could justifiably be reproduced here. However, the one chosen is the description of the development, at the Lister Institute by Leslie Collier, of dried and heat-resistant smallpox vaccine [11].

Historical perspective

From the very early days of vaccination there was the need to transport and preserve vaccine material. Initially, in temperate climates and for short distances and unpredictable periods, this was achieved by drying vesicle fluid on threads or ivory points or by sealing it between glass plates. For long distances in tropical climates vaccine was maintained arm-to-arm through a sequence of susceptible individuals.

Towards the end of the 19th century vaccine, grown in animals, was suspended in glycerol. Originally used empirically, glycerol was later shown to have some antibacterial activity by S. Monckton Copeman. With developments in refrigeration, vaccine in 40–50% glycerol could be kept liquid at -10 to $-20\,^{\circ}C$. Thereafter glycerolated vaccine sealed in glass or plastic capillary tubes and kept at $<0\,^{\circ}C$ became the standard product wherever a satisfactory cold chain was available. However, the requirement for a vaccine that would maintain its potency in primitive tropical conditions was acute.

Vaccine development

Apparently successful vaccines dried *in vacuo* from the liquid state had been developed in the 1920s but Collier's attempts to reproduce these were 'disappointing' [12]. However, the increasing availability of effective freeze-drying techniques made this approach more attractive. Almost coincidentally with Collier's work, a combined yellow fever–smallpox vaccine prepared in this way had maintained its infectivity at 0 °C for 16 months, but still required a cold chain [13]. Collier's intention was to develop a dried vaccine which, as well as meeting the then current British requirements of stability at 37 °C for at least 1 month, could be reconstituted easily and used by semi-skilled workers.

The paper reproduced here documents in detail all the variables tested in the vaccine's development, e.g. glycerol *vs*. lanolin [Table 2]; addition of 'protective' constituents such as peptones, serum, etc. [Tables 15–17]; effect of phenol [Tables 3–5]; storage under vacuum or nitrogen [Tables 9, 10]; technicalities of freeze-drying [Tables 7, 8]; batch-to-batch variation [Tables 6, 20], and bacteriological quality [Table 22]. The final 'Lister' vaccine was a partially purified suspension of virus in 5% peptone, sealed in ampoules under nitrogen after drying from the frozen state. The paper also correlated the infectivity for the CAM against clinical effect, and in preliminary results was fully effective after at least 6 months at 37 °C and at least 12 months at 22 °C [Table 18].

The vaccine in use

Independent comparative trials involving laboratory titrations and vaccination of RAF personnel showed the superiority of the Lister vaccine (coded P in ref. [14]).

Subsequently the vaccine was found to be fully effective after storage at 45 °C for up to 2 years. Astonishingly, the dried vaccine also retained a clinically effective titre after being immersed at 100 °C for 2 h [15]. Collier's method became the standard for vaccine production during the Smallpox Eradication Campaign.

The vaccine in perspective

When assessing this study, one should remember that attenuated vaccines for poliomyelitis, measles, rubella and mumps were still to be developed; indeed measles and rubella viruses had not been grown *in vitro* at the time. As for the future, if recombinant vaccinia vaccines are used to vector other immunizing antigens in tropical climates the technology developed by Collier will be used.

D. A. Henderson, Chief of the WHO Smallpox Eradication Unit during the important early years listed three developments which, in chronological order, laid the groundwork for the ultimate eradication of smallpox. The first was the bulk production of vaccine in animals. Second was Monckton Copeman's development of glycerolated vaccine. Third was Collier's development of freeze-drying of the vaccine, which permitted the vaccine to be distributed throughout the tropics in potent form. 'Copeman and Collier made an enormous contribution for which neither, in my opinion, ever received due credit' (D. A. Henderson, personal communication, 2000).

References

1. **Westwood JCN, Phipps PH, Boulter EA.** The titration of vaccinia virus on the chorio-allantoic membrane of the developing chick embryo. J Hyg 1957; **55**: 123–139.
2. **Boulter EA.** The titration of vaccinial neutralizing antibody on chorio-allantoic membranes. J Hyg 1957; **55**: 502–512.
3. **Downie AW, McCarthy K.** The antibody response in man following infection with viruses of the pox group. III Antibody response in smallpox. J Hyg 1958; **56**: 479–487.
4. **Bedson HS, Dumbell KR.** The effect of temperature on the growth of pox viruses in the chick embryo. J Hyg 1961; **59**: 457–469.
5. **Chantrey J, Meyer H, Baxby D, et al.** Cowpox: reservoir hosts and geographic range. Epidemiol Infect 1999; **122**: 455–460.
6. **Horgan ES.** The experimental transformation of variola to vaccinia. J Hyg 1938; **38**: 702–715.
7. **Marshall ID, Fenner F.** Studies on the epidemiology of infectious myxomatosis of rabbits. V. Changes in the innate resistance of Australian wild rabbits exposed to myxomatosis. J Hyg 1958; **56**: 288–302.
8. **Marshall ID, Fenner F.** Studies on the epidemiology of infectious myxomatosis of rabbits. VII. The virulence of strains of myxoma virus recovered from Australian wild rabbits between 1951 and 1959. J Hyg 1960; **58**: 485–488.
9. **Fenner F, Fantini B.** Biological control of vertebrate pests. The history of myxomatosis an experiment in evolution. Wallingford; , 1999.
10. **Ross J, Sanders MF.** Innate resistance to myxomatosis in wild rabbits in England. J Hyg 1977; **79**: 411–416.
11. **Collier L.** The development of a stable smallpox vaccine. J Hyg 1955; **53**: 76–101.

12. **Collier LH.** The preservation of vaccinia virus. Bact Rev 1954; **18**: 74–86.
13. **Hahn RG.** A combined yellow fever–smallpox vaccine for cutaneous application. Am J Hyg 1951; **54**: 50–70.
14. **Cockburn WC, Cross RM, Downie AW, et al.** Laboratory and vaccination studies with dried smallpox vaccines. Bull WHO 1957; **16**: 63–77.
15. **Cross RM, Kaplan C, McClean D.** The heat resistance of dried smallpox vaccine. Lancet 1957; **1**: 446–448.

https://doi.org/10.1017/S0950268805004280 Published online by Cambridge University Press