J. Hyg., Camb. (1974), **73**, 35
Printed in Great Britain

A possible relation between human pathogenicity of smallpox vaccines and virus growth at elevated temperatures

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(*Received* 31 *October* 1973)

SUMMARY

Seven smallpox vaccines of known human pathogenicity were tested for their ability to produce pocks on the chick chorioallantois at 39·7° C. Significant differences were found and the more pathogenic strains produced pocks with greater efficiency at 39·7° C. than did strains of average or low pathogenicity.

INTRODUCTION

Clinical trials have shown differences in the human pathogenicity of smallpox vaccine strains. For instance Polak *et al.* (1963) showed that the order of human pathogenicity of 4 vaccines was Lister < Ecuador < Bern < Copenhagen. More recent trials have shown the attenuated strain, CV-1 to be less pathogenic than the Lister and Wyeth strains (Kempe, 1968; Ducksbury *et al.* 1972; J. G. Galasso, I. Tagaya, personal communication).

Little progress has been made in the search for simple laboratory markers which correlate with human pathogenicity. Bektemirov, Shenkman & Marennikova (1971) showed a correlation between interferon resistance and pathogenicity for mice and rats. Various studies, however, have shown that when strains are listed in their order of pathogenicity for laboratory animals, the order determined is not the same as the order of pathogenicity for man (e.g. Turner, 1967; Anderson, 1969; John, 1969; S. S. Marennikova, personal communication).

The present paper suggests a possible relation between the human pathogenicity of smallpox vaccine strains and their ability to produce pocks at elevated temperatures on the chick chorioallantoic membrane (CAM).

MATERIALS AND METHODS

Virus strains

In all 7 vaccines were tested, the Lister, Copenhagen, Bern, Ecuador, CV-1, Tashkent and Wyeth strains.

Quantitative ceiling temperatures

After inoculation onto the CAM, groups of fertile chick embryos were held at either 35° C. or at various experimental temperatures in special incubators with constant recording thermometers and thermostats accurate to $\pm 0{\cdot}1°$ C. (Baxby

Table 1. *Relationship between pock production and human pathogenicity of smallpox vaccines*

Vaccine	% pock suppression at 39·7° C (± S.D.)	Relative human pathogenicity: 'Index of* Pathogenicity'	Relative human pathogenicity: Fever† 38·3° C (%)	Relative human pathogenicity: Malaise‡ (%)
Copenhagen	13·5 ± 4·1	100	—	—
Tashkent§	26 ± 5·4	—	—	—
Bern	30 ± 5·1	94	—	—
Ecuador	50 ± 7·7	57	—	—
Wyeth	59 ± 8·1	—	45	—
Lister	63 ± 8·4	37	—	80
CV-1	76 ± 9·2	—	12	32

* From Polak *et al.* (1963). Figure given is days with fever > 38·9° C. Copenhagen = 100 %, others adjusted accordingly.

† From Kempe (1968). Figure is % vaccinees with fever > 38·3° C.

‡ From Ducksbury *et al.* (1972). Figure is % vaccinees with 'malaise' ('Fretfulness, irritability, anorexia and restlessness').

§ 'Highly pathogenic' (Marennikova *et al.* (1969)).

1969). Pocks were counted after 48 hr. and the degree to which pock production was decreased at the experimental temperature was assessed.

RESULTS

As a result of preliminary experiments 39·7° C. was selected as the test temperature. With higher temperatures chick embryo deaths increased (Bedson & Dumbell, 1961) and the pocks produced by some strains, notably Wyeth, CV-1 and Copenhagen strains, changed to a very flat grey type which was sometimes difficult to count; the problems of changes in pock character at different temperatures have been discussed elsewhere (Baxby, 1969).

The results obtained at 39·7° C. are shown in Table 1, the vaccine strains being placed in order of efficiency of pock production at that temperature. It can also be seen that the same order is maintained when the strains are listed in order of human pathogenicity. The differences obtained in pock reduction tests with different strains were not great but, with attention to inoculation technique and temperature control, were very reproducible. Pock production by the least pathogenic strain CV-1 was reduced by about 75 %, that by the most commonly used strains, Wyeth and Lister, by about 60 %, whilst that by the most pathogenic strains was reduced by 25–30 % for Tashkent and Bern, and by 13 % for Copenhagen.

The data on human pathogenicity are drawn principally from the extensive trial of Polak *et al.* (1963) together with more limited trials which have compared CV-1 with either Wyeth or Lister vaccines. An extensive American trial, still in progress, should also provide valuable information. The results so far indicate the attenuated nature of the CV-1 strain and show the Wyeth and Lister vaccines to be similar to each other, the exact values for morbidity being dependent on titre of vaccine and route of inoculation (J. G. Galasso, personal communication).

DISCUSSION

The results presented here suggest a possible relation between the human pathogenicity of smallpox vaccines and growth on the chick chorioallantois at elevated temperatures. It is of interest that a similar relation is suggested by the work of Nizamuddin & Dumbell (1961) and Bedson, Dumbell & Thomas (1963) on different strains of smallpox virus.

A suitable smallpox vaccine must of course offer considerable protection against smallpox. This is usually estimated by serological studies and/or revaccination. Although early work with CV-1 suggested a high rate of seroconversion (Kempe, 1968) more recent studies suggest that seroconversion and resistance to revaccination is lower than it is with Lister and Wyeth vaccines (Ducksbury *et al.* 1972; J. G. Galasso, I. Tagaya, personal communication).

Despite the success of the WHO smallpox eradication campaign it is possible that smallpox vaccine development will continue. The selection of strains which show reduced pock production at elevated temperatures should provide a convenient initial stage in the development of further vaccines.

I would like to thank Drs H. G. S. Murray, R. Gispen, J. Leerhoy, A. J. Lee and G. Appleyard for providing virus strains, and Drs J. G. Galasso, H. Tint, M. F. Polak, K. McIntosh and I. Tagaya for providing information in advance of publication.

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