



EPIDEMIOLOGICAL IMPLICATIONS OF THE TYPING OF VARIOLA ISOLATES

by

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INTRODUCTION

Outbreaks of smallpox vary in their severity, and this is influenced by many factors. De Korte in 1904 described a distinct, mild form of variola, which he called amaas, and since then it has become well recognized that there are two epidemiological varieties of smallpox. These are variola major or asiatic smallpox and variola minor or alastrim. They give complete cross immunity, one against the other and both are prevented by recent vaccination. The principal difference between them is not in the extent of the rash, but in the overall figures for mortality in unvaccinated people. The mortality rate in variola major may be 20-40% while that for variola minor is quoted as less than 1% (Dixon, 1962).

It might be expected that the viruses causing these two conditions would be very similar, and this proved to be so true that it was not until 1956 that any difference was found between them. Dinger (1956) was the first to make any observation of this kind and he was closely followed by Helbert, who in 1957 showed that viruses from the two types of smallpox had also differing pathogenicity for the chicken embryo. Nizamuddin & Dumbell in 1961 described a much simpler test. If the chicken embryos were inoculated on the chorioallantois (C.A.M.) and then incubated at 38.3°C, variola major virus produced pocks which were smaller and less numerous than those at the optimum temperature of 35°C, but variola minor virus produced no pocks at all at the higher temperature. Nizamuddin and Dumbell verified this observation for 24 strains of variola, but all their 10 strains of variola minor were taken from two epidemics in Europe. It was later confirmed that 23 strains of variola minor virus from Brazil failed to grow on chick chorioallantois in eggs incubated at 38.3°C (Downie et al., 1963). Variola isolates from East Africa were not so easily dealt with. Bedson, Dumbell & Thomas (1963) showed that a group of variola strains from Tanzania occupied an intermediate position; they produced pocks on chick chorioallantois at 38.3°C but were clearly more sensitive to this temperature than the classical strains of variola major originating in India or Pakistan.

It was hoped that studies of this kind might throw light on the reasons why closely similar viruses should differ so much in human pathogenicity. What was clearly needed was additional characters of the viruses, which could be determined in the laboratory and which would distinguish between variola major and variola minor viruses.

Dumbell, Huq and Wells have found a new character in which variola major viruses differ from variola minor. Their results will be published in detail elsewhere and only a very brief description is necessary here. This character depends on haemadsorption by human embryo skin and muscle cell cultures which have been inoculated with sufficient variola virus to infect all the cells and then incubated at 40°C for 48 hours. One of three results can be obtained. Haemadsorption can be confluent or nearly so. A virus giving this result is put in Group A. Group C contains those variola viruses which give no haemadsorption. There is a third group of viruses (Group B) in which haemadsorption is partial; some foci of haemadsorption are seen in every microscopic field but the majority of the cells do not haemadsorb.

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Over 200 strains of variola virus have been examined by two or more of the methods described. They were isolated from cases in Asia, Africa and South America and from certain outbreaks of smallpox in Europe, resulting from known importations. Most of the strains were isolated between 1960 and 1970 but the collection also contains some strains of earlier and some of more recent date. All experiments were made with virus stocks which had had between one and nine passages in chick chorioallantois; most of them were in the second or third egg passage. Virus stocks were preserved in buffered glycerol at -20°C .

RESULTS

Fourteen strains of variola from six outbreaks of major smallpox in Britain between the years 1946 and 1959 were all found to be Group A strains. Five isolates were available from the outbreak of 1962. One of these was from the Cardiff area and was in Group A; the other four were from cases in London and the Midlands and all these were in Group B. This evidence that at least two distinct viruses were involved in the 1962 outbreak is in accord with the epidemiological evidence (Ministry of Health, Smallpox 1961-1962). All outbreaks in 1962 were started by people who had arrived from Pakistan and it might be asked if Group B strains were known there. Sixteen isolates from cases in Karchi and Lahore were tested and of these 15 were in Group A and one was in Group B. There was no doubt about the virulence of the virus responsible for the Midlands focus of smallpox in 1962, where six people died of the 14 infected. The placing of an isolate in Group B, therefore, does not imply any necessary reduction in its virulence. In fact this particular grouping appears to be unrelated to the human pathogenicity of the strain, for among 25 undoubted alastrim isolates obtained from Brazil, there were five which were in Group B; the other 20 were in Group C (Table 1). It is of some further interest that all the five Group B strains were among the 11 isolates available from the north-eastern provinces of Pernambuco, Paraiba and Bahia. All the isolates from the central provinces, Sao Paulo and Minas Gerais, were in Group C as were also the isolates from the European outbreaks of alastrim in 1953, 1954 and 1966. There is good evidence then that a variola strain in Group B may be either virulent or avirulent, and that a minority of strains, both from South American alastrim and Asian variola major fall into this group.

The situation was very different in Africa. The majority of isolates from a number of African countries have been found to be Group B. This is shown in Table 1. If Kenya be excepted, Group B includes well over 90% of all the African isolates examined. The different state of affairs among the Kenyan isolates may be accounted for by the frequent importation of smallpox from Asian sources which has occurred in the past (Seymour-Price et al., 1960).

An "African" type of variola virus

The dominance of Group B strains among the available isolates from Tanzania and Western Africa raised the question whether the peculiarities noted by Bedson et al. (1963) among Tanzanian isolates were also to be found in the isolates from the countries in the west of Africa.

The results are summarized in Tables 2 and 3 and it can be seen that in these tests as well, West African and Tanzanian isolates appear to belong to the same family.

It is therefore proposed that there is a variety of smallpox virus, endemic in many parts of Africa which is detectably different in three laboratory tests from the smallpox viruses of South America and from those of asiatic origin imported into Britain. This African strain is relatively avirulent for the chick embryo, is more sensitive than variola major to a temperature of 38.3°C during growth in the chick C.A.M. and is in Group B by the new haemadsorption test.

DISCUSSION

The sources of smallpox outbreaks

The ability to distinguish a number of different types within the general character of a particular species of micro-organism can be used to good effect in tracing the source of outbreaks. Variola is no exception to this, although there are but few examples to quote. It has been mentioned above that typing of variola strains revealed at least two different viruses in the outbreak of smallpox in Britain in 1962. This is in keeping with the epidemiological evidence. It is known that there were five separate importations of smallpox into Britain at that time. The exact source of the cases in South Wales was never proven, though there was circumstantial evidence that they resulted from one particular importation; the cases in the Midlands definitely derived from a separate importation (Smallpox 1961-1962, Her Majesty's Stationery Office, 1963).

There was smallpox in London in 1963, for which there were three possible sources (Report of Committee of Enquiry). Evidence has been presented elsewhere (Dumbell, 1974) which links the virus causing the outbreak to one only of the three possibilities, a standard strain of variola major. The other two possible sources were two viruses which cannot be distinguished from variola by laboratory tests, but which were isolated from apparently healthy monkeys (Gispen & Brand Saathof, 1972). It would have been a blow to the smallpox eradication campaign if either of these viruses had been implicated in human smallpox.

Ten isolates were available from three outbreaks of alastrim in Europe. Four came from the outbreak at Rochdale (Innes, 1953), four were from the outbreak at the Hague (Blomhert, 1955) and two were from the outbreak in the English Midlands (Gordon et al., 1966). In the present study no differences were found between any of these 10 isolates and the isolates from central Brazil in each of the three laboratory tests. This observation, by itself, proves nothing, but there is a further point. All the many African isolates examined have been different from South American variola, and yet some of them have come from cases confidently called variola minor.

Variola major and variola minor

The differences between variola major and variola minor viruses which have been described (Dinger, 1956; Helbert, 1957; Nizamuddin & Dumbell, 1961) have been based on isolates of variola minor from the two outbreaks at Rochdale and the Hague plus one additional strain from the Sudan which was used by Helbert. The differences between the groups of viruses being compared will therefore certainly include differences of geographical origin as well as differences of pathogenicity. It is entirely possible that some of the differences described might be determined more by the area of endemicity from which the virus came than by its degree of pathogenicity. The present study shows that different patterns of results in these tests are characteristic of the variola found in different regions of the world. It has also been suggested (Shafa, personal communication) that the separation of variola strains into two distinct categories of major and minor is an oversimplification. Recent observations during the smallpox eradication campaign fit in better with the idea of a spectrum of variola viruses of differing pathogenicity, ranging from a minimum in Brazil to a maximum in Bangladesh. It is hoped to clarify further the relationship between the laboratory type and the human pathogenicity of variola viruses.

CONCLUSIONS

Study of the behaviour in laboratory tests of 200 strains of variola from Asia, Africa and South America, has shown a characteristic African pattern which differs from both the variola major associated with India and Pakistan and from the variola minor endemic until recently in South America.

The differences between the viruses of variola major and variola minor which have previously been reported may not arise entirely from their differing pathogenicities; but may be a reflection of their origin from different areas where variola had a long-established endemicity.

ACKNOWLEDGEMENT

The work reported herein was supported by grants from the World Health Organization to whom acknowledgement is gratefully made.

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TABLE 1. RESULTS OF HAEMADSORPTION TESTS ON HUMAN EMBRYO CELL CULTURES INFECTED AT 40°C

Source of virus	Number of isolates in Group		
	A	B	C
6 outbreaks of variola major in Britain 1946-1959	14	0	0
Britain 1962 - South Wales	1	0	0
Britain 1962 - Midlands	0	4	0
Pakistan - Karchi and Lahore	15	1	0
Brazil - Sao Paulo and Minas Gerais	0	0	14
Brazil - Bahia, Paraiba and Pernambuco	0	5	6
Sierra Leone and Guinea	0	6	0
Niger and Upper Volta	0	5	0
Togo	0	6	0
Nigeria	0	10	0
Zaire	2	12	0
Tanzania	2	20	0
Kenya	25	18	0

TABLE 2.

Source of virus	Number of strains with a difference in titre on C.A.M. at 35° and 38.3° of	
	0.5 or less	more than 0.6
Tanzania	5	15
West Africa	4	26
Variola major	10	0

TABLE 3.

Source of virus	Number of strains giving a mean survival time following inoculation of 100 000 pk.f.u. on C.A.M. of	
	less than 5 days	5 days and longer
Tanzania	3	8
West Africa	3	8
Variola major	10	1