

A note on two attenuated strains of myxoma virus isolated in Great Britain

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INTRODUCTION

It has been said that myxomatosis presents a unique opportunity for the study of the effect of a virus disease on a virgin population and it has often been assumed that the Australian course of events would be repeated here. This is not necessarily true as the principal vector is different. Since 1958 very little work has been carried out in Great Britain on the virus strains causing myxomatosis in the field. It was in 1954, approximately 2 years after the initial epizootic, that the first attenuated strain of myxoma virus was isolated in Britain (Hudson, 1954, unpublished; Andrewes, Muirhead-Thomson & Stevenson, 1956; Fenner & Marshall, 1955) in Sussex.

Recently, we have been investigating various aspects of the myxoma virus including an examination of strains isolated from wild rabbits sent to the Central Veterinary Laboratory, Weybridge. This present communication describes the behaviour of two strains isolated in this manner and discusses the implications of the findings in relation to the strains of myxoma virus previously described in this country.

MATERIALS AND METHODS

Virus strains

These are set out in Table 1 together with origin and number of passages.

Rabbits

Domestic rabbits were obtained from Allington Farm, Porton, and a local breeder. Animals were chosen which weighed 2–2½ kg. Rabbits were fed on a diet of pellets and water and were housed in cages in rooms which were heated.

Virus titrations

Virus suspensions were titrated on the chorioallantoic membrane of 11-day-old chick embryos according to the technique of Westwood, Phipps & Boulter (1957).

Virus diluent

This consisted of 200 ml. McIlvaine's citric acid/di-sodium phosphate buffer (Clarke, 1928) at 0.004 M concentration and pH 7.2. To this buffer was added 2 ml.

of skim milk and 0.2 ml. of a penicillin and streptomycin solution which gave a final concentration of 100 units/ml. of penicillin and 100 $\mu\text{g.}/\text{ml.}$ of streptomycin.

Preparation of virus strains for virulence tests

The material from which the suspensions of different strains of virus were prepared included animal material (Durham, Brecon, KM 13 and Glenfield) and egg pass material (Cornwall and A & H).

The materials containing KM 13, Glenfield, Cornwall and A & H strains were used directly, without pre-treatment, as sources of virus. The materials containing the two field strains (Durham and Brecon) were small pieces of eyelid and lung and were received in 50% (v/v) glycerol saline. The materials were well washed with cold saline and then ground in a cold pestle and mortar with sterile sand. The ground-up tissues were suspended in the special diluent used in the egg titrations and lightly centrifuged to remove debris and sand. The supernatants containing the virus were stored at -70°C.

Table 1. *Virus strains*

| Name | Synonyms and first description | Origin | Number of laboratory passages |
|-----------|---|---|-------------------------------|
| Cornwall | England/Cornwall/4-54/1 | Diseased wild rabbit | Several |
| Glenfield | Australia/Dubbo/2-51/1, Fenner & Marshall (1957) | Naturally infected wild rabbit | c. 100 |
| KM 13 | Aus./Corowa/12-52/2, Fenner & Marshall (1957) | From pool of <i>Anopheles annulipes</i> | 1 |
| A & H | Andrewes & Harisijades (1955) | Mouse brain adapted (30 passages) | Several (after adaptation) |
| Brecon | 4298 (Weybridge 1961) | Diseased wild rabbit | 0 |
| Durham | 4297 (Weybridge 1961) | Diseased wild rabbit | 0 |

Method of inoculating rabbits

The selected rabbits were inoculated intradermally with a needle contaminated with virus material, in the manner used by Fenner and his co-workers in their more recent experiments (Fenner 1962). Groups of five rabbits were employed for each strain of virus.

Reading of results

The following were recorded in tabular form for each rabbit inoculated:

- (1) type of virus;
- (2) Date of appearance of primary lesion;
- (3) Date of appearance of secondary lesion;
- (4) appearance of the primary lesion at the advent of secondaries (*a*) whether flat or raised, and (*b*) lesion colour and whether necrotic;
- (5) date of involvement of head and/or eyelids;
- (6) date eyes closed;
- (7) date of death;
- (8) survival time in days.

EXPERIMENTAL RESULTS

Comparison of strains

The two field strains of virus, one from Durham and the other from Brecon, were each inoculated into groups of 5 rabbits and the course of the disease was followed. At the same time known virus strains were inoculated into groups of 5 rabbits for comparison purposes. The results are summarized in Table 2. It became obvious early on that the two field strains were different from the classical virulent English strain (Cornwall), the virulent Australian strain (Glenfield) and the mouse brain adapted strain (A & H). At the same time it was apparent that there was a close similarity between the clinical symptoms produced by the field strains and by the attenuated strain KM 13.

The first part of Table 2 shows a comparison between the times taken for the appearance of the primary and secondary lesions for all the virus strains tested. This table shows that there is no significant difference between the times for the virulent and attenuated strains of virus. However, the very real difference between the primary lesions caused by virulent and attenuated strains of virus is indicated in the last column of Table 2 and shown by Pl. 1, figs. 1*a*, *b*.

It was not possible to distinguish between strains at the advent of the primary lesions. However, as the primaries developed and secondary lesions appeared it became obvious that there was a marked difference between the attenuated and the fully virulent strains. About 6–7 days after inoculation and approximately at the time secondary lesions appeared the primary lesions of the fully virulent strains were raised (about 2–3 cm.) with a somewhat smaller base than the greatest diameter of the lesions, which was of the order of 5 cm. These primary lesions had a very dark red, frequently purple and necrotic centre. In contrast the primary lesions of the attenuated strains were only slightly raised (0.5–1 cm.) and were pink to red in colour. The edges of the lesions were diffuse, the overall diameters were difficult to measure and had a much greater individual variation than those of lesions produced by the fully virulent strains. At 13–15 days most of the rabbits infected with the virulent strains were dead and showed few well-developed secondary lesions. There was, however, involvement of the head with very marked oedema; the eyes were closed and there was a copious purulent discharge from eyes and nose. There was also a discharge from the anus. There were many more secondary lesions at this time in rabbits infected with the attenuated strains. These lesions tended to be much smaller (*c.* 5 mm.) and more nodular. The head and eyelids were not so markedly affected and the discharges from nose, eyes and anus were less than in the rabbits with the virulent strains. The extremities (feet and ears) were affected. There was generally more edema and less localization of reaction in the rabbits given the virulent strains of virus.

Figures 1*a*, *b* show the differences at 15 days. The first series of photographs taken at 6 days were spoilt by the processing laboratory and we did not feel it was justifiable to inoculate another series of rabbits simply for photographs. However a photograph was taken of the 6 day primary lesion of the Brecon strain (Pl. 1, fig. 2), which was the subject of subsequent experiments.

Table 2 also shows the time taken for the production of secondary lesions due

Table 2. *Summary of results*

| Virus strain | Those showing disease/ Number of rabbits in a group | Mean; plus range (in days) | | | | Mean survival time | Lesion shape and colour at 13 days |
|--------------------------------------|--|--|--|---|---------------------------------|---|------------------------------------|
| | | Time taken for appearance of primary lesions | Time taken for appearance of secondary lesions | Time taken for serious head involvement | | | |
| Cornwall (English-virulent) | 4/5 | 4·5; 3-6 | 7·25; 7-8 | 9; 8-10 | 14; 13-15 | Raised, necrotic and purple | |
| Glenfield (Australian-virulent) | 5/5 | 2·8; 2-3 | 6·2; 6-7 | 7·6; 7-8 | 15·4; 13-24 | Raised, necrotic and dark red | |
| A & H (mouse brain adapted-virulent) | 5/5 | 3·6; 3-6 | 6·8; 6-7 | 9; 8-10 | 15; 13-21 | Raised, necrotic and dark red (2 were purple) | |
| KM 13 (Australian-attenuated) | 5/5 | 5; 5 | 6·8; 6-7 | 17; 14-20 | 19·3; 16-22 (with 2 recoveries) | Flat and red | |
| Durham (British field strain) | 3/5 | 5; 3-6 | 7·66; 7-8 | 14·5; 14-15 | All recovered | Flat and red | |
| Brecon (British field strain) (1) | 5/5 | 5; 5 | 7; 6-8 | 13·3; 9-15 | 29·85; 19-47 (3 recovered) | Flat and red | |
| N | 5/5 | Not later than 5 days | 6; 6 | | | | |
| 0·05 | 5/5 | Not later than 5 days | 5·8; 5-6 | 12·4; 12-14 | 21·4; 17-26 | Flat and red | |
| 6 | 5/5 | Not later than 5 days | 5·6; 5-6 | Killed at 6 days | | | |

to the Brecon strain when the simple transmission type of inoculation was used (lesion to skin by needle prick) shown under Brecon (1), Brecon N and Brecon 6, and after an intradermal inoculation of 0.05 ml. of the original virus material (Brecon 0.05). The original material contained approximately 1×10^3 p.f.u./ml. so that a 0.05 ml. quantity gave 50 p.f.u. The quantity of virus which is normally transmitted by an insect bite has been calculated to be somewhere in the region of 2 pock forming units (or 5 rabbit infectious doses) (Fenner & Marshall, 1957; Mykytowycz, 1956).

By an examination of the time taken for the serious involvement of the head (closing of the eyes, severe discharge from eyes and nose) (Table 2) it is immediately apparent that there is a considerable difference between the virulent and attenuated strains of virus in this respect.

Examination of one of the field strains

Having deduced, from the comparison of survival times and clinical symptoms of all the virus strains examined, that the field strains (Brecon and Durham) were attenuated, a further examination of the Brecon strain was carried out. It was considered necessary to see whether the survival times and clinical symptoms obtained in the first experiment could be repeated and also whether the virus could be serially passaged. Two groups of five rabbits were inoculated intradermally by a needle which had been contaminated with lesion material from a rabbit infected with the Brecon strain. Those of the first group were killed 6 days after inoculation (Brecon 6 in Table 2). The second was allowed to proceed normally, i.e. to death or recovery (Brecon N in Table 2). A further group of five rabbits were given 0.05 ml. of the original virus material intradermally and the disease allowed to proceed normally (Brecon 0.05 in Table 2).

A close watch was kept on the rabbits and the timing and nature of the clinical symptoms were recorded. It was apparent from the summarized results (Table 2) that there was no significant difference between those obtained in the first experiment (see comparison of strains) and those obtained by serial passage together with reference back to the original material.

DISCUSSION

Previously there has been a report of two strains of attenuated myxoma virus in Great Britain (Fenner & Marshall, 1957). In 1954 a strain of virus was obtained from Sussex which produced a mean survival time of approximately 22 days and which led to some recoveries (no mortality rate is available). In 1955 a sample of myxoma virus was isolated in the Nottingham area which in the hands of Fenner & Marshall (1957) was shown to be a mixture of two strains. The first was very attenuated having a mean survival time of approximately 121 days and there were recoveries. This had a mortality rate of 23%. The second strain was fully virulent having a survival time of approximately 12 days and there were no survivors.

From the description of the effects of the Sussex strain (Andrewes *et al.* 1956), the attenuated Nottingham strain and the Australian attenuated KM 13 strain

(Fenner & Marshall 1957), it would appear that the field strains described in this paper are more closely related to the Sussex and KM 13 strains than the Nottingham strain, on the basis of clinical symptoms and mean survival time. The mean survival time of the KM 13 and the Sussex strain are identical (21.5 days in laboratory rabbits), but, whereas the two field strains have in general identical clinical symptoms, there is a marked difference in the mean survival time. The Durham strain infected only 3 out of 5 rabbits and the two unaffected ones were found to be susceptible on reinoculation with a virulent strain. The 3 infected rabbits all recovered. In contrast the five rabbits inoculated at the same time with the Brecon strain all became infected and only one recovered. Of a total of 15 rabbits infected with the Brecon strain all but 2 died, this indicates a mortality rate of 87%. The mortality rate of the KM 13 strain is 88% (Fenner & Marshall 1957). The mean survival time with the Brecon strain is 26.3 days (mean of all rabbits inoculated with this strain) whereas the m.s.t. for KM 13 is 21.5. However, the mean survival time for the Glenfield strain, as determined in our hands at the same time, was 15 days while in the hands of Fenner & Marshall (1957) it was 10.2 days. A difference in the room temperature could explain this discrepancy. Certainly, Parker & Thompson (1942) showed that continued high temperature gave infected animals a prolonged survival time. Marshall (1959) demonstrated that the temperature of housing after inoculation had a profound effect on the response of the rabbits inoculated in the standard manner. The mean survival time of the Glenfield strain, as determined during a subsequent experiment at the Infestation Control Laboratory, (carried out by P. J. C.) was found to be 11 days. The difference between this result and that obtained at M.R.E. might be explained by the difference in temperature of the two animal houses. However, it should be pointed out that differences in the strain of rabbits might also influence the survival time (see note at the end).

Approximately 8 years have now elapsed since the initial outbreak of myxomatosis in this country. It is very interesting to note that a strain with a high mortality can still be isolated. It has been suggested by Andrewes, Thomson & Mansi (1959) that myxomatosis need not necessarily follow the same pattern in this country as in Australia. That there are differences it cannot be denied, but it is significant that one of the strains of virus described in this paper is almost identical with the Australian KM 13 strain which is the prototype of the Grade III virulence group of virus strains (Fenner & Marshall, 1957) and this is the group which is predominant in Australia at this time and has been for the last 5 years (Fenner 1962). The isolation of these two attenuated strains is interesting on two counts the first of which has already been outlined, the second concerns the difference noted by Fenner & Marshall (1957) between the Australian and the European strains of virus. It was suggested that the European strains produced a much more nodular disease than their Australian counterparts. That the Brecon and Durham strains produce flat lesions may be very significant in the evolution of the virus in Britain. It was on this supposed difference between Australian and European strains that the proposed grading of virulence for the latter was based.

A survey of virus strains present in this country is being made to determine the virulence and clinical symptoms of the predominant strains, to observe whether there is any change from year to year, and to make a comparison with the Australian strains.

SUMMARY

Two strains of myxoma virus isolated from naturally infected wild rabbits have been described. They showed similar clinical symptoms, the most striking feature being the flat primary lesion. In fifteen rabbits the Brecon strain gave a mean survival time of 26.3 days with a range of 17–47 days, and one recovery. Of 5 rabbits inoculated with the Durham strain three became clinically infected and two remained healthy, the latter were found to be fully susceptible on reinoculation. The three infected rabbits all recovered.

The field strains of virus were compared with known myxoma strains—Glenfield, Cornwall, A & H and KM 13. The first three are fully virulent, while KM 13 is attenuated and characteristic of the field strains now being isolated in Australia. The similarity between the clinical symptoms and mean survival time of KM 13 and the Brecon strain was noted.

It is suggested that the two field strains described in this paper may be the first indication that the evolution of myxoma virus in this country is similar to the evolution of the virus in Australia.

One of us (P. J. C.) is greatly indebted to the Director for allowing this work to be carried out at M.R.E. whilst laboratory accommodation was being prepared at Worplesdon. We are grateful for the help and encouragement of Dr J. C. N. Westwood of M.R.E. To Prof. F. Fenner who gave us the Australian strains of myxoma virus and to Mr J. H. Darbyshire of the Central Veterinary Laboratory who supplied the tissues from which the two attenuated strains were isolated, we extend our thanks.

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EXPLANATION OF PLATE

Lesions in rabbit after inoculation with two strains of myxoma virus.

Fig. 1. (a) A & H strain, 15th day, rabbit died at 21 days. (b) Brecon strain 15 day, rabbit recovered.

Fig. 2. Brecon strain, 6th day.

ADDITIONAL NOTE BY P. J. C.

Subsequent experiments at the Ministry of Agriculture's Field Research Station gave shorter survival times for all strains tested. In the case of Glenfield the mean survival time (M.S.T.) was 11 days (range 8–13) on one occasion and 11·6 (range 9–14) on another. The Brecon strain gave a M.S.T. of 16 days (range 12–18) and the Durham strain a M.S.T. of 18·4 (range 16–20). The symptoms remained the same. In the case of the two field strains the primary lesions were flat and red while the Glenfield strain primary lesion was very raised with a purple necrotic centre. There were no survivors in any of the groups of five rabbits which were used for testing each strain.

The differences in the survival times found at the two establishments are not surprising if the following factors are borne in mind. The animal house at M.R.E. has a fairly high (c. 18·5° C. (65° F.)) temperature which is easily maintained through the medium of air conditioning, and double glazing. At the Field Research Station the animal house is a wooden structure, with a certain amount of insulation built in, heated by tubular electric heaters, but with the windows left open to provide ventilation. Therefore, it is much more susceptible to the vagaries of the external environment and in addition the thermostat is set at 15·6° C. (60° F.).

The animals used in the experiments at the field station came from a different source than those used at M.R.E.

That both these factors can influence the survival times has been very well shown by Marshall (1959).

It is interesting to note that the M.S.T. for the Glenfield strain, as determined at the Field Research Station is almost the same as that obtained by Fenner & Marshall (1957)—who quote a figure of 10·2 days (range 9–12).

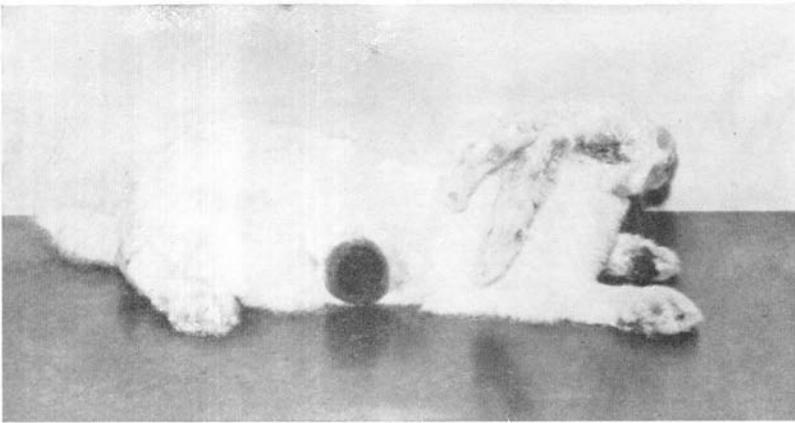


Fig. 1 (a)

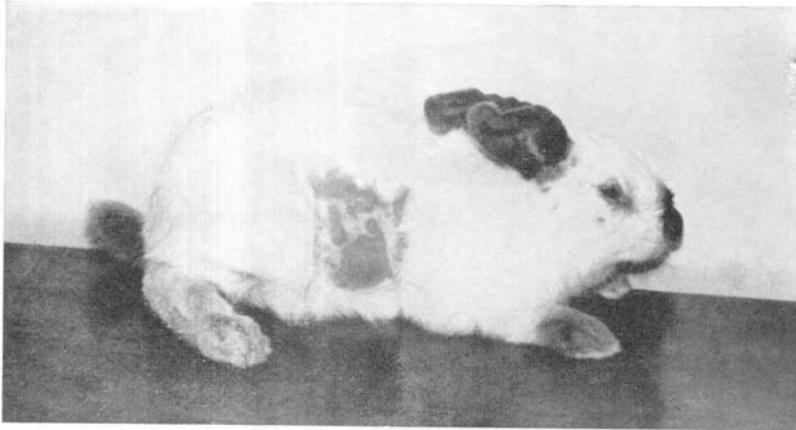


Fig. 1 (b)

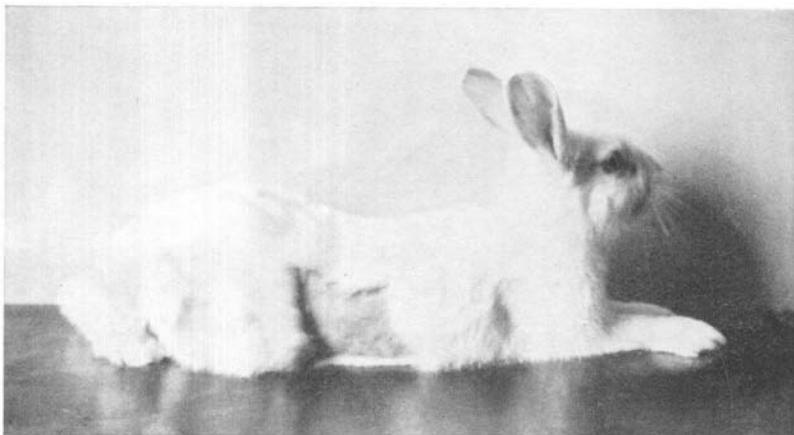


Fig. 2