STUDIES IN THE EPIDEMIOLOGY OF INFECTIOUS MYXOMATOSIS OF RABBITS

VI. THE EXPERIMENTAL INTRODUCTION OF THE EUROPEAN STRAIN OF MYXOMA VIRUS INTO AUSTRALIAN WILD RABBIT POPULATIONS*

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(With Plate 4 and 2 Figures in the Text)

Three different groups of myxoma virus strains can be differentiated on the basis of the symptomatology of the disease they cause in *Oryctolagus* rabbits (Fenner & Marshall, 1957). These are the Brazilian strains and their immediate derivatives in Europe (prototype: Brazil/Campinas/1949/1 (Lausanne)), the standard laboratory strain and its attenuated derivatives (prototypes: standard laboratory strain, Aust./Corowa/12–52/2 (KM 13), Aust./Uriarra/2–53/1, and neuromyxoma), and the Californian strains (prototype: U.S.A./San Francisco/1950/1 (MSW)).

The major epizootics of myxomatosis in *O. cuniculus* have been those occurring in Australia since the introduction of the standard laboratory strain of virus into the wild rabbit population in 1950, and in Europe since the introduction of the Lausanne strain in June, 1952. One important difference in the behaviour of the disease in Europe and in Australia in the first two years after introduction was the uniform high virulence of strains recovered from the field in Europe, and the almost invariable attenuation of strains recovered from naturally infected wild rabbits in Australia, a difference all the more striking in view of the repeated large-scale introductions of the virulent standard laboratory strain by inoculation campaigns in Australia, and the absence of such reintroduction of the virulent virus in Europe. One possible explanation for this difference was that the Lausanne strain was intrinsically more stable in its high virulence than the standard laboratory strain. The maintenance of high virulence was of such great importance in the Australian rabbit destruction campaigns that an experimental small-scale introduction of the European strain of virus was carried out at Lake Urana, New South Wales. A subsidiary object of this experiment was to determine whether it was in fact possible to introduce a strain artificially when a naturally occurring enzootic strain was circulating in the rabbit population under study. Doubts of the possibility of effectively introducing the highly virulent standard laboratory strain under such circumstances had been entertained from the beginning of the Australian

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inoculation programmes, but it was impossible to differentiate recently introduced virus from naturally occurring strains, except by virulence. Examination of large numbers of strains by standard virulence tests was impractical because of the large numbers of experimental rabbits required, and there was no way of distinguishing a recently attenuated variant of the artificially introduced standard laboratory strain from an attenuated enzootic strain. The strikingly different symptomatology associated with infections caused by the Lausanne strain, and the standard laboratory strain and its derivatives, was made the basis of a screening test which permitted recognition of the Lausanne-type infections when several strains were tested in a single rabbit.

The location of Lake Urana, its physiography, and the history of myxomatosis there up to March, 1953, have already been reported (Myers, Marshall & Fenner, 1954).

MATERIALS

Virus strains

Standard laboratory strain, the material used to initiate the Australian outbreaks.

France/Dordogne/11–53/1 (*French*) was derived from a wild rabbit in Périgeux, in the Dordogne, in November, 1953, and kindly sent to us by Dr P. Lépine. A stock of freeze-dried virus, of which the titre on reconstitution was about 10^4 rabbit infectious doses per ml., was prepared by the Commonwealth Serum Laboratories from virus material prepared according to the method described in the appendix to Fenner & Woodroofe (1954).

Aust./Urana/11–54/1 (*Urana*) was derived from a naturally infected wild rabbit on the experimental site at Lake Urana early in November, 1954, just before the introduction of the French strain.

France/Loiret/4–55/1 (*Loiret* 55), the first attenuated strain recognized in Europe (Jacotot, Vallée & Virat, 1955*b*; Fenner & Marshall, 1955). This strain was provided by Dr H. Jacotot, Pasteur Institute, Paris.

England/Sussex/9–54/1, an attenuated strain recovered from Newhaven, England, in September, 1954 (Fenner & Marshall, 1957). This strain was sent to us by Mr J. R. Hudson.

Rabbits

Laboratory rabbits were bred in the Australian National University Animal Breeding Establishment, and used at the age of 4 months.

METHODS

Virus titrations were carried out by pock-counting on the chorioallantois of the developing chick embryo (Lush, 1937; Fenner & McIntyre, 1956).

Sera were tested for antibody by complement-fixation and neutralization tests (Fenner, Marshall & Woodroofe, 1953).

EXPERIMENTAL RESULTS

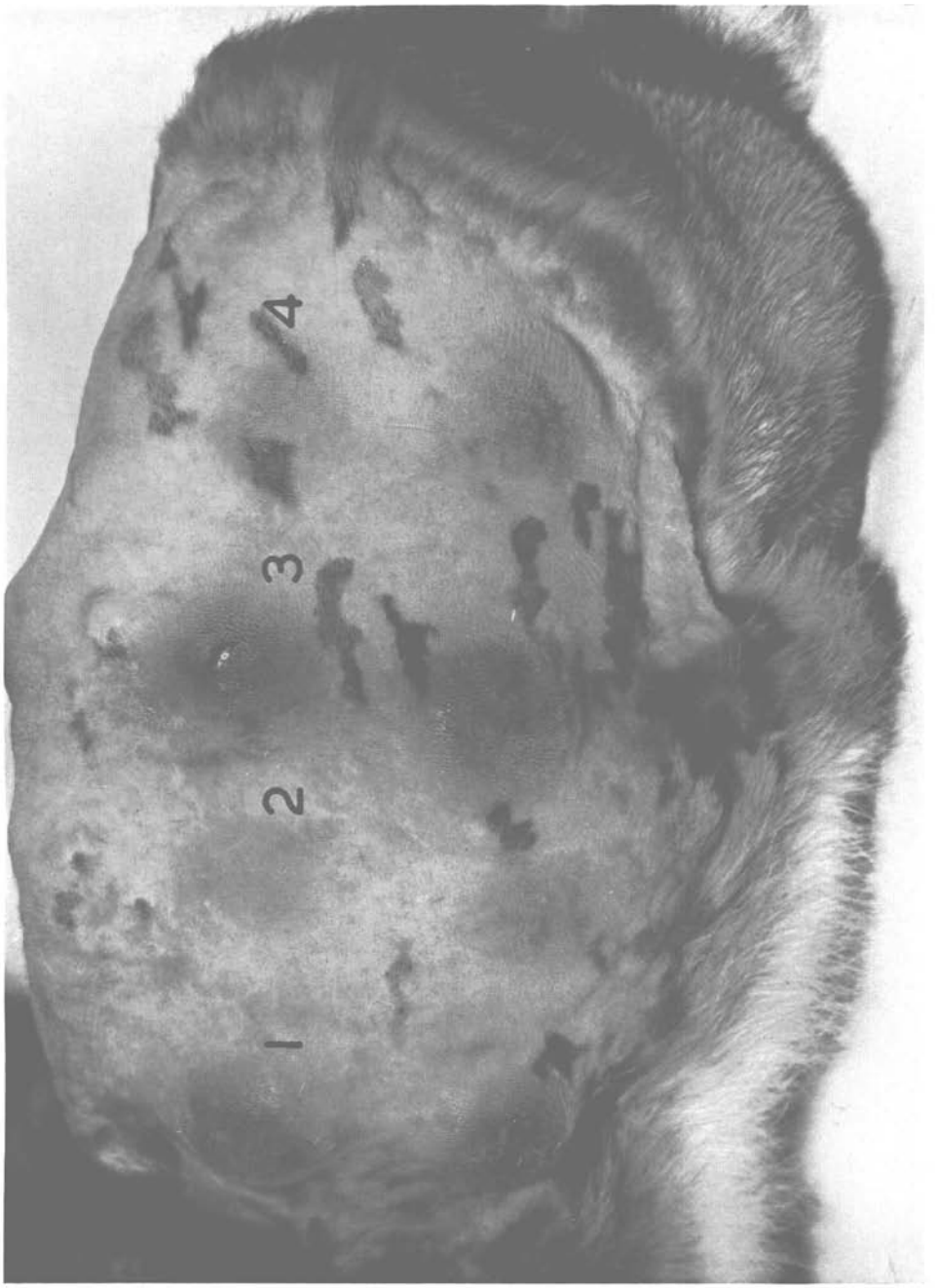
A screening method for distinguishing the French strain from the standard laboratory strain and from naturally occurring Australian strains of virus

We have already described the clinical features of myxomatosis due to the intradermal inoculation of rabbits with small doses of various representative strains of myxoma virus (Fenner & Marshall, 1957). All strains of virus obtained from Europe up to April, 1955* produced a similar clinical picture in infected rabbits (Fenner & Marshall, 1955), and this contrasted strongly with that found with all the Australian strains. In order to test large numbers of strains of virus it was necessary to devise a screening method which would permit several strains to be tested on a single rabbit. The difference in the appearance of the primary lesions developing at the inoculation sites after intradermal inoculation of the French and Urana strains was so great that comparisons were made of the lesions resulting from multiple intradermal inoculations in the back of a single rabbit. The results obtained with comparable doses of the French strain, Urana, and the standard laboratory strain are illustrated in Pl. 4. The differences were even more pronounced than are shown by the black and white photograph, for lesions due to the French strains were large, raised, hard and deep purple in colour, whereas the lesions produced by the naturally occurring Australian strains were flat, had indistinct margins, and were at most pink in the centre. The standard laboratory strain produced lesions intermediate in character between these extremes, more prominent than those due to attenuated Australian strains, but much less protuberant than those produced by virulent French strains. Subsequent experiments showed that these differences in character were independent of the dosage of virus, although the lesions caused by large doses were considerably larger than those due to small doses.

The technique finally adopted for screening tests was as follows. Lesion material received from the field was stored in a deep freeze cabinet until it was convenient to test it. Portions of the material were then ground in a chilled mortar and pestle with alundum, taken up in gelatin saline containing penicillin (500 units per ml.) and streptomycin (4 mg. per ml.), and ampouled. Six strains were tested on one rabbit, and simultaneously a rough check of their titre was obtained by chorioallantoic inoculation of the same suspensions, undiluted and at 1/100 dilution. Each of two sites on the shaved back of a rabbit was inoculated intradermally with 0·1 ml. of each unknown strain, and as controls two sites were always inoculated with the French strain and two with the Urana strain. Readings were made 5 and 6 days later, and the results were unequivocal. Occasionally large flat lesions occurred in sites inoculated with high concentrations of slightly attenuated Australian strains of virus, but they could not be confused with the large raised purple lesions caused by the French strain.

The first attenuated strains obtained from Europe were not available until the conclusion of the field experiment described in this paper. The symptomatology

* Subsequently, strains of virus recovered from Sussex in September and October 1954 were found to be attenuated (Fenner & Marshall, 1957).



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of infections with these strains, and especially the rate of development of lesions, differed considerably from that of the virulent European strains (Fenner & Marshall, 1957). Further experiments were therefore made to determine whether the intradermal screening test could be used to diagnose variants such as the attenuated European strains. It was found that the attenuated strains France/Loiret/4–55/1 and England/Sussex/9–54/1 produced primary lesions which were indistinguishable from those produced by attenuated Australian strains throughout the 10 days which the rabbits survived (they had also been infected with virulent French type virus as a control of their reactivity). Thus the intradermal screening test could be used to distinguish between virulent French type strains, and the standard laboratory strain and its attenuated field derivatives, but did not permit differentiation of some of the attenuated European strains.

The rabbit situation at the experimental site before the introduction of virus

The epizootic history of myxomatosis at Lake Urana up to March, 1953 has been described in a previous paper (Myers *et al.*, 1954). The experimental site used for the present experiments was on the western side of the lake about 5 miles distant from the sites described in that report, but its epizootic history in 1952 and 1953 was essentially the same.

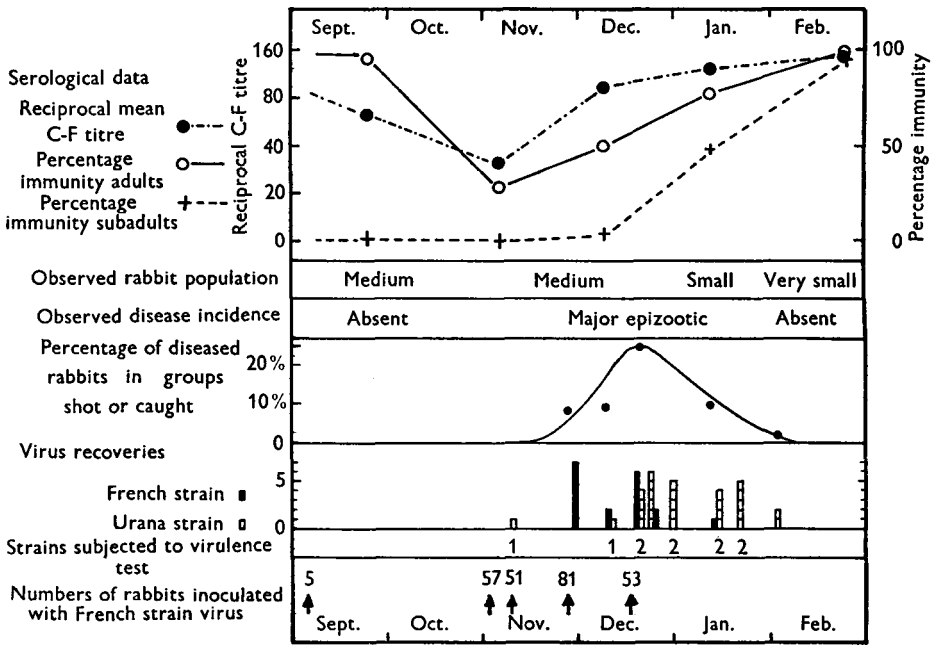
Contrary to the experience in 1951 and 1952, there was no early summer (November–December) epizootic in 1953, and a large rabbit population was present in February, 1954. At this time, however, a sharp epizootic occurred, and caused a considerable reduction of the population. Serological tests on 202 sera collected in March, 1954 showed that the survivors consisted almost entirely of immune rabbits. One sample of virus was obtained on 10 April, 1954, and this showed the same virulence as the strains recovered in 1952, i.e. a mean survival time of about 20 days (Fenner & Marshall, 1957). By September, 1954 there had been a considerable increase in the rabbit population due to breeding, and 350 live rabbits were captured by netting at night with a spotlight. All the younger rabbits were taken to Canberra for testing the innate resistance of rabbits whose parents had been exposed to several epizootics of myxomatosis. One hundred and six animals were judged to be too large to have been born since the epizootic, and on 11 September they were inoculated intradermally with the French strain of virus. Only five contracted generalized myxomatosis, and these were released again at the trial site. The percentage of animals shown to be immune by challenge inoculation was thus virtually the same as that determined serologically at the same time (Text-fig. 1).

Serum surveys were continued at approximately monthly intervals, with the results shown in Text-fig. 1. As at Texas and Dunroy (Marshall, Dyce, Poole & Fenner, 1955) the trend in the titre of complement-fixing antibody was the same as the trend in the proportions of positive sera in the adult rabbit population.

On 2, 9, 27–29 November, and 15–17 December groups of 50–80 juvenile rabbits captured by netting were inoculated with the French strain of virus, ear-marked, tattooed with a serial number, and released again. Between the beginning of

November and the end of February all rabbits seen to be suffering from myxomatosis were captured or shot, and virus-containing material collected from them. The strains of virus recovered from naturally occurring cases were then tested by intradermal inoculation in rabbits, as described earlier.

Naturally infected cases of myxomatosis, as distinct from those infected by inoculation, were first seen on 10 November. The epizootic reached a peak in mid-December, and gradually diminished until it appeared to be over by the end of January. By then the rabbit population was reduced to a very low level. The disease incidence corresponds with the incidence of vector mosquitoes (*Anopheles annulipes* and *Culex annulirostris*) feeding on rabbits, for Myers (1955) showed that at the experimental site these numbers rose sharply from mid-October to a peak in November, and fell off to a low level by the beginning of February. Except at the peak of the epizootic in mid-December, when only a proportion of cases were sampled for virus, virus-containing material was collected from all diseased rabbits captured or shot.



Text-fig. 1. The epizootic which followed the introduction of the French strain of myxoma virus into the wild rabbit population at Lake Urana—September, 1954 to February, 1955. Graphs show the incidence of the disease, the size and immune status of the rabbit population, the type of virus as determined by the intradermal screening test, the strains selected for virulence tests and the schedule of inoculation of the French strain of myxoma virus.

The results are shown diagrammatically in Text-fig. 1. The first natural case of myxomatosis was seen in 10 November, and the strain of virus obtained from this animal (Aust./Urana/11–54/1; called 'Urana' in this paper) proved to be indistinguishable from strains obtained from the area in December, 1952 and March, 1954. It is probable that this strain of virus had survived in the area at a sub-observational level since the epizootic of the previous summer. Although no diseased

rabbits were captured during the winter months occasional animals with clinical and serological signs of recent recovery from myxomatosis were captured during August and September, 1954. At the beginning of November, 1954 myxomatosis had not occurred on a large scale in that part of Australia, and inoculation campaigns with the standard laboratory strain of virus had not been commenced, so that movement of the virus into Lake Urana from elsewhere was unlikely.

The next collection of virus samples was made on 28–30 November, when all of the seven strains obtained (out of 88 rabbits captured for inoculation) were of the French type. Five sick rabbits were obtained on 9 December, out of 58 shot. Two of these were infected with the French strain of virus and one with the local strain, and no virus was recovered from material derived from the other two. Between 17 and 19 December cases of myxomatosis were much more common, 18 out of 73, or 25% of the rabbits captured for inoculation being active cases. Virus was recovered from 18 of the 22 cases taken at this time (18 netted, 4 shot). Eight of these proved to be the French strain and the other 10 the local strain. The epizootic was waning by the end of December, and only one of the 21 strains recovered on the five expeditions organized between 30 December and 2 February was of the French type. All the others were indistinguishable from the local Urana strain.

A serological survey in February, 1955 showed that almost all (82 out of 84) of the few remaining rabbits had recovered from myxomatosis.

The scanty rabbit population increased somewhat due to breeding in the autumn and spring. The vector mosquito population rose to a very high level by late October, 1955, due to heavy late winter and spring rains, and diminished again by the end of November (Myers, personal communication). Myxomatosis broke out in October. A sharp epizootic in the small rabbit population was virtually over by the first week of December. None of the seventeen samples of virus recovered from this epizootic was of the virulent French type.

Further testing of strains collected at Lake Urana

Diagnosis of the types of virus occurring during the 1954–55 and November, 1955 epizootics at Lake Urana was based on the intradermal screening test described earlier. To check this, and to differentiate possible attenuated French type strains from attenuated Australian strains, certain specimens were subjected to more detailed investigation, three to five adult laboratory rabbits being inoculated intradermally with about 5 rabbit-infectious doses of virus (Fenner & Marshall, 1957). The strains selected from those obtained in the 1954–55 outbreak are indicated in Text-fig. 1. The preponderance of attenuated Australian strains amongst those subjected to detailed investigation was due to deliberate selection of strains which had produced large but flat lesions in the screening tests. The skin lesions produced by the virulent French strain were so distinctive that they could not have been mistaken, and therefore only a few were tested any further. The results are given in Table 1. They confirm the diagnosis of strain made on the basis of the screening tests, and indicate that, as far as the limited sampling could indicate, the two strains, attenuated Australian and French type, did not alter appreciably in their virulence during the course of the epizootic.

Table 1. *Survival times of rabbits inoculated intradermally with 5 rabbit-infectious doses of strains of myxoma virus obtained from naturally occurring cases at Lake Urana and Merricumbene*

(Cases are classified as 'attenuated Australian' or French type, according to the symptomatology.)

Date of collection	Strain	Survival time (days)	Average survival time*	Type of disease	
				Screening test	Virulence test
Lake Urana 1954–55					
10. iv. 54	Aust./Urana/4–54/1	16, 17, 18, 24, 28	19·8	Attenuated Australian	Attenuated Australian
10. xi. 54	Aust./Urana/11–54/1	16, 16, 18, 20, 27	18·8	Attenuated Australian	Attenuated Australian
9. xii. 54	Aust./Urana/12–54/1	14, 16, 20, 28	18·5	Attenuated Australian	Attenuated Australian
17. xii. 54	Aust./Urana/12–54/2	15, 15, 17, 27	17·6	Attenuated Australian	Attenuated Australian
17. xii. 54	Aust./Urana/12–54/3	18, 20, 28, S‡	23·0	Attenuated Australian	Attenuated Australian
30. xii. 54	Aust./Urana/12–54/4	16, 17, 18, 20	17·6	Attenuated Australian	Attenuated Australian
30. xii. 54	Aust./Urana/12–54/5	11, 20, 23, 43	19·7	Attenuated Australian	Attenuated Australian
11. i. 55	Aust./Urana/1–55/1	15, 15, 17, 27	17·6	Attenuated Australian	Attenuated Australian
11. i. 55	Aust./Urana/1–55/2	10, 11, 12, 12, 13	11·4	French	French
20. i. 55	Aust./Urana/1–55/3	13, 18, 21, 28	18·7	Attenuated Australian	Attenuated Australian
20. i. 55	Aust./Urana/1–55/4	17, 32, S	—	Attenuated Australian	Attenuated Australian
Urana North Station 1954–55					
12. i. 55	Aust./Urana North/1–55/1	11, 11, 13, 13	11·9	French	French
12. i. 55	Aust./Urana North/1–55/2	20, 24, 29, 41	27·1	Attenuated Australian	Attenuated Australian
14. i. 55	Aust./Urana North/1–55/3	10, 11, 11, 11, 12	10·9	French	French
Merricumbene 1954–55					
9. xi. 54	Aust./Merricumbene/11–54/1	22, 26, 26, 33, S	28·2	Attenuated Australian	Attenuated Australian
23. iii. 55	Aust./Merricumbene/3–55/1	17, 20, S, S, S	—	Attenuated Australian	Attenuated Australian
21. iii. 55	Aust./Merricumbene/3–55/2	10, 10, 11, 12, 12	10·8	French	French
9. iii. 55	Aust./Merricumbene/3–55/3	12, 13, 14, 27	14·9	Attenuated Australian	Attenuated Australian
12. iv. 55	Aust./Merricumbene/4–55/1	17, 20, 30	21·3	Attenuated Australian	Attenuated Australian
12. iv. 55	Aust./Merricumbene/4–55/2	17, 25, 41, S	27·9	Attenuated Australian	Attenuated Australian

Lake Urana 1955–56					
27. x. 55	Aust./Urana/10–55/1	30, S, S, S, S	—	Attenuated Australian	Attenuated Australian
27. x. 55	Aust./Urana/10–55/2	13, 19, 20, 25, 29	19·9	Attenuated Australian	Attenuated Australian
27. x. 55	Aust./Urana/10–55/3	20, 31, S, S, S	—	Attenuated Australian	Attenuated Australian
28. x. 55	Aust./Urana/10–55/4	38, S, S, S, S	—	Attenuated Australian	Attenuated French?
28. x. 55	Aust./Urana/10–55/5	15, S, S, S, S	—	Attenuated Australian	Attenuated Australian
28. x. 55	Aust./Urana/10–55/6	15, 19, 26, 30, S	24·4	Attenuated Australian	Attenuated Australian
28. x. 55	Aust./Urana/10–55/7	25, 27, 31, 32, 32	29·2	Attenuated Australian	Attenuated Australian
28. x. 55	Aust./Urana/10–55/8	24, 25, 25, 36, 37	28·6	Attenuated Australian	Attenuated Australian
28. x. 55	Aust./Urana/10–55/9	23, 26, 33, S, S	37·3	Attenuated Australian	Attenuated Australian
4. xi. 55	Aust./Urana/11–55/1	12, 12, 12, 14, 22	13·6	Attenuated Australian	Attenuated Australian
7. xii. 55	Aust./Urana/12–55/1	24, 31, S, S, S	—	Attenuated Australian	Attenuated Australian
7. xii. 55	Aust./Urana/12–55/2	20, 21, 21, 25, 25	22·2	Attenuated Australian	Attenuated Australian
7. xii. 55	Aust./Urana/12–55/3	9, 9, 10, 12, 23	10·6	Attenuated Australian	Attenuated Australian
7. xii. 55	Aust./Urana/12–55/4	11, 17, 22, 22, 25	17·8	Attenuated Australian	Attenuated Australian
26. i. 56	Aust./Urana/1–56/1	19, 21, 22, 24, S	22·1	Attenuated Australian	Attenuated Australian
26. i. 56	Aust./Urana/1–56/2	S, S, S, S, S	—	Attenuated Australian	Very attenuated Australian
Merricumbene 1955–56					
18. iv. 56	Aust./Merricumbene/4–56/1	15, 16, 17, 21, 25	18·2	Attenuated Australian	Attenuated Australian
Standard laboratory strain		43 rabbits†	10·8	—	—
Virulent European strains		66 rabbits†	12·0	—	—
Attenuated Australian (moderate virulence—KM 13)		77 rabbits†	21·5	—	—
Attenuated Australian (low virulence—Uriarra)		45 rabbits†	26·2	—	—

* Calculated from the transformed ($\log_{10}$ (survival time in days − 8)) survival times, using Sampford's (1954) method when there were survivors (see Fenner & Marshall, 1957).

† From Fenner & Marshall (1957).

‡ S = rabbit recovered from infection.

None of the strains recovered from the 1955–56 epizootic was of the virulent French type, as judged by the intradermal screening test. When subjected to the standard virulence test these strains exhibited great variability in their behaviour (Table 1). Using the grading of virulence set out earlier (Fenner & Marshall, 1957), two strains were highly virulent (grade I or grade II), five were moderately virulent (grade III—like KM 13), five were of low virulence (grade IV), and four were very attenuated (grade V). As inspection of Table 1 will indicate, some of the last group were of much lower virulence than any strains previously recovered from Australian wild rabbits. Furthermore, the skin lesions associated with some of these very attenuated strains, such as Aust./Urana/10–55/4, were very similar to those observed in infections with some of the highly attenuated European strains, like England/Nottingham/4–55/1 (attenuated). It is possible, therefore, that a highly attenuated variant of the virulent French strain persisted at Lake Urana. Whether this happened or not, the heterogeneity of strains recovered from this fifth epizootic contrasts with the homogeneity of those recovered previously (1954–55—Table 1 of this paper; and 1952–53—Myers *et al.*, 1954).

Experiments with the French strain elsewhere in New South Wales

Rabbits were inoculated with the French strain of virus at a few other localities, one near Lake Urana, and another at Merricumbene, near the south-east coast of New South Wales. The information obtained was not as complete as at the Lake Urana site, but merits a brief description.

North Urana Station

There was a large rabbit population on the sandy pine ridges on this station, which is situated to the east of the lake, about 5 miles from the experimental site at Lake Urana. On 3 December 38 rabbits were inoculated with the French strain of virus. An epizootic developed about a month later. Four virus samples were collected on 12 January and 15 samples on 14 January, when the epizootic was at its height. A further 21 samples were taken on 19 January, when the rabbits yielding the samples were the only live animals seen. Most appeared to be in the late stages of the disease, or in the early stages of recovery from it. Thirty strains were recovered from these 40 samples, and all except two, recovered on 12 January, proved to be of the local Australian type. The virulence of three strains was tested, with the results shown in Table 1.

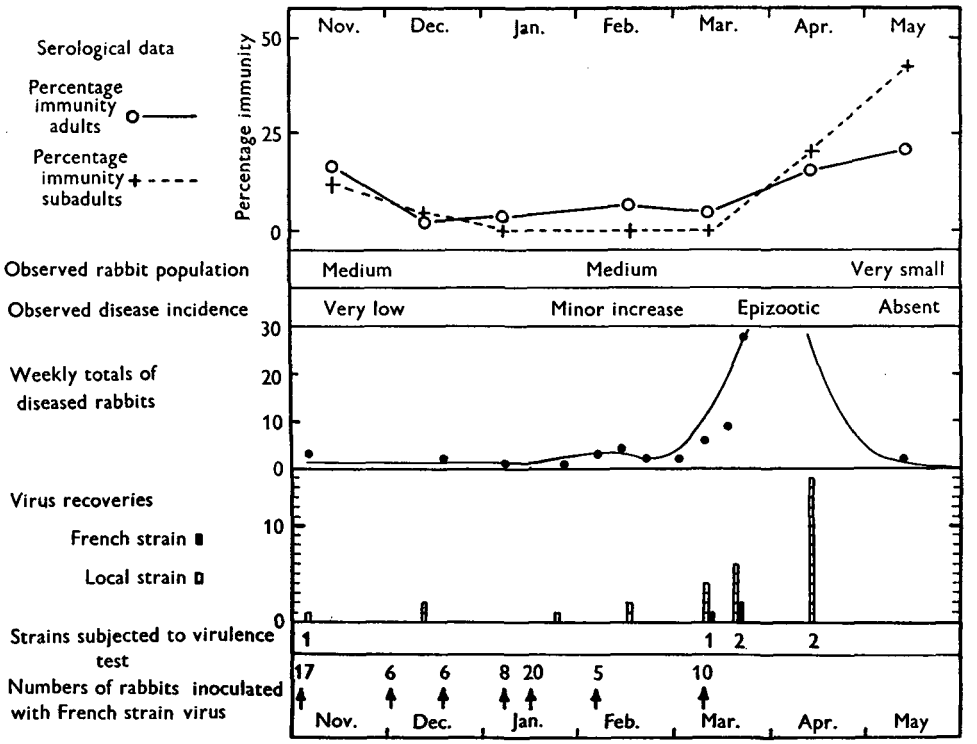
Merricumbene

This study area, about 2000 acres in extent, is about 180 miles south of Sydney, and 25 miles from the coastal town of Moruya. It consists of an open glade on the Moruya River surrounded by forested hills. Descriptions of its physiography and of investigations of mosquito ecology and rabbit breeding behaviour at Merricumbene will be published elsewhere.

Local farmers reported that they had observed myxomatosis in the area intermittently since it was introduced artificially in the autumn of 1951, but in spite of

repeated small-scale reintroduction of the standard laboratory strain appreciable kills did not occur.

At Lake Urana 242 rabbits were captured, inoculated and released over a period of 6 weeks. Few landholders in Australia would undertake introductions of this magnitude, and at Merricumbene the experimental release of the French strain was so designed that the method used could be widely practised if the results justified widespread introduction of the French strain. Rabbits were infected by inoculation and released; and also with a gin trap modified so that when sprung the rabbit was inoculated subcutaneously and intradermally with the virus and then escaped (Anonymous, 1942). Bull & Mules (1944) found that about 75% of rabbits which sprang this modified trap became infected with myxomatosis, and this figure has been used to calculate the numbers of rabbits artificially infected at Merricumbene.



Text-fig. 2. The epizootic which followed the introduction of the French strain of myxoma virus into the wild rabbit population at Merricumbene—November 1954 to May 1955. Graphs show the incidence of the disease, the size and immune status of the rabbit population, the type of virus as determined by the intradermal screening test, the strains selected for virulence tests, and the schedule of inoculation of the French strain of myxoma virus.

The epizootic history during the summer of 1954–55 is illustrated in Text-fig. 2. About 70 rabbits were infected with the French strain of virus over a period of 4½ months. The weekly sightings of diseased rabbits shown in Text-fig. 2 include all sick rabbits seen by the research team or by landholders in the area. An occasional

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sick animal was seen between September and the middle of February. The number of cases then increased considerably and diseased animals were numerous during the last week of March and the first week of April. During the collection of sera in mid-April many of the rabbits shot were apparently recovering from myxomatosis, or had recovered completely.

Virus was recovered from all diseased rabbits captured or shot, except at the collection in mid-April when lesion material was taken from only a proportion of the available obviously infected rabbits. All strains recovered were tested by the intradermal screening test and several specimens, indicated in Text-fig. 2, were also tested for virulence (Table 1).

An attenuated Australian strain was obtained at the time of the first release of French virus, early in November, 1954, and an occasional rabbit infected with the local strain of virus was captured during the next 4 months. The first case naturally infected with the French strain was obtained on 10 March, 33 days after the last introduction of that virus, so that at least three or four serial transmissions occurred before the presence of the strain was recognized. Two other cases infected with the French strain were obtained just before the peak of the epizootic. All 15 samples tested from rabbits taken in mid-April, during the decline of the epizootic, were of the attenuated Australian type.

Following the sharp outbreak there was a considerable fall in the rabbit population, and this was accentuated by subsequent systematic sampling for studies of reproductive physiology by one of us (W.E.P.). In August, 1955 it was impossible to obtain a large enough sample for a serological survey. In spite of the great fall in rabbit numbers only about half the survivors shot at the end of the epizootic had recovered from myxomatosis; the rest had escaped infection.

DISCUSSION

The decision to introduce the French strain of virus experimentally into Australian wild rabbit populations was based primarily on the stability of high virulence which had been observed in Europe at the time of initiation of the experiment (Fenner & Marshall, 1955; Jacotot, Vallée & Virat, 1955*a*). Since then several slightly, and some greatly attenuated, variants have been recovered in France and England (Fenner & Marshall, 1955, 1957; Hudson, Thompson & Mansi, 1955) so that the presumed advantage of the French strain over the standard laboratory strain may have been illusory. However, the experiments provided information on another interesting problem, namely, the possibility of introducing a strain of virus artificially into a rabbit population enzootically infected with another strain. The development of the intradermal screening test made it possible to determine the changing incidence of the enzootic attenuated strain and the virulent introduced strain.

The field situation was particularly satisfactory for this purpose both at Lake Urana and at Merricumbene, for an attenuated strain was found to be enzootic in each area just before the first artificial introduction of the French strain.

At Lake Urana a total of 18 cases were diagnosed as French type and 28 as

attenuated Australian. However, the great majority of the strains recovered in the early part of the epizootic were of the French type, and it can be calculated (Appendix) that by 21 December about 65 % of the initial population was killed by infection with the French type of virus (assuming that this had a case-mortality rate of about 99·5 %). The relatively small number of samples of virus recovered, and the approximate nature of the estimates of disease activity, preclude a more precise estimate of the relative importance of the French and Urana strains.

In spite of the initial high density of cases artificially and then naturally infected with the French strain of virus, the attenuated strain itself caused a minor epizootic and was the only virus recovered during the concluding stage of the outbreak. Although strains of virus recovered from the spontaneous outbreak at Lake Urana a year later were highly variable in their virulence, none resembled the virulent French strain. The symptomatology in a few of the rabbits infected with the very attenuated strains was rather like that occurring in animals infected with highly attenuated European strains, so that it is possible that a greatly attenuated derivative of the introduced French strain did persist at Lake Urana.

With the smaller rabbit and mosquito populations at Merricumbene, and the lower intensity of artificial inoculation, cases of myxomatosis infected with the French strain were less common than at Lake Urana, only three out of thirteen cases taken at the period of maximum incidence of new cases being due to the French strain. Any estimate of the relative importance of the French type and the local strain of virus at Merricumbene is guesswork. Since the French strain was recovered primarily during the early stages of the epizootic, and since the case mortality rate associated with infections due to the French strain was about 99·5 % and due to the local strain 70–80 % (Fenner & Marshall, 1957), it is possible that between one-third and one-half of the fatal cases at Merricumbene were due to the French strain. As at Lake Urana, all strains recovered after the peak of the epizootic were of the attenuated Australian type.

The results provide an experimental confirmation of the views expressed earlier (Fenner, Day & Woodroofe, 1956), in a consideration of the epidemiological consequences of the mechanical transfer of myxomatosis by mosquitoes. By providing a relatively large reservoir of animals infected with the French strain it proved possible to introduce this virulent strain into wild rabbit populations in which the local attenuated strain was circulating at a very low level of activity. The majority of rabbits were infected with the virulent French strain, and this probably accounts for the much greater population reduction after this epizootic compared with an outbreak associated with an equally intense infection rate during the previous season. Nevertheless, the enzootic attenuated strain was able to spread and finally become dominant. It was the dominant but not the only strain recognized in the outbreak which occurred spontaneously a year later, in October–November, 1955. The more prolonged survival of rabbits in a condition highly infectious for mosquitoes almost certainly accounts for the successful emergence of the attenuated Australian strain, in spite of the early preponderance of cases infected with the highly virulent French strain.

The practical importance of these experiments resides in the demonstration that it is possible to kill many rabbits by artificially introducing a highly virulent strain of virus into the population at about the time of the expected summer epizootic. It is probable that the results obtained with the French strain would be duplicated with the standard laboratory strain. If the attenuated strain actually died out in an area, as must frequently happen, the introduction of the virulent strain would be much more effective, although our experience so far shows that it would probably not survive unchanged until the next epizootic. The increase in innate resistance which has recently been demonstrated in Australian wild rabbits (Fenner & Marshall, unpublished) has been accelerated by the dominance of attenuated strains of virus, and can be retarded only by destruction by other methods of the survivors of epizootics, or by the activity on a large scale of more virulent strains of virus.

The decision as to whether the French strain or the standard laboratory strain is the better virulent strain for large-scale introductions in Australia depends upon factors not considered in this paper. The fact that one naturally occurring derivative of the French strain (England/Nottingham/4–55/1 (attenuated)) is much less lethal than the most attenuated natural variant of the standard laboratory strain (Fenner & Marshall, 1957) suggests that the present policy, i.e. use of the standard laboratory strain, should be maintained, and this opinion is reinforced by the suspicion that some of the highly attenuated strains recovered from Lake Urana in 1955–56 were derived from the French type of virus.

SUMMARY

The primary lesions produced in rabbits by the intradermal inoculation of the virulent French strain of myxoma virus were clearly distinguishable from those produced by the standard laboratory strain or by attenuated Australian field strains. Based on this fact a screening test was developed which allowed classification of large numbers of samples of virus into French or attenuated Australian types.

The virulent French type of virus was introduced into the wild rabbit population at Lake Urana by the inoculation of 242 rabbits over a period of 6 weeks, the attenuated Australian strain of virus having been recovered from a naturally infected rabbit at the beginning of the series of inoculations. A severe outbreak of myxomatosis occurred in which an estimated 70 % of cases were caused by the French strain of virus. In spite of the early predominance of cases due to this introduced strain the majority of samples obtained in the latter half of the outbreak were of the attenuated Australian type, and this was probably the only strain which survived through the following winter and caused an intense epizootic in the spring.

A similar picture of early establishment of the artificially introduced virulent French strain, and its subsequent replacement by the naturally occurring attenuated Australian strain, was seen at two other study sites, North Urana Station and Merricumbene.

We are greatly indebted to colleagues on the staff of the Wildlife Survey Section at Albury, especially Mr M. P. Hines, for assistance in the field work, and to Mr P. Bentley, the manager of Cocketgedong Station, on which the experimental site is situated. We have profited from numerous discussions with Mr F. N. Ratcliffe, and are grateful to Dr G. S. Watson for assistance in the statistical treatment of the results.

APPENDIX

Calculation of the proportion of rabbits killed by the French strain of myxoma virus at Lake Urana

The average survival time of rabbits infected with attenuated Australian strains was about 23 days, compared with 12 days in the case of the French strain. With both strains animals could be recognized as suffering from myxomatosis by the seventh or eighth day, so that the relative lengths of time during which rabbits suffered from clinically diagnosable infections were about 15 and 5 days. If selection of diseased rabbits was random, therefore, there was three times the opportunity of capturing a rabbit infected with the attenuated Australian strain compared with the French strain.

Making a correction for the greater chance of capturing a rabbit infected with the attenuated Australian strain, we obtain the following figures for the relative abundance of French (F) and Australian (A) strains: 27–29 November, $\infty:1$; 9 December, 6:1; 15 December, 5:1. Up to 15 December the epizootic was due, to all intents and purposes, wholly to French type virus.

Let $N(t)$ = total population size at time t,

$i(t)$ = number of rabbits infected per unit time,

S = average length of period when symptoms were apparent,

L = average length of latent period,

m = mortality rate for French strain ($\approx 99{\cdot}5\,\%$),

t = days from 1 November,

$f(t)$ = proportion observed with symptoms at time t.

Then (1) proportion of population with symptoms at time t

$$= \frac{1}{N(t)} \int_{t-L-S}^{t-L} i(t)\,dt.$$

$$\approx \frac{Si(t-L-\tfrac{1}{2}S)}{B(t)}$$

$$\approx \frac{Si(t-9)}{N(t)};$$

$$(2) \quad \frac{dN(t)}{dt} = -mi(t-9),$$

i.e. rate of decrease of population at time t = rate of fatal infection at time $t-9$.

Hence (1) and (2) give

$$\frac{d(N)\,t}{dt} + \frac{m}{S} f(t)\,N(t) = 0.$$

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Then $$N(t) = N(0) \exp\left(-\frac{m^t}{s_0} f(t)\,dt\right).$$

The area under the graph of $f(t)$ up to 15 December was found to be 10/2, i.e.

$$\frac{N(45)}{N(0)} = \exp\left(-\frac{0{\cdot}995}{6} \times 5\right) \approx \exp\left(-\tfrac{5}{6}\right) = 0{\cdot}44.$$

Thus by 15 December the French strain had actually killed 56 % of the initial population. Already infected with this strain and destined to die within 6 days of December 15 was the proportion $0{\cdot}44 \times 0{\cdot}25 \times 0{\cdot}83 \approx 0{\cdot}09$.

$0{\cdot}56 + 0{\cdot}09 = 0{\cdot}65$, i.e. by 21 December 65 % of the initial population would have died due to infection with the French strain of virus.

After this date the proportion of isolations of the French strain diminished greatly. As the sampling errors are large and virtually unknown it is reasonable to conclude that about 70 % of the initial rabbit population was killed by infection with the French strain of virus.

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EXPLANATION OF PLATE

The differentiation of Australian and European strains of myxoma virus by the intradermal inoculation of rabbits. The appearance of skin lesions 6 days after the inoculation of doses of about 100 rabbit-infectious doses of: (1) French type (France/Dordogne/11–53/1), (2) attenuated Australian (Aust./Urana/11–54/1), (3) prototype French type (Brazil/Campinas/1949/1 (Lausanne), and (4) the standard laboratory strain.

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