A COMPARISON OF THE VIRULENCE FOR EUROPEAN RABBITS (*ORYCTOLAGUS CUNICULUS*) OF STRAINS OF MYXOMA VIRUS RECOVERED IN THE FIELD IN AUSTRALIA, EUROPE AND AMERICA*

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INTRODUCTION

Virulence is one of the most loosely used words in microbiology (Dubos, 1945; Miles, 1955), hence it is necessary at the outset to define the term as it is used in this paper. Myxomatosis is one of the few natural infections in which lethality can be equated with virulence in both the field and the laboratory. Here we use the term virulence as a quantitative characteristic of myxoma virus strains, using a standardized dose and method of inoculation in standardized host animals. Under these conditions significant variations in the mortality rates occur with different strains of virus, and these are correlated with variations in the severity of the disease and the mean survival times of groups of rabbits. Such differences are also correlated with differences in the case-mortality rates in natural outbreaks.

Whether myxomatosis is used as a method of rabbit control, as in Australia, or whether its presence is regarded as a serious threat to the rabbit industry, as in

* Aided by grants from the Rural Credits Development Fund of the Commonwealth Bank of Australia.

parts of Europe, the most important character of the virus is its virulence, and the most important feature of the disease its case-mortality rate. Other writers (Berry, 1939; Smith, 1952), when considering the fibroma-myxoma group of viruses, have commented upon the wide range in virulence encountered when the whole group of related viruses is considered. In the present report attention will be confined to myxoma virus, and it will be shown that within the limits of this species virus strains occur which range in virulence from extremely high (almost always lethal) to very low (very rarely lethal).

Prior to 1953 five strains of myxoma virus of differing virulence had been described. Almost all laboratory work has been carried out with a South American strain recovered from a naturally infected *Oryctolagus* rabbit by Dr A. Moses of the Oswaldo Cruz Institute in Brazil (Moses, 1911), and sent by him to the Rockefeller Institute in 1924 (Rivers, 1930). It was subsequently sent from the Rockefeller Institute for Medical Research to England, and was there designated 'strain B' by Martin (1936). Later it was sent to Australia (Bull & Mules, 1944), where it was ultimately used to initiate the widespread epizootics which broke out in 1950. It has been maintained for many years by serial passage in laboratory rabbits. The sub-line of this virus maintained in our laboratories has been designated the standard laboratory strain. Martin (1936) also used a 'strain A' derived from dried material sent to him from South America by Aragão in 1934. This had not been subjected to serial passage in the laboratory, and in Martin's hands it was less virulent than strain B, the survival times of rabbits infected by contact being slightly longer and the case-mortality rate slightly lower.

Two greatly attenuated strains have been described, Hurst's 'neuromyxoma' (Hurst, 1937) and Berry's '80A mutant' (Berry, 1938). Neuromyxoma was recovered by serial intracerebral passage of the South American (Moses) strain in rabbits. Hurst reported that 16 out of 21 rabbits inoculated intradermally in a single area with a large dose of neuromyxoma virus recovered after a relatively mild illness. Rhodes (1938) observed changes in the histological picture after serial intracerebral inoculation of myxoma virus but no change in virulence. The '80A mutant' (Berry, 1938) was obtained during a transformation experiment in which the transforming agent had been heated to 80° C. for 30 min., conditions which usually inactivate its transforming capacity. It remained stable for 23 rabbit passages over a period of 13 months, and caused about a 15% case-mortality rate in laboratory rabbits. It is not now available. Haagen & Du (1938) reported that serial passage of the Moses strain on the chorioallantois of developing chick embryos resulted in an increase of its virulence for the chick embryo and a decrease in its virulence for rabbits. Lush (1937), on the other hand, found no change in virulence of the South American (Moses) strain after 26 passages on the chorioallantoic membrane.

The existence of strains of diminished virulence in wild rabbits in Australia was first reported by Mykytowycz (1953), and later by Myers, Marshall & Fenner (1954) and Marshall, Dyce, Poole & Fenner (1955). More recently, attenuated strains have been recovered from naturally infected wild rabbits in France and England (Jacotot, Vallée & Virat, 1955*b*; Hudson, Thompson & Mansi, 1955;

Fenner & Marshall, 1955). In the present paper additional evidence is presented on the distribution in space and time of attenuated strains in Australia, and comparisons are made with strains isolated from outbreaks in South and North America, France and England. A detailed description is given of the symptomatology associated with infections with several 'prototype' strains.

MATERIALS AND METHODS

Virus strains

The origins of the virus strains employed in the present investigation are set out in Table 1.

The following convention has been adopted in designating strains of virus recovered from the field—country of origin/locality/month–year/serial number. Laboratory strains, and a few field strains which have been extensively used, are sometimes described by a shorter name: thus Aust/Corowa/12–52/2 is the KM13 strain; and Aust/Uriarra/2–53/1 is the Uriarra strain.

Rabbits

Laboratory rabbits from the Australian National University Animal Breeding Establishment, and during 1952 and 1953 from the Walter and Eliza Hall Institute for Medical Research, and from the Baker Institute of Medical Research, were employed when they were about 4 months old and weighed $2\frac{1}{2}$–3 kg. During 1953 Australian wild rabbits were obtained from various parts of eastern Australia, principally from the Australian Capital Territory. Wild rabbits which had recovered from myxomatosis were excluded on the basis of serological tests. It has been found (Fenner & Marshall, unpublished observations) that a demonstrable increase in the genetic resistance of wild rabbits to infection with an attenuated strain of myxoma virus occurred within a few years in an area where there were annual severe outbreaks of the disease. The wild rabbits used in the present study had not been subjected to this degree of selection, but the widespread and variable occurrence of changes in the genetic resistance of Australian wild rabbits that has now occurred (1956) renders them unsuitable for further use as experimental animals for the assessment of the virulence of myxoma virus strains.

Rabbits were housed in individual cages in animal rooms which were heated in winter, and were fed on a diet of pellets and water, supplemented daily with green feed.

Virus titrations

Virus suspensions were titrated on the chorioallantoic membrane of 11- to 12-day-old chick embryos (Lush, 1937). It has been shown for six different strains of myxoma virus that the titre in rabbits inoculated intradermally (calculated as rabbit-infectious doses per ml.) was 2·5 times higher than the egg titre (pock-producing particles per ml.) (Fenner & McIntyre, 1956). Throughout this paper virus concentrations have been expressed as rabbit-infectious doses, using where necessary the conversion factor 2·5.

Table 1. *The origin of strains of myxoma virus examined and the results of virulence tests carried out by inoculating rabbits intradermally in one flank with about 5 rabbit-infectious doses of each strain*

Strain no. (A)	Source (B)	No. of laboratory passages (C)	Date of last introduction of virulent virus to district (D)	Rabbits inoculated: Type (E)	Rabbits inoculated: No. (F)	Rabbits inoculated: No. of survivors (G)	Range of survival times (days) (H)	Mean of transformed survival time ± S.E. (I)	Mean survival time in days and its 95 % fiducial range (J)
				LABORATORY STRAINS					
1	Standard laboratory strain. For history see text. Used to initiate Australian myxomatosis outbreaks	Numerous	—	Laboratory	43	0	8–15	0·45 ± 0·03	10·5 **10·8** 11·2
				Wild	15	0	10–14	0·56 ± 0·03	11·2 **11·6** 12·2
2	Batch no. 30 (1953) of freeze dried virus prepared from seed standard laboratory strain by Commonwealth Serum Laboratories for distribution in the field (Fenner & Woodroofe, 1954)	Numerous	—	Laboratory	5	0	10–14	0·53 ± 0·08	10·3 **11·4** 12·9
3	Freeze dried material prepared from standard laboratory strain by the Institute of Medical and Veterinary Science, Adelaide, for field distribution in South Australia (D. Surrey Dane)	Numerous	—	Laboratory	5	0	9–13	0·52 ± 0·13	9·8 **11·3** 14·0
4	Same as standard laboratory strain, but maintained at Rockefeller Institute, New York. Received in Australia 24 Sept. 1951 (R. E. Shope)	Numerous	—	Laboratory	9	0	10–12	0·41 ± 0·04	10·1 **10·5** 11·1
5	Aust/Dubbo/2–51/1, originally obtained from naturally infected wild rabbit near Dubbo, N.S.W., shortly after the commencement of the first Australian epizootic. Maintained by rabbit passage at the Veterinary Research Station, Glenfield, for preparation of dried material for field distribution in N.S.W. (G. Edgar)	35	—	Laboratory	5	0	9–12	0·34 ± 0·10	9·4 **10·2** 11·5
6	Neuromyxoma. Derived from 8th serial passage of standard laboratory strain by intracerebral inoculation of rabbits (Hurst, 1937)	Numerous	—	Laboratory	22	22	—	—	—
7	Brazil/Campinas/1949/1 (Lausanne). For history, see text. Used to initiate European myxomatosis outbreaks (G. Bouvier)	5	—	Laboratory	5	0	11–16	0·69 ± 0·07	11·5 **12·9** 14·8

FIELD STRAINS FROM AUSTRALIA

(*a*) Queensland

8	Aust/Texas/5–52/1, from diseased rabbit (Marshall, Dyce, Poole & Fenner, 1955)	1	Dec. 1951	Laboratory	4	0	12–21	0·83 ± 0·11	12·1	**14·8**	19·2
				Wild	4	0	12–16	0·75 ± 0·06	12·3	**13·6**	15·4
9	Aust/Texas/5–52/2, from diseased rabbit (Marshall *et al.* 1955)	1	Dec. 1951	Laboratory	6	0	11–17	0·69 ± 0·06	11·7	**12·9**	14·5
				Wild	4	0	10–13	0·44 ± 0·09	9·8	**10·8**	12·2
10	Aust/Texas/8–52/1, from diseased rabbit (Marshall *et al.* 1955)	1	Dec. 1951	Laboratory	5	1	12–S	1·24 ± 0·21	14·6	**25·2**	53·7
				Wild	4	0	11–16	0·96 ± 0·16	12·4	**17·1**	27·1
11	Aust/Texas/11–52/1, from diseased rabbit (Marshall *et al.* 1955)	1	Nov. 1952	Laboratory	4	0	16–19	0·99 ± 0·03	16·5	**17·8**	19·2
				Wild	4	0	11–25	0·96 ± 0·17	12·2	**17·1**	28·0
12	Aust/Texas/4–53/1, from diseased rabbit (Marshall *et al.* 1955)	0	Nov. 1952	Laboratory	4	0	19–54	1·29 ± 0·13	18·7	**27·5**	43·5
				Wild	4	0	14–35	1·07 ± 0·16	13·6	**19·7**	32·5
13	Aust/Texas/4–53/2, from diseased rabbit (Marshall *et al.* 1955)	0	Nov. 1952	Laboratory	5	0	15–27	1·04 ± 0·09	15·2	**19·0**	24·6
				Wild	3	0	23–34	1·28 ± 0·07	21·8	**27·1**	34·9

(*b*) New South Wales

14	Aust/Bathurst/2–51/1, from diseased rabbit. Natural spread from initial escape on Murray River, Dec. 1950 (Ratcliffe, Myers, Fennessy & Calaby, 1952)	1	—	Laboratory	5	0	10–13	0·54 ± 0·07	10·5	**11·5**	12·8
15	Aust/Dunroy/5–52/1, from diseased rabbit (Marshall *et al.* 1955)	1	Nov. 1951	Laboratory	8	1	13–S*	1·11 ± 0·13	15·1	**21·0**	31·8
				Wild	3	0	20–31	1·18 ± 0·09	18·0	**23·1**	30·9
16	Aust/Dunroy/12–52/1, from diseased rabbit (Marshall *et al.* 1955)	1	Nov. 1951	Laboratory	10	0	12–28	1·02 ± 0·12	14·0	**18·5**	26·2
				Wild	4	0	12–23	0·77 ± 0·14	11·1	**13·9**	19·2
17	Aust/Urana/11–52/1, from pool of *Anopheles annulipes* (Myers, Marshall & Fenner, 1954)	1	Oct. 1952	Laboratory	6	0	15–23	1·01 ± 0·05	16·1	**18·2**	20·9
				Wild	2	0	16, 25	1·07		—	
18	Aust/Urana/11–52/2, from pool of *Anopheles annulipes* (Myers *et al.* 1954)	1	Oct. 1952	Laboratory	5	0	18–21	1·08 ± 0·03	18·5	**20·0**	21·8
19	Aust/Urana/11–52/3, from pool of *Anopheles annulipes* (Myers *et al.* 1954)	1	Oct. 1952	Laboratory	5	0	13–25	0·98 ± 0·09	14·3	**17·5**	22·5
20	Aust/Urana/11–52/4, from pool of *Anopheles annulipes* (Myers *et al.* 1954)	1	Oct. 1952	Laboratory	8	0	12–45	1·06 ± 0·12	14·6	**19·5**	27·9
				Wild	3	0	15–19	0·93 ± 0·06	14·5	**16·5**	19·2
21	Aust/Urana/11–52/5, from pool of *Culex annulirostris* (Myers *et al.* 1954)	1	Oct. 1952	Laboratory	6	1	13–S	1·27 ± 0·17	16·5	**26·8**	49·3
				Wild	3	0	14–27	1·09 ± 0·16	13·9	**20·3**	33·7
22	Aust/Urana/11–52/6, from pool of *Aedes theobaldi* (Myers *et al.* 1954)	1	Oct. 1952	Laboratory	6	0	10–21	0·86 ± 0·13	12·0	**15·2**	21·2

* S = Rabbit recovered from infection.

Table 1 (*continued*)

Strain no. (A)	Source (B)	No. of laboratory passages (C)	Date of last introduction of virulent virus to district (D)	Rabbits inoculated: Type (E)	No. (F)	No. of survivors (G)	Range of survival times (days) (H)	Mean of transformed survival time ± S.E. (I)	Mean survival time in days and its 95 % fiducial range (J)
23	Aust/Urana/12–52/1, from pool of *Anopheles annulipes* (Myers *et al.* 1954)	1	Oct. 1952	Laboratory	6	0	15–28	1·09 ± 0·06	17·3 **20·3** 24·2
				Wild	2	0	14, 18	0·89	—
24	Aust/Urana/12–52/2, from pool of *Anopheles annulipes* (Myers *et al.* 1954)	1	Oct. 1952	Laboratory	6	0	13–20	0·87 ± 0·06	13·6 **15·4** 16·8
				Wild	2	0	12, 14	0·69	—
25	Aust/Urana/4–54/1, from diseased rabbit during autumn outbreak (W. E. Poole)	1	Oct. 1952	Laboratory	5	0	16–28	1·07 ± 0·09	15·8 **19·8** 25·8
26	Aust/Urana/11–54/1, from diseased rabbit before major epizootic (Fenner, Poole, Marshall & Dyce, 1957)	0	Oct. 1952	Laboratory	5	0	16–20	1·03 ± 0·07	15·8 **18·8** 22·8
27	Aust/Urana/11–54/2, from diseased rabbit during major epizootic (Fenner *et al.* 1957)	0	Nov. 1954 (French)	Laboratory	5	0	11–13	0·62 ± 0·04	11·5 **12·1** 13·0
28	Aust/Urana/1–55/1, from diseased rabbit at conclusion of major epizootic (Fenner *et al.* 1957)	0	Nov. 1954 (French)	Laboratory	5	0	15–27	0·95 ± 0·09	13·9 **17·0** 21·5
29	Aust/Corowa/12–52/1, from pool of *Anopheles annulipes* (Myers *et al.* 1954)	1	Dec. 1950	Laboratory	6	1	11–S	0·97 ± 0·19	12·0 **17·4** 30·3
				Wild	2	0	18, 25	1·12	—
30	Aust/Corowa/12–52/2 (KM 13), from pool of *Anopheles annulipes* (Myers *et al.* 1954)	1	Dec. 1950	Laboratory	77	9	13–S	1·13 ± 0·03	19·7 **21·5** 23·5
				Wild	58	7	13–S	1·24 ± 0·03	23·2 **25·4** 27·9
31	Aust/Corowa/12–52/3, from pool of *Anopheles annulipes* (Myers *et al.* 1954)	1	Dec. 1950	Laboratory	6	0	10–19	0·66 ± 0·13	10·5 **12·6** 16·3
				Wild	2	0	15, 24	1·02	—
32	Aust/Corowa/12–52/4, from pool of *Anopheles annulipes* (Myers *et al.* 1954)	1	Dec. 1950	Laboratory	5	1	15–S	1·14 ± 0·11	16·6 **21·9** 30·6
33	Aust/Corowa/2–54/1, from diseased rabbit (W. E. Poole)	0	Dec. 1950	Laboratory	5	0	15–39	1·12 ± 0·11	15·9 **21·2** 29·9
34	Aust/Corowa/3–55/1, from diseased rabbit (W. E. Poole)	0	Dec. 1954	Laboratory	5	0	13–25	1·04 ± 0·08	15·6 **19·1** 23·8
35	Aust/Colo Vale/3–53/1, from pool of *Aedes alboannulatus* and *Aedes queenslandis* (A. L. Dyce)	1	Dec. 1952	Wild	5	0	16–28	1·05 ± 0·07	16·1 **19·2** 23·5
36	Aust/Colo Vale/2–55/1, from diseased rabbit (A. L. Dyce)	0	Dec. 1952	Laboratory	5	0	13–20	0·95 ± 0·07	14·5 **17·0** 20·3

37	Aust/Colo Vale/4–55/1, from diseased rabbit (A. L. Dyce)	0	Dec. 1952	Laboratory	5	1	16–S	1·34 ± 0·15	19·1	**29·9**	51·5
38	Aust/Sydney/3–53/1, from naturally infected laboratory rabbit, Royal North Shore Hospital	2	—	Laboratory	3	0	15–20	0·94 ± 0·07	14·3	**16·7**	20·0
				Wild	3	0	11–26	0·88 ± 0·22	10·8	**15·6**	28·9
39	Aust/Merricumbene/11–54/1, from diseased rabbit (Fenner *et al.* 1957)	0	—	Laboratory	5	1	22–S	1·31 ± 0·15	18·1	**28·3**	48·6
40	Aust/Merricumbene/3–55/1, from diseased rabbit (Fenner *et al.* 1957)	0	Nov. 1954 (French)	Laboratory	5	3	17–S	—		—	
41	Aust/Merricumbene/3–55/2, from diseased rabbit (Fenner *et al.* 1957)	1	Nov. 1954 (French)	Laboratory	5	0	10–12	0·46 ± 0·06	10·2	**11·0**	11·8
			(*c*) Australian Capital Territory								
42	Aust/Uriarra/2–53/1, from diseased rabbit (R. Mykytowycz, 1953)	3	Dec. 1952	Laboratory	45	19	11–S	1·26 ± 0·04	23·1	**26·2**	29·9
				Wild	26	9	12–S	1·42 ± 0·07	27·0	**34·6**	44·3
43	Aust/Uriarra/8–54/1, from diseased rabbit (R. Mykytowycz)	0	Dec. 1952	Laboratory	5	0	13–19	0·82 ± 0·06	13·0	**14·5**	15·7
44	Aust/Uriarra/1–55/1, from diseased rabbit (A. L. Dyce)	0	Dec. 1952	Laboratory	5	0	14–19	0·90 ± 0·04	14·6	**16·0**	17·5
45	Aust/Canberra/2–53/1, from diseased rabbit (R. Mykytowycz)	1	Jan. 1953	Laboratory	5	1	11–S	0·77 ± 0·11	11·6	**13·9**	17·9
46	Aust/Angle Bend/6–53/1, from diseased rabbit (R. Mykytowycz)	4	Nov. 1952	Laboratory	9	4	18–S	1·34 ± 0·07	26·4	**29·8**	33·7
			(*d*) Victoria								
47	Aust/Pyramid/4–52/1, from diseased rabbit (Fenner, Marshall & Woodroofe, 1953)	1	Nov. 1951	Wild	5	0	12–19	0·79 ± 0·09	12·1	**14·2**	17·3
48	Aust/Pyramid/4–52/2, from diseased rabbit (Fenner *et al.* 1953)	1	Nov. 1951	Laboratory	5	0	10–14	0·48 ± 0·11	9·8	**11·0**	13·0
49	Aust/Yarram/11–53/1, from diseased rabbit (G. Douglas)	1	Jan. 1953	Laboratory	5	2	15–S	1·43 ± 0·20	18·5	**35·0**	77·1
50	Aust/Piangil/2–54/1, from diseased rabbit (G. Douglas)	0	Jan. 1954	Wild	5	0	19–26	1·13 ± 0·04	19·2	**21·5**	24·2
51	Aust/Piangil/2–54/2, from diseased rabbit (G. Douglas)	0	Jan. 1954	Wild	6	0	13–26	1·04 ± 0·08	16·3	**19·0**	22·5
52	Aust/Werrimal/2–54/1, from diseased rabbit (G. Douglas)	0	Jan. 1954	Wild	5	1	13–S	1·18 ± 0·16	15·3	**23·0**	39·1

Table 1 (*continued*)

Strain no. (A)	Source (B)	No. of laboratory passages (C)	Date of last introduction of virulent virus to district (D)	Rabbits inoculated: Type (E)	No. (F)	No. of survivors (G)	Range of survival times (days) (H)	Mean of transformed survival time ± S.E. (I)	Mean survival time in days and its 95% fiducial range (J)
53	Aust/Werrimal/2–54/2, from diseased rabbit (G. Douglas)	0	Jan. 1954	Laboratory	10	0	11–15	0·65 ± 0·03	11·9 **12·5** 13·1
54	Aust/Ouyen/3–55/1, from diseased rabbit (G. Douglas)	1	Nov. 1954	Laboratory	5	0	15–38	1·25 ± 0·12	18·2 **25·8** 38·9
55	Aust/Bacchus Marsh/8–54/1, from diseased rabbit (G. Douglas)	0	Mar. 1954	Laboratory	5	0	11–19	0·68 ± 0·10	11·0 **12·8** 15·6
56	Aust/Bacchus Marsh/1–55/1, from diseased rabbit (T. Pearce)	0	Mar. 1954	Laboratory	5	1	16–S	1·35 ± 0·15	19·2 **30·6** 53·9
57	Aust/Gunbower/3–52/1, from diseased rabbit (Fenner *et al.* 1953)	1	Nov. 1951	Laboratory	5	0	12–20	0·81 ± 0·09	12·3 **14·4** 17·8
58	Aust/Gunbower/1–53/1, from diseased rabbit (B. V. Fennessy)	1	Nov. 1951	Laboratory	5	2	16–S	1·10 ± 0·08	16·5 **20·5** 26·5
59	Aust/Gunbower/3–54/1, from diseased rabbit (B. V. Fennessy)	0	Dec. 1953	Laboratory	5	0	12–23	0·80 ± 0·12	11·6 **14·3** 20·0
60	Aust/Nhill/6–55/1, from diseased rabbit	0	Dec. 1954	Laboratory	5	0	13–21	0·94 ± 0·07	14·3 **16·7** 20·0
			(*e*) South Australia						
61	Aust/Peake/10–53/1, from diseased rabbit (E. Waterhouse)	1	Dec. 1951	Laboratory	5	0	13–27	1·06 ± 0·10	15·2 **19·5** 26·2
62	Aust/Dismal Swamp/2–54/1, from diseased rabbit (E. W. Lines)	1	Dec. 1953	Laboratory	5	0	11–18	0·73 ± 0·11	11·2 **13·3** 16·9
63	Aust/Robe/2–54/1, from diseased rabbit (E. W. Lines)	1	Dec. 1953	Laboratory	5	0	13–20	0·83 ± 0·08	12·7 **14·8** 17·8
64	Aust/Clare/3–55/1, from diseased rabbit (D. Surrey Dane)	2	—	Laboratory	5	1	22–S	1·31 ± 0·05	23·8 **28·2** 33·8
			(*f*) Western Australia						
65	Aust/East Narrogin/3–54/1, from diseased rabbit (D. Gooding)	1	Jan. 1952	Laboratory	5	0	12–15	0·72 ± 0·04	12·4 **13·2** 14·3

			(*g*) Tasmania								
66	Aust/Evandale/9–54/1, from diseased rabbit (T. M. Alexander)	0	Sept. 1953	Laboratory	5	0	12–17	0·73 ± 0·04	12·5	**13·3**	14·5
67	Aust/Colebrook/9–54/1, from diseased rabbit (A. F. Ryan)	0	—	Laboratory	5	0	21–27	1·17 ± 0·03	20·9	**22·7**	25·0
68	Aust/Cressy/10–54/1, from diseased rabbit (A. F. Ryan)	0	—	Laboratory	5	0	19–28	1·19 ± 0·04	20·9	**23·5**	26·6
			Field strains from Europe								
			(*a*) France								
69	France/Dordogne/11–53/1, from diseased rabbit (P. Lépine)	4	—	Laboratory	5	0	12–15	0·69 ± 0·05	11·9	**12·9**	14·2
70	France/Loir et Cher/11–53/1, from diseased rabbit (P. Lépine)	4	—	Laboratory	5	0	11–12	0·58 ± 0·02	11·5	**11·8**	12·2
71	France/Vienne/4–54/1, from diseased rabbit (P. Lépine)	1	—	Laboratory	5	0	11–16	0·69 ± 0·07	11·5	**12·9**	14·9
72	France/Indre/4–54/1, from diseased rabbit (P. Lépine)	1	—	Laboratory	5	0	11–12	0·55 ± 0·03	11·1	**11·5**	12·1
73	France/Eure/4–54/1, from diseased rabbit (P. Lépine)	1	—	Wild	5	0	12–14	0·68 ± 0·03	12·2	**12·8**	13·5
74	France/Seine Inférieure/4–54/1, from diseased rabbit (P. Lépine)	1	—	Wild	5	0	12–14	0·68 ± 0·03	12·2	**12·8**	13·5
75	France/Loiret/4–54/1, from naturally infected domestic rabbit (H. Jacotot)	1	—	Laboratory	5	0	11–14	0·59 ± 0·06	11·0	**11·9**	13·1
76	France/Loiret/4–55/1, from naturally infected domestic rabbit (H. Jacotot)	1	—	Laboratory	29	10	19–S	1·40 ± 0·04	29·2	**33·1**	37·6
77	France/Sologne/8–55/1, from diseased rabbit (H. Jacotot)	4	—	Laboratory	5	1	13–S	1·39 ± 0·23	16·6	**32·5**	76·3
			(*b*) United Kingdom								
78	England/Kent/10–53/1, from diseased rabbit during first confirmed outbreak in England (J. R. Hudson)	1	—	Laboratory	5	0	10–13	0·51 ± 0·07	10·3	**11·2**	12·5
79	England/Cornwall/4–54/1, from diseased rabbit (J. R. Hudson)	Several	—	Laboratory	5	0	12–14	0·66 ± 0·11	10·8	**12·5**	15·6
80	England/Sussex/9–54/1 (MXE 46), from diseased rabbit (J. R. Hudson)	0	—	Laboratory	10	2	18–S	1·13 ± 0·04	19·5	**21·5**	23·8
81	England/Sussex/10–54/1, from mosquito pool (*Anopheles maculipennis atroparvus*) (Andrewes, Muirhead-Thomson & Stevenson, 1956)	—	—	Laboratory	5	0	18–23	1·06 ± 0·03	18·0	**19·5**	21·2

Table 1 (*continued*)

Strain no. (A)	Source (B)	No. of laboratory passages (C)	Date of last introduction of virulent virus to district (D)	Rabbits inoculated: Type (E)	No. (F)	No. of survivors (G)	Range of survival times (days) (H)	Mean of transformed survival time ± S.E. (I)	Mean survival time in days and its 95% fiducial range (J)		
82	England/Surrey/12–54/1, from diseased rabbit (J. R. Hudson)	0	—	Laboratory	5	0	12–13	0·66 ± 0·02	12·2	**12·6**	13·0
83	Ireland/County of Armagh/1–55/1, from diseased mountain hare (*Lepus timidus*) (J. R. Hudson)	1	—	Laboratory	5	0	11–14	0·54 ± 0·06	10·6	**11·4**	12·6
84	England/Suffolk/3–55/1 (MXE 134), from diseased rabbit (J. R. Hudson)	1	—	Laboratory	5	0	10–13	0·58 ± 0·02	11·5	**11·8**	12·2
85	England/Nottingham/4–55/1 (MXE 155), from diseased rabbit (J. R. Hudson)	1	—	Laboratory	10	6	11–S	See Table 9 and text			
86	England/Nottingham/4–55/1, attenuated. Derived from 85 (see text)	2	—	Laboratory	39	30	14–S	2·05 ± 0·09	82·8	**121·4**	179·8
87	England/Nottingham/4–55/1, virulent. Derived from 85 (see text)	2	—	Laboratory	11	0	11–14	0·62 ± 0·02	11·8	**12·2**	12·7
		STRAINS FROM SOUTH AMERICA									
88	Uruguay/Department of Colonia/1947/1, from naturally infected domestic rabbit (*Oryctolagus cuniculus*). (B. Szyfres, see Epstein, Reissig & de Robertis, 1952)	44	—	Laboratory	5	0	9–12	0·34 ± 0·06	9·7	**10·2**	10·9
89	Brazil/Campo Grande, Rio de Janeiro/12–53/1, from naturally infected domestic rabbit (*Oryctolagus cuniculus*). Probably infected directly from *Sylvilagus* rabbit (H. de B. Aragão)	3	—	Laboratory	5	0	9–12	0·30 ± 0·05	9·6	**10·0**	10·5
90	Brazil/Jacarépaguá, Rio de Janeiro/4–54/1, from naturally infected domestic rabbit (*Oryctolagus cuniculus*) (H. de B. Aragão)	4	—	Laboratory	5	0	12–14	0·66 ± 0·01	12·4	**12·5**	12·9
		STRAINS FROM NORTH AMERICA									
91	U.S.A./San Francisco/1950/1 (MSW), from naturally infected domestic rabbit (D. G. McKercher)	8	—	Laboratory	16	0	7–14	0·09 ± 0·10	8·8	**9·2**	10·0
92	U.S.A./San Diego/1949/1 (MSD), from naturally infected domestic rabbit (D. G. McKercher)	5 (rabbit) 93 (eggs) 2 (rabbit)	—	Laboratory	15	0	10–22	0·67 ± 0·08	11·2	**12·6**	14·8

S = Rabbit recovered from infection.

Preparation of virus strains for virulence tests

The material from which the different strains of virus were recovered included collections of wild caught mosquitoes, lesion material from diseased rabbits, and freeze-dried or glycerolated preparations. Prior to the virulence tests many specimens were passed at least once in laboratory rabbits, by intradermal inoculation. The subcutaneous tissue of the local lesion was removed aseptically about a week later and ground with sand in a cold mortar and pestle, suspended in gelatin saline containing antibiotics, and distributed in several 1 ml. glass ampoules. These were stored at $-70°$ C., and later one ampoule from each group was thawed and titrated on the chorioallantoic membrane. Other ampoules of the same batch were assumed to have the same titre. The dilution required for inoculation of rabbits was calculated from this result, the aim being to inoculate about 5 rabbit-infectious doses of virus. Where known, the number of passages in laboratory rabbits after recovery of the virus from a naturally infected animal is shown in Table 1.

Statistical methods

The principal criterion adopted for differentiation of the virulence of different strains of virus was the survival time of rabbits infected in a standard manner. Since the distribution of survival times is not normal, and their variances are not homogeneous, a normalizing and scedasticity transformation of the data is required prior to applying standard statistical tests. In practice a logarithmic transformation was found to be best suited to this purpose, and the data were treated in accordance with the expression $y = \log (U - U_0)$. Using doses of the size employed in these experiments we have never observed the death of an adult rabbit from typical uncomplicated myxomatosis in less than 8 days. This period was therefore taken as U_0 and subtracted from the observed survival time (U). If some animals recovered from the challenge infection, the data were treated by the method of Sampford (1954, paragraph 3·4). The number of rabbits in standard groups (five) is very small for the application of Sampford's method, and if more than two animals survived in a group of five it was impossible to use the survival time as a method of comparison. However, this result occurred very rarely, and in these cases the virulence of the virus could be satisfactorily classified on the basis of the severity of the clinical symptoms, and the fact that several rabbits recovered from the infection.

Calculations of the means and variance, and comparisons of different groups, were made with the transformed figures. In most tables the parameters have also been expressed in terms of the original data.

EXPERIMENTAL RESULTS

Choice of dosage and route of inoculation

Field observations at Lake Urana showed that under natural conditions the standard laboratory strain of myxoma virus, spreading among Australian wild rabbits not previously exposed to myxomatosis, caused epizootics in which the

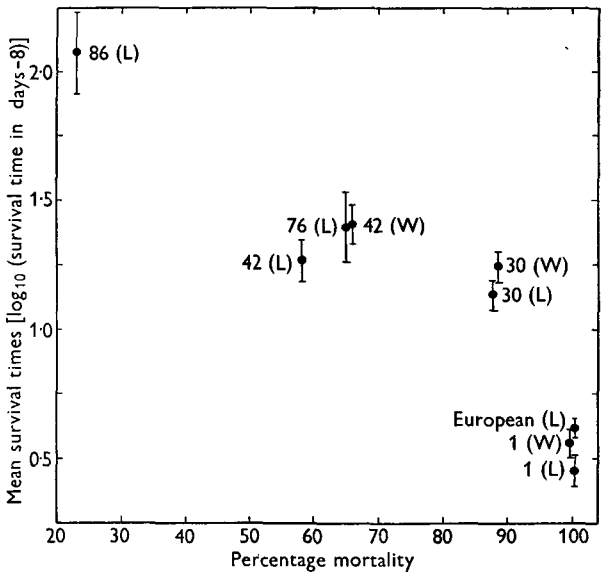
case-mortality rate exceeded 99·5 %. Strains recovered from the field a year later were associated with epizootics in which the case-mortality rate was about 90 % (Myers, *et al.*, 1954). Assuming that this difference was due solely to a reduction in the virulence of the virus strains involved in the two epizootics, the problem arose of defining such small fluctuations in virulence.

An essential requirement was to simulate as closely as possible the natural means of infection which obtained during Australian outbreaks, i.e., mechanical transmission by mosquito biting (Fenner, Day & Woodroofe, 1952). It has been shown (Fenner, Day & Woodroofe, 1956) that the dose inoculated in this way is quite small (usually between 1 and 10 rabbit-infectious particles) and this dose is probably wiped off on the epidermal cells rather than inoculated intravenously (as viruses carried in the salivary glands might be injected). For these reasons the standard means of infection adopted consisted of the intradermal inoculation into one flank of 0·1 ml. of a preparation which was estimated to contain about 5 rabbit-infectious doses of virus, the titre being checked by titration on the chorioallantois at the time of inoculation.

The limited numbers of rabbits available precluded the use of a test which could detect significant differences in the mortality rate with strains of virus which produced 90 and 99·5 % case-mortality rates. At the 5 % level of significance one would need to employ 45 rabbits in each group to detect such a difference. The first tests carried out with field strains and the standard laboratory strain, in groups of five rabbits, showed that although the disease was almost always fatal, reproducible differences occurred in the progress of the symptoms and in the survival times of the rabbits. It is on these differences that reliance has been placed in assessing the virulence of the strains tested. The most objective single criterion was the survival time.

Extensive series of inoculated rabbits are available for six strains: the standard laboratory strain, Aust/Corowa/12–52/2 (KM13), Aust/Uriarra/2–53/1 (Uriarra), France/Loiret/4–55/1 (Loiret 55), England/Nottingham/4–55/1 (attenuated), and Brazil/Campinas/1949/1 (Lausanne) and its virulent field derivatives from Europe; and examination of these series allows a decision to be made about the validity of using the survival time as an index of the virulence expressed as case-mortality rate. The relevant data were extracted from Table 1, and are shown in Text-fig. 1.

There is clearly a correlation between mortality rate and survival time, but the available results do not justify an attempt to fit a curve to the points in Text-fig. 1. The main problem lay in differentiating strains in the 80–100 % mortality-rate range and here the use of transformed survival times allowed ready differentiation of the virulence (killing capacity) of different strains. As the number of survivors increased to 50 %, or thereabouts, the mean survival time became less useful, but the frequent occurrence of two or more survivors in small groups of rabbits, and clear-cut differences in symptomatology, allowed the mortality rate and symptomatology to be used rather than survival time to characterize these more attenuated strains.



Text-fig. 1. The relationship between mean survival times and mortality rates with several strains of myxoma virus of widely differing virulence. Transformed means shown with ± twice the standard error. W = wild rabbits used for test. L = laboratory rabbits used for test. Data from Table 1. 1 = standard laboratory strain. European = composite group of all highly virulent European strains. 30 = Aust/Corowa/12–52/2 (KM13). 42 = Aust/Uriarra/2–53/1 (Uriarra). 76 = France/Loiret/4–55/1 (Loiret 55). 86 = England/Nottingham/4–55/1 (attenuated).

Effects of varying the dosage and route of inoculation

The standard method of inoculation, viz., intradermal inoculation of a small dose (5 rabbit-infectious units), was adopted so that the method of natural infection would be closely imitated. Experiments reported by Mykytowycz (1956) showed that the severity of the disease and the mortality rate in wild rabbits

Table 2. *The mortality rates of wild rabbits infected with the Aust/Uriarra/2–53/1 (Uriarra) strain of myxoma virus by various routes of inoculation*

(Data from Mykytowycz, 1956.)

Method of inoculation	No. of rabbits	Percentage mortality
Intradermal	26	65
Mosquito bite	45	69
Intramuscular	5	0
Contact infection from intradermally inoculated rabbits	101	19

infected with the Uriarra strain by mosquito bite, or by the intradermal inoculation of small doses of virus, were very similar, whereas wide differences occurred in animals infected by other routes (Table 2). Experiments with more virulent strains confirmed the differences due to route of inoculation (Table 3). Using the KM13 and standard laboratory strains inoculated intradermally in small or large

doses, the differences in mean survival times were found to be small (Table 4), but the rate of progression of symptoms was much more rapid in the animals inoculated with the large doses. In all subsequent experiments rabbits were inoculated intradermally in one flank with 0·1 ml. of saline containing about 5 rabbit-infectious doses of virus.

Table 3. *Survival times of laboratory rabbits inoculated simultaneously by various routes with the same small dose of the Aust/Corowa/12–52/2* (KM13) *strain of myxoma virus*

Route	Survival times (days)	Mean ± S.E.*	Mean survival time in days and its 95% fiducial range
Intradermal	12, 14, 15, 15, 16, 16, 17, 17, 18, 19, 21, 23, 24, 30, 32	1·003 ± 0·06	15·6 **18·07** 21·3
Subcutaneous	12, 23, 24, 25, 27	1·098 ± 0·12	15·2 **20·53** 29·8
Intramuscular	16, 22, 23, 29, 29, 38, S,† S, S, S	1·389 ± 0·09	24·4 **32·5** 44·6
Intravenous	17, N.I.,‡ N.I., N.I., N.I.	—	—

* Expressed as $\log_{10}$ (survival time in days − 8).
† S = rabbit recovered from infection.
‡ N.I. = rabbit not infected.

Table 4. *The survival times of groups of rabbits inoculated with small and large doses of two strains of myxoma virus*

Strain of virus	Dose (rabbit-infectious doses)	Survival times (days)	Mean ± S.E.*
Standard laboratory strain	10	11, 12, 12, 13	0·60 ± 0·05
	10^5	9, 9, 9, 9, 10	0·06 ± 0·06
Aust/Corowa/12–52/2 (KM13)	5	14, 15, 15, 16, 16, 19, 21, 24, 30, 32	1·04 ± 0·07
	10^5	13, 14, 16, 16, 29	0·92 ± 0·11

* Expressed as $\log_{10}$ (survival time − 8 days).

The age and physical condition of the rabbits

Our experience accords with that of Houlihan & Derrick (1945) that rabbits in poor physical condition or suffering from other infections respond in an abnormal way to the inoculation of myxoma virus. Such animals were therefore not used in the virulence tests

The survival times of young rabbits infected with the standard laboratory strain by mosquito bite or by intradermal inoculation are much shorter than those of adult animals, mean values being 5·4 days at the age of 9–11 days, 6·0 days at the age of 21–27 days, and 7·3 days at the age of 47 days, compared with 10·8 days in adult rabbits (Fenner & Marshall, 1954). To avoid this age effect no rabbit was used in the virulence tests until it was at least 4 months old, and the great majority of animals were between 4 and 5 months old when inoculated.

Comparability of results of different tests

Seasonal effects. Experiments by Mykytowycz (1956) showed that among susceptible wild rabbits maintained in cages in unheated animal quarters in Canberra there was a seasonal variation in the mortality rate when the animals were infected with the attenuated Uriarra strain of myxoma virus, either by contact or by mosquito bite, many more rabbits dying during the winter months. Sobey (unpublished observations) has also observed a higher mortality rate during winter in experiments in unheated animal houses in Sydney, using the KM13 strain of virus. All animals used in the experiments described in this paper were kept in heated quarters in the winter. With every batch of virus strains tested a control group of rabbits was inoculated with the standard laboratory strain of virus. The dates of the inoculations and the survival times of rabbits inoculated with the standard laboratory strain are shown in Table 5. An analysis of variance

Table 5. *Dates of inoculation and survival times of rabbits inoculated with about 5 rabbit-infectious doses of the standard laboratory strain of myxoma virus*

Date	Survival time (days)	Mean survival time* ± S.E.
	Laboratory rabbits	
24. iv. 53	10, 10, 10, 11	0·345 ± 0·04
24. xi. 53	8, 10, 12, 13, 15	0·489 ± 0·15
26. i. 54	10, 10, 11, 12, 13	0·476 ± 0·08
15. iv. 54	10, 12, 12, 13	0·551 ± 0·09
26. vii. 54	10, 10, 10, 10, 11	0·336 ± 0·03
31. viii. 54	10, 10, 11, 11, 12	0·432 ± 0·06
25. i. 55	10, 11, 12, 12, 13	0·536 ± 0·07
22. vi. 55	11, 12, 12, 12, 13	0·596 ± 0·04
2. ix. 55	9, 10, 10, 10, 13	0·320 ± 0·11
	Wild rabbits	
26. vi. 53	12, 12, 12, 12	0·602 ± 0·00
2. ix. 53	11, 12, 12	0·560 ± 0·04
9. x. 53	10, 10, 12	0·401 ± 0·10
22. vi. 53	11, 11, 12, 13, 14	0·606 ± 0·06

* Expressed as $\log_{10}$ (survival time in days − 8).

was carried out on the transformed data, with the results shown in Table 6 (A and B). No significant differences in survival times were found, either amongst the wild or the laboratory rabbits, due to the time of the year at which the tests were made. The results of experiments conducted at different times during the last three years are directly comparable.

Comparison of the response of laboratory and wild rabbits

Since seasonal effects can be disregarded, a comparison of the results obtained with laboratory and wild rabbits is possible. As shown in Table 6C there was a possibly significant longer survival time among the wild rabbits, compared with that of the laboratory rabbits.

Table 6. *Analysis of variance of data presented in Table 5, using transformed survival time* $= \log_{10}$ (*survival time in days* $- 8$)

Source	D.F.	Sum of squares	V	F	
		A. Laboratory rabbits and dates of inoculation			
Dates	8	0·3931	0·0491	1·45	$(1{\cdot}49)0{\cdot}20 < P$
Error	34	1·1529	0·0339		
Total	42	1·5460			
		B. Wild rabbits and dates of inoculation			
Dates	3	0·0931	0·0310	2·40	$(2{\cdot}66)0{\cdot}10 < P < 0{\cdot}20(1{\cdot}83)$
Error	11	0·1423	0·0129		
Total	14	0·2354			
		C. Laboratory rabbits and wild rabbits			
Rabbits	1	0·1140	0·1140	3·58	$(4{\cdot}02)0{\cdot}05 < P < 0{\cdot}10(2{\cdot}80)$
Error	56	1·7815	0·0318		
Total	57	1·8955			

It can be seen from Text-fig. 1 that the survival times of wild rabbits are consistently slightly longer than those of laboratory rabbits, whether the virus used was of high (standard laboratory strain), moderate (KM 13 strain) or low virulence (Uriarra strain).

Because of this slight difference in the response of laboratory and wild rabbits, data from tests with wild rabbits have been recorded separately from those obtained with laboratory rabbits (Table 1). Control groups of either wild or laboratory rabbits were set up whenever a batch of field strains was tested, so that direct comparisons of the field and standard laboratory strains could be made in the appropriate host animals.

The symptomatology of myxomatosis due to different strains of the virus

The foregoing discussion establishes the basis for the comparison of the virulence of different strains of myxoma virus. The principal criterion adopted for comparison is the mean survival time of groups of five or more 4- to 6-month-old laboratory rabbits, inoculated intradermally with about 5 rabbit-infectious doses of virus contained in 0·1 ml. of saline.

Before discussing the application of this criterion to the ninety-two strains listed in Table 1, it is desirable to describe the clinical features of infection due to different strains of the myxoma virus. Eight 'prototype' strains have been selected as representative of fairly clearly defined groups. Especially among the attenuated Australian field strains the selection of certain strains as typical is somewhat arbitrary, as there was a gradation of severity of symptoms between something slightly less rapidly progressive than infections with the standard laboratory strain and the type characterized here as Uriarra. The descriptions should be read in conjunction with the illustrations (Pls. 2 and 3). In Text-fig. 2 we have attempted to represent diagrammatically the differences in the appearance of the primary

lesion and the eyelids at various intervals after the infection of rabbits with standard doses of the eight prototype strains. The figures given below for survival times and mortality rates refer to laboratory rabbits infected in the standard manner.

(1) *The standard laboratory strain* (Pl. 2, fig. 1). Mean survival time 10·8 days. Range 8–15 days. Mortality rate 100%. This is the reference strain (no. 1 of Table 1) for the present investigation, and is the strain which was used to initiate the Australian epizootics and which has been repeatedly artificially reintroduced into the Australian wild rabbit population.

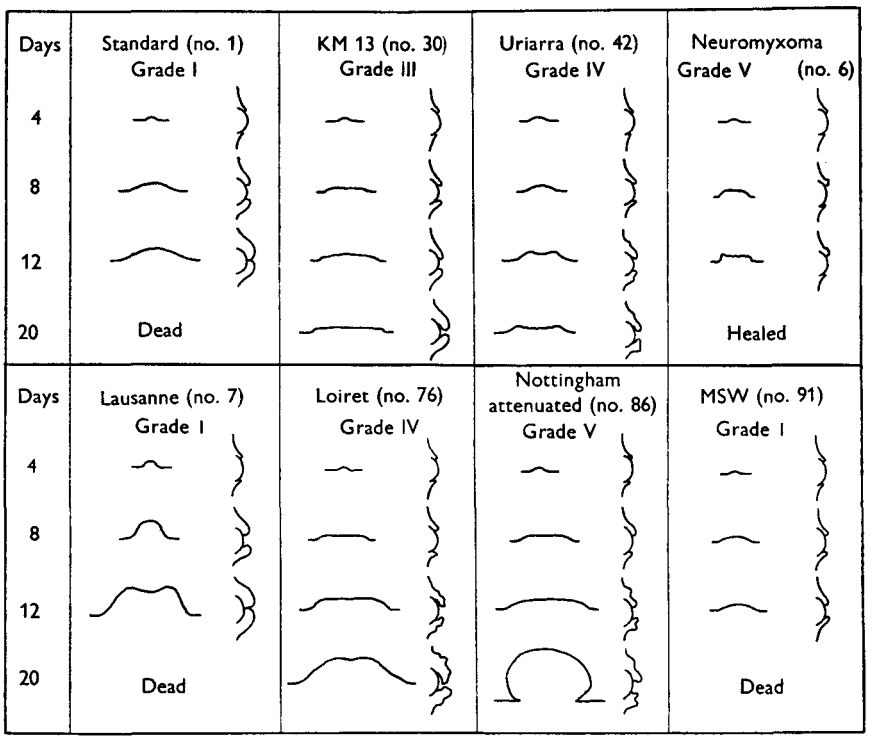
The lump produced at the inoculation site is first seen on the third day and later becomes large, hard and convex. The roughly circular margin merges gradually into the surrounding skin, there being no sharp demarcation between the tumour and normal skin. The skin over the primary lesion remains unbroken throughout the disease. Secondary skin lesions are recognizable by the sixth and seventh day, and by the ninth day they are well distributed over the body and ears. Lumps occur on the limbs only in cases which survive longer than usual. Thickening of the eyelids is first seen on the sixth or seventh day, and the eyes are usually completely closed by the ninth day. There is an opalescent discharge from the eyes which becomes copious and more turbid during the last 2 or 3 days of life. Oedematous swelling of the head, base of the ears, and perineum becomes pronounced in the later stages of the disease, and the slow respiration is often accompanied by a semipurulent nasal secretion. The rabbit usually continues to eat and drink until shortly before death, and body fat is usually abundant at the time of death.

The virus attains a high concentration in primary and secondary skin lesions, and mosquito transmission occurs readily (Fenner *et al.*, 1956).

(2) *KM* 13 *strain* (*Aust/Corowa*/12–52/2) (Pl. 2, fig. 2). Mean survival time 21·5 days. Range 13 days to recovery. Mortality rate 88%. This strain (no. 30 of Table 1), a field derivative of the standard laboratory strain, has been selected as representative of Australian field strains of moderate virulence.

The primary lesion at the inoculation site appears on the third or occasionally the fourth day, and becomes large, flat and soft. It sometimes becomes harder and slightly convex in the later stages of a fatal infection. Occasionally it never progresses beyond a thickening of the skin. The margin is irregular, and in the later stages becomes clearly demarcated from the surrounding skin, especially in cases which live for longer periods than usual. The secondary skin lesions are of the same general character as the primary lesion, but may become more nodular, particularly on the legs. The first appearance of various symptoms coincides with the time pattern observed with the standard laboratory strain, but the subsequent development is much more gradual. The eyes are rarely closed before the fourteenth to sixteenth day, and an occasional animal dies or recovers without the eyes closing completely. The eyelids are sometimes irregularly distorted rather than generally thickened, but discrete nodules are rare. The oedematous swelling of the head is less pronounced than in infections with the standard laboratory strain, but in advanced severe cases there may be numbers of semi-confluent tumours over the head and around the mouth. Occasionally the whole course of the disease is relatively mild,

but the severe case which survives for much longer than 20 days presents a wretched picture in the later stages. There is a purulent nasal discharge and the breathing is laboured, the animal is emaciated, the eyes are closed and bulbous, with a purulent discharge, usually associated with secondary bacterial infection, and strings of mucus are passed with the faeces. There is bulbous oedematous swelling of the perineum, the ears are pendulous, and the head, legs and body are covered with small, sometimes nodular, lumps.



Text-fig. 2. Diagrammatic representation of the progressive development of the primary lesion and swelling of the eyelids in rabbits infected with the prototype strains of myxoma virus. The bracketed numbers refer to the strain number in Table 1. The grades of severity follow the definitions given in Table 11.

In animals which recover the skin over the flat tumours produced by the KM 13 strain becomes dry and scaly, but the lump itself remains fleshy for a considerable period before it dries out and sloughs. Virus has been recovered from tissue slices of such secondary tumours up to 60 days after infection, and successful mosquito transmission has been recorded 40 days after inoculation (Fenner *et al.*, 1956).

(3) *Uriarra strain* (*Aust/Uriarra*/2–53/1) (Pl. 2, fig. 3). Mean survival time 26·2 days. Range 15 days to survival. Mortality rate 58 %. This strain (no. 42 of Table 1) has been selected as representative of the Australian field strains of relatively low virulence.

Mykytowycz (1956) has described the characteristics of infections induced in wild rabbits by contact and by mosquito bite, and Jacotot, Vallée & Virat (1955*a*)

have described the symptomatology in laboratory rabbits infected by injection. The following description applies to laboratory rabbits infected by the intradermal inoculation of about 5 rabbit-infectious doses. The local lesion at the inoculation site appears on the third or fourth day and soon becomes hard, red and slightly convex. The colour often deepens to purple about the ninth or tenth day. The irregular margin at first merges gradually into the surrounding skin, but except in acutely fatal cases it becomes clearly demarcated by about the twelfth day. In some rabbits the centre of the flat primary lesion becomes necrotic and forms a black scab during the third week of the disease, but in other animals this process is deferred for another week or even longer. The central scab sloughs out during the fifth week in animals which recover. Secondary skin lesions are usually numerous, relatively flat, red in colour, and have very clearly demarcated margins. In milder cases there are discrete nodules on the eyelids instead of a general oedematous thickening. Discharge from the eyes and nose is less than in infections with more virulent strains, but there is often extensive perineal oedema.

The virus content of secondary skin lesions was found to vary considerably from one animal to another (Fenner *et al.*, 1956). It was always high between the eighth and fourteenth days, but in some animals it then fell precipitately, and in others remained high for a further 2 weeks.

(4) *Neuromyxoma strain* (Pl. 2, fig. 4). All rabbits survive infection with small doses. This is the most attenuated strain of myxoma virus known (no. 6 of Table 1).

The local lesion produced at the inoculation site is hard, red, and convex. The centre becomes depressed and purple very early and the periphery is well demarcated by the fifth day. Regression and scabbing follow soon afterwards. Secondary skin lesions are scanty, and consist of small nodules usually less than 5 mm. in diameter. These may occur anywhere on the body, or on the eyelids, or perineum. Generalized oedema of the head and perineum is never seen in infections with small doses of virus, and the general health of the rabbit is hardly affected.

The virus content of skin lesions reaches its peak about the sixth day, but at its highest is well below the level observed with the more virulent strains. The clinical regression of the skin lesions after the seventh day is accompanied by a pronounced fall in the virus concentration of the lesions (Fenner *et al.*, 1956). Virus is only occasionally recovered, and then in low concentration, from the bloodstream.

(5) *Lausanne strain* (*Brazil/Campinas*/1949/1) (Pl. 3, fig. 5). Mean survival time 12·9 days. Range 10–16 days. Mortality rate 100 %. This is the strain (no. 7 of Table 1) which was used to initiate the European epizootics in June 1952, and is representative of strains recovered from Europe up to September 1954. The symptomatology of rabbits infected with this strain differs appreciably from that observed in infections with the standard laboratory strain and all derivatives of it (nos. 1–4 above), and is characterized by the great proliferation of the skin lesions, so that they rapidly become large hemispherical tumours.

A hard, protuberant convex tumour is produced at the site of inoculation. It reaches its maximum size by the tenth day, when the shining skin over it breaks down and oozes serous fluid. This is followed by ulceration of the centre of the lesion. The colour also differs from that of lesions produced by the derivatives of

the standard laboratory strain, being purple from the fourth day onwards and later becoming black. The protuberance of the tumour gives the impression that it is clearly demarcated from the surrounding skin, but in fact the edges merge gradually into normal skin.

Generalization is more florid and proliferative than in infections with the standard laboratory strain, but follows the same time sequence. Secondary lesions appear on the body, legs and feet, but rarely on the ears. The head and perineal region are extremely swollen and there is a copious semipurulent discharge from eyes and nose. Death occurs before the rabbit loses much weight.

The virus content of the skin lesions follows much the same time-sequence as in infections with the standard laboratory strain, and mosquitoes readily transmit after biting through skin lesions produced by the Lausanne strain (Day, Fenner, Woodroofe & McIntyre, 1956).

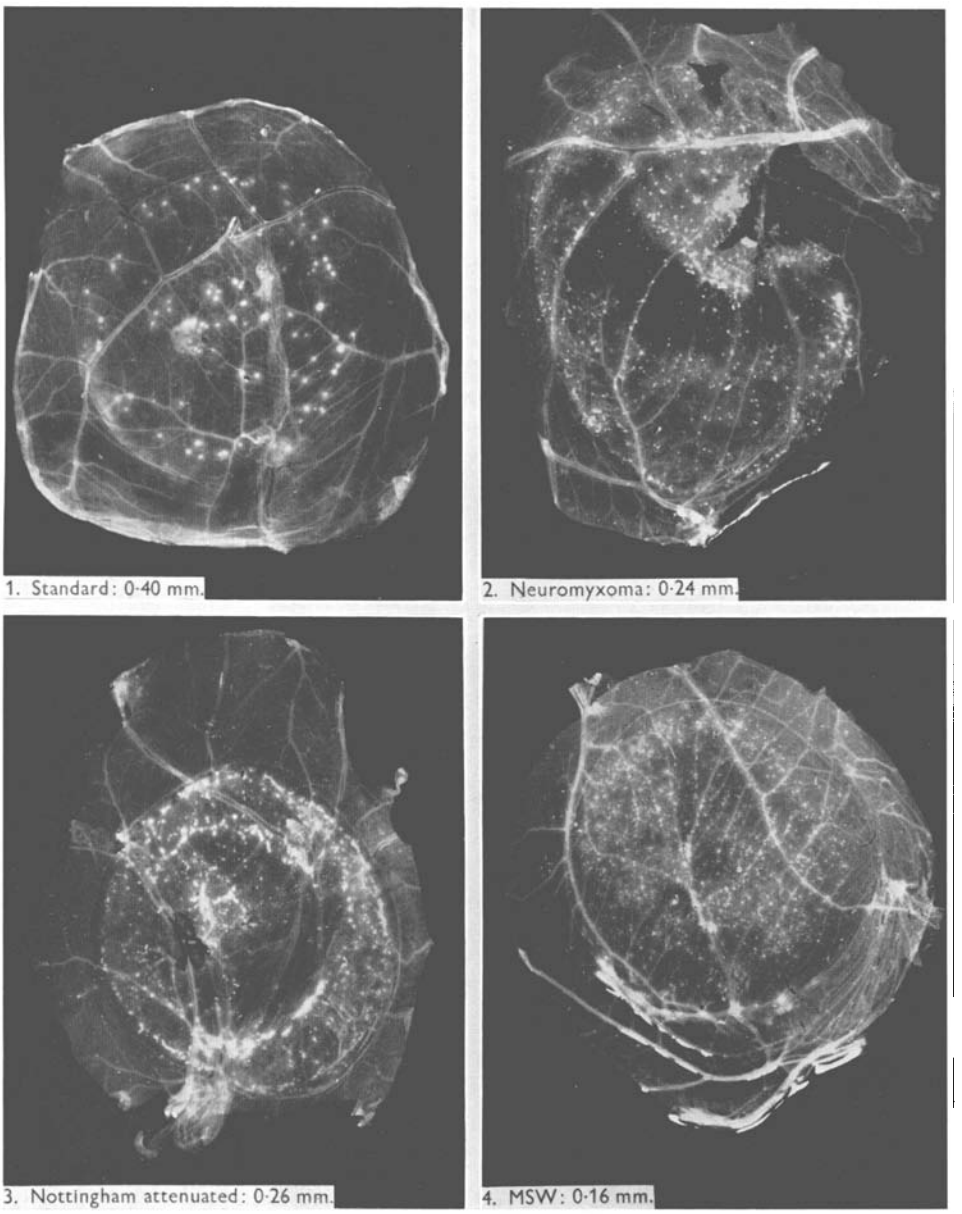
(6) *Loiret 55 strain* (*France/Loiret/4–55/1*) (Pl. 3, fig. 6). Mean survival time 33·1 days. Range 19 days to recovery. Mortality rate 65 %. This strain (no. 76 of Table 1) was the first attenuated strain recognized from Europe.

Although attenuated, its clinical characteristics resemble those of infections due to the Lausanne strain much more closely than any of the Australian strains. Differences from the Lausanne strain reside principally in the rate of development of the symptoms. The primary lesion usually appears on the third day, but remains red in colour and relatively flat until about the eighth day. Proliferation is progressive, however, so that by the fourteenth day the primary lesion, and the very numerous secondary skin lesions, are large and prominent, but rarely become purple in colour. The edges are more clearly demarcated from the surrounding skin than in infections with the Lausanne strain, and the eyelids are grossly deformed rather than included in a general extreme oedema of the head. In the later stages the rabbit becomes emaciated and covered with large red protuberant skin lesions, which occur all over the body, and on the ears. There is gross oedematous swelling of the perineum and genitalia, which, in bucks, frequently leads to bursting of the scrotum and consequent exposure to secondary infection.

The virus content of the skin lesions rises rather more slowly than with the Lausanne strain, but attains the same high level and persists at a high level for a long time (Fenner *et al.*, 1956).

(7) *Nottingham 55 attenuated strain* (*England/Nottingham/4–55/1 (attenuated)*) (Pl. 3, fig. 7). Range of survival times 14 days to recovery. Mortality rate 23 %. This strain (no. 86 of Table 1) is the attenuated component of what appears to be a mixture of strains recovered from a naturally infected rabbit in Nottinghamshire, England in April, 1955. Details of this strain will be discussed in a later section.

The local lesion does not appear at the inoculation site until the fifth day, and remains relatively flat and rubbery in consistency until about the twelfth day. At this stage the lesion either begins to regress and form a scab, or it rapidly proliferates into a large, hard, protuberant tumour which is purple in colour and clearly demarcated from the surrounding skin. The latter type of tumour often persists into the fifth week of the disease, before it gradually regresses and scabs. Secondary lesions appear on the ninth day and are nodular and clearly demar-



1. Standard: 0·40 mm.

2. Neuromyxoma: 0·24 mm.

3. Nottingham attenuated: 0·26 mm.

4. MSW: 0·16 mm.

(*Facing p.* 168)

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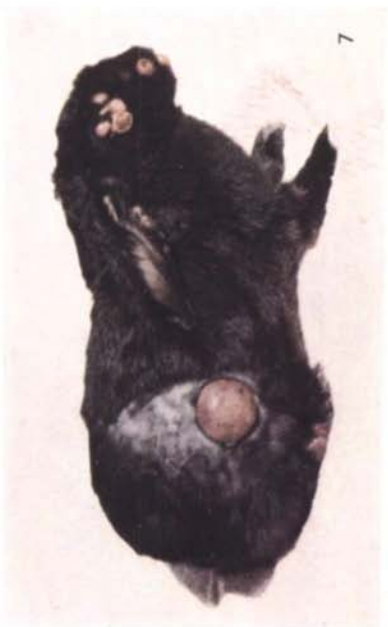

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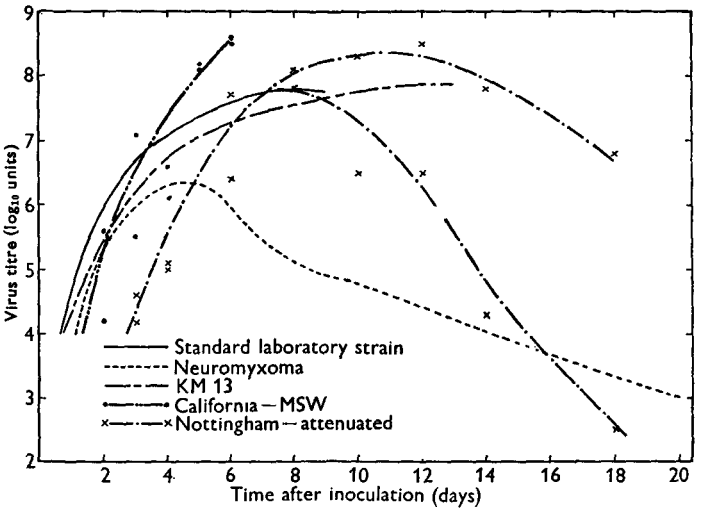
cated. There is severe oedema of the perineum, but elsewhere oedema is almost absent, and lesions of the eyelids consist of localized nodules. The disease develops slowly and runs a relatively benign course, rather like a generalized fibromatosis.

Study of the virus content of the tumours show that it rises much more slowly than occurred with other strains, but by the eighth day the titre attained is similar to that found with most other strains (Text-fig. 3). After the eighth day the virus content usually diminishes fairly rapidly but in a few rabbits it remains at a high level for a prolonged period.



Text-fig. 3. Concentration of various strains of myxoma virus in the epidermis of rabbits infected by the intradermal inoculation in several sites of large doses of virus (about $10^{4 \cdot 3}$ rabbit-infectious doses). Curves for standard laboratory strain, neuromyxoma and KM13 from Fenner *et al.* (1956). Two curves are shown for the Nottingham attenuated strain, the upper one derived from an animal with persistent fleshy tumours, and the lower from an animal in which the skin lesions healed rapidly (see text).

(8) *California—MSW* (*U.S.A./San Francisco*/1950/1 (*MSW*)) (Pl. 3, fig. 8). Mean survival time 9·2 days. Range 7–14 days. Mortality rate 100%. Myxomatosis has been enzootic in California since 1930 (Kessel, Prouty & Meyer, 1930). The MSW strain (no. 91 of Table 1) was derived from a domestic rabbit naturally infected in 1950.

It is highly lethal for laboratory rabbits, but the progress of the infection differs from that seen with any other of the strains examined, except the other strain from California (U.S.A./San Diego/1949/1 (MSD)). The primary lesion appears on the third day and slowly increases in size, but never becomes prominent. It is rubbery in consistency and the edges merge gradually into the normal skin. Swelling of the eyelids is first apparent on the seventh day, but never progresses to closure of the eyes. It was rarely more severe than shown in Pl. 3. In contradistinction to other strains (except neuromyxoma) the conjunctival discharge is light in amount and thin and milky in nature. Skin secondaries and swelling in

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the anogenital region appear about the ninth day, but are often absent at the time of death. Neither symptom progresses to an advanced stage. Manifestations of involvement of the central nervous system occurred more commonly in rabbits infected with this strain than with any other studied, and consisted of a very rapid tremor or convulsions.

In the dead animal there was often a bloody discharge from the nose or the anus, and post-mortem examinations showed haemorrhages in the skin, stomach, intestines, lungs and other viscera to be more frequent than in fatal cases of myxomatosis due to other strains of the virus.

So many rabbits died before symptoms of advanced myxomatosis appeared that we suspected contamination of the myxoma virus with another pathogen. However, no bacteria could be isolated from suspensions of the virus, and previous infection of rabbits with fibroma, or other strains of myxoma virus protected them from generalized myxomatosis due to the MSW strain. Specific soluble antigen (Fenner & Woodroofe, 1953) was present in the serum at death, even when the external symptoms were slight. Two out of sixteen rabbits inoculated with this strain died on the seventh day after inoculation. The occurrence of these two deaths was not thought sufficient reason to alter the general formula used for the calculation of transformed mean survival times of other groups of rabbits ($y = \log_{10}$ (mean survival time $- 8$ days)).

The multiplication curve of virus in the skin after the inoculation of moderate doses intradermally resembled those of the standard laboratory and Lausanne strains, usually rising to an even higher level (Text-fig. 3), but the local lesions were never large and secondary skin lesions were usually very slightly developed at the time of death.

Variations in the character of lesions produced on the chorioallantois

All strains of myxoma virus examined were titrated on the chorioallantoic membrane of developing chick embryos. Four recognizably different types of lesions were produced. The great majority of virus strains—all the Australian field strains, all the Brazilian strains, and all the virulent European strains—produced similar lesions. These are exemplified by the pocks produced by the standard laboratory strain, which are illustrated in Pl. 1, fig. 1. The three variations from this usual appearance were the small pocks produced by neuromyxoma, by the Californian strains (MSW and MSD—nos. 91 and 92 of Table 1), and by the attenuated component of England/Nottingham/4–55/1. The former were uniformly small, and each lesion appeared to consist of a central opaque plaque surrounded by a faint 'halo' (Pl. 1, fig. 2). The pocks due to the attenuated component of the Nottingham 55 strain were smaller than those caused by the standard laboratory strain, but were otherwise similar to them (Pl. 1, fig. 3). The two Californian strains produced minute pocks, with no 'halo' (Pl. 1, fig. 4).

Groups of pocks were measured with an ocular micrometer, using a binocular dissecting microscope with 5× eyepieces and 4:1 objective. The dimensions of the pocks are shown in Table 7 and Text-fig. 4. The measurements confirm the general observation that the size of pocks produced by each of these three strains is

significantly different from the usual size of pocks produced by myxoma virus (characterized by the standard laboratory strain).

Table 7. *The diameter of pocks produced by three strains of myxoma virus grown on the chorioallantoic membrane of* 12*-day-old chick embryos for* 3 *days at* 35° C.

No. (Table 1)	Virus strain designation	No. of pocks measured	Mean diameter ± S.D. (mm.)
1	Standard laboratory strain	99	0·40 ± 0·122
6	Neuromyxoma	100	0·24 ± 0·062
91	U.S.A./San Francisco/1950/1 (MSW)	72	0·16 ± 0·042
85	England/Nottingham/4–55/1, original	269	0·27 ± 0·112
86	England/Nottingham/4–55/1, attenuated	359	0·26 ± 0·084
87	England/Nottingham/4–55/1, virulent	105	0·37 ± 0·111

Neutralization tests were carried out with strains of virus representative of the four pock types and a myxoma antiserum produced in rabbits against the standard laboratory strain. High percentage reductions of pock numbers were obtained with each of the virus strains tested, but that attained with England/Nottingham/4–55/1 (attenuated) was lower than those with the other strains (Table 8). This result was obtained in three separate experiments, and is the only consistent evidence of antigenic difference found in many cross-neutralization tests carried out with strains of myxoma virus of widely differing origin and homologous and various heterologous antisera (Marshall, unpublished).

Table 8. *The results of neutralization tests with myxoma-immune rabbit serum (produced against standard laboratory strain), and five strains of myxoma virus*

Virus strain		Pock type (mean diameter, Table 7) (mm.)	Percentage reduction of pock count on chorioallantoic membrane
No. (Table 1)	Designation		
1	Standard laboratory strain	0·40	97
7	Brazil/Campinas/1949/1 (Lausanne)	0·40	98
6	Neuromyxoma	0·24	99
91	U.S.A./San Francisco/1950/1 (MSW)	0·16	97
86	England/Nottingham/4–55/1, attenuated	0·26	83

The 'purity' of attenuated strains of myxoma virus

At the time of the initial description of an attenuated field strain of myxoma virus (Aust/Uriarra/2–53/1), Ratcliffe (Mykytowycz, 1953) suggested that the wide variation in symptomatology seen in rabbits infected with this strain by contact or by intradermal inoculation might be due to the fact that it was a relatively stable mixture of approximately equal proportions of a highly virulent and an attenuated strain of virus. Different rabbits were assumed to react differently because they were infected predominantly, or solely, with one or other component of the mixture. Jacotot and his collaborators (Jacotot *et al.*, 1955*a*, *b*)

have recently stated explicitly that they regard the Australian Uriarra (Aust/Uriarra/2–53/1) and French Loiret 55 (France/Loiret/4–55/1) strains as mixtures of this type. Hudson & Mansi (1955) reported that virus recovered from rabbits infected with the Nottingham 55 strains (England/Nottingham/4–55/1) was highly virulent if obtained early in the course of the disease, and of reduced virulence if obtained later in the course of the infection.

There is no reason to doubt that the standard laboratory strain, the Lausanne strain and neuromyxoma are 'pure' strains. The type of lesion produced, the rate of progress of the disease, and the final outcome are remarkably uniform from one rabbit to another. Large numbers of rabbits infected with small doses of each of these strains of virus by the standard methods have now been observed. The narrow range of survival times with the standard laboratory strain (mean 10·8 days, range 8–15 days) and the Lausanne and related strains (mean 12·1 days, range 10–16 days), and the invariable survival and mild symptomatology in rabbits infected with small doses of neuromyxoma attest this uniformity.

Wide variation in symptomatology on its own is not an adequate basis for the supposition that the causative infections are due to mixtures of viruses of differing virulence. Variations in host resistance are obscured by the overwhelming virulence of the standard laboratory, and the Lausanne strains and are difficult to detect in the mild disease caused by neuromyxoma. One might expect differences in the innate resistance of different rabbits to find their most obvious expression in infections with viruses of intermediate virulence.

The issue was so important that it was decided to test it by the comparison of several 'pure clones' of the KM 13, Uriarra, Loiret 55 and Nottingham 55 strains. The procedure adopted was as follows. Large numbers of eggs were inoculated with doses of the strain under study which would produce single pocks on some of the eggs and no lesions on the others. The chorionic surface of the membrane was carefully examined in the intact, opened egg, with a dissecting microscope, on the third day after inoculation. Five or more single pocks were removed from eggs on which only one pock was seen, ground individually, and ampouled. The statistical justification for regarding a pock as the result of infection of the chorioallantoic membrane by a single infectious virus particle has been presented elsewhere (Fenner & McIntyre, 1956), and the virus particles arising in the membrane from the multiplication of the single infectious virus particle which produced the pock are regarded as components of a pure clone. Several of the suspensions derived from single pocks were inoculated intradermally into groups of five laboratory rabbits, together with a similar dose of the parent strain used to initiate the pure clones. The symptomatology of each group of rabbits was observed carefully, and the mean survival times are recorded in Table 9. In order to carry out analysis of variance of the transformed survival times shown in Table 9 it was necessary to allot an arbitrary survival time to the survivors. The period of 60 days (transformed = 1·716) was selected because animals never died of uncomplicated myxomatosis after the fiftieth day and with the KM 13 strain, at least, had rarely recovered before the fortieth day. As can be seen from Table 9 comparison of transformed mean survival times calculated by Sampford's method, and by allotting survivors

a survival time of 60 days, showed that the latter figure was probably an overestimate and biased the analysis somewhat in the direction of demonstrating differences between single pock derivatives.

With the KM 13 strain the range of symptoms seen in each group was much the same. All animals became very sick. Some, in both the pure clone groups and in the parent material, died relatively early, and some survived for several weeks or recovered. Analysis of variance disclosed no significant differences between the survival times in different groups ($P > 0{\cdot}20$).

Table 9. *Infection of laboratory rabbits with small doses of the parent strain and single pock derivatives (pure clones) of strains Aust/Corowa/12–52/2* (KM 13), *Aust/Uriarra/2–53/1 (Uriarra), France/Loiret/4–55/1 (Loiret* 55), *and England/Nottingham/4–55/1*

Strain of virus	Individual rabbits	Survival times: Transformed mean ± S.E. A*	Survival times: Transformed mean ± S.E. B†	Survival times: Mean survival time in days and its 95% fiducial range†
Aust/Corowa/12–52/2 (KM 13)				
Parent strain	13, 15, 17, 18, 19, 22, 26, 27, S‡	1·10 ± 0·10	1·07 ± 0·08	16·3 **19·8** 24·7
Single pock: No. 1	20, 22, 27, 34, 41	1·29 ± 0·08	1·29 ± 0·08	21·3 **27·4** 36·2
No. 2	13, 18, 20, 24, S	1·14 ± 0·17	1·11 ± 0·14	14·9 **21·0** 32·3
No. 3	21, 22, 23, 26, 29	1·20 ± 0·04	1·20 ± 0·04	21·4 **23·9** 27·0
No. 4	17, 24, 40, S, S	1·42 ± 0·15	1·41 ± 0·16	20·0 **33·5** 62·1
No. 5	17, 20, 22, 22, 27	1·12 ± 0·05	1·12 ± 0·05	18·4 **21·2** 24·7
Aust/Uriarra/2–53/1				
Parent strain	17, 18, 31, 31, 34, S, S, S, S, S, S	1·49 ± 0·09	1·45 ± 0·09	26·2 **36·0** 50·9
Single pock: No. 1	22, 32, 33, S	1·41 ± 0·11	1·42 ± 0·11	23·6 **34·1** 51·7
No. 2	13, 17, 18, 49, S	1·20 ± 0·20	1·28 ± 0·25	14·1 **27·2** 68·5
No. 3	18, 25, S, S, S	1·47 ± 0·15	—	—
No. 4	16, S, S, S, S	1·55 ± 0·16	—	—
No. 5	32, 45, S, S, S	1·62 ± 0·07	—	—
France/Loiret/4–55/1 (Loiret 55)				
Parent strain	20, 22, 27, 27, 29, 38, S, S, S, S	1·44 ± 0·08	1·34 ± 0·05	25·2 **29·8** 35·6
Single pock: No. 1	48, S, S, S, S	1·69 ± 0·02	—	—
No. 2	26, 27, 31, 32, S	1·40 ± 0·08	1·33 ± 0·03	26·6 **29·4** 32·5
No. 3	28, 33, 38, 43	1·43 ± 0·05	1·43 ± 0·05	29·1 **34·9** 42·3
No. 4	19, 19, 28, 42, S	1·33 ± 0·14	1·35 ± 0·15	19·2 **30·4** 52·7
England/Nottingham/4–55/1				
Parent strain	11, 11, 13, S, S, S, S, S, S, S	—	—	—
Single pock: No. 1	S, S, S, S, S	1·72	—	—
No. 2	39, S, S, S, S	1·67 ± 0·04	—	—
No. 3	11, 12, 12, 12, 13	0·60 ± 0·01	0·60 ± 0·01	10·7 **11·9** 13·8

* Calculated on assumption that survival time of survivors = 60 days (see text).
† Calculated by Sampford's method. ‡ S = Rabbit recovered from infection.

The symptomatology of all rabbits in the six groups infected with the Uriarra strain or single pock derivatives of this strain differed from that seen in rabbits infected with the KM 13 strain, and conformed with the clinical description set out earlier. Again, the range of severity in each group was uniform. With this more attenuated strain a few animals sustained relatively mild infections not involving

closure of the eyes. Some of these occurred in groups infected with pure clone material and others among the rabbits inoculated with the parent material. Again, there were no significant differences between the survival times of rabbits of different groups ($P > 0{\cdot}20$).

These two experiments show conclusively that the range of symptoms encountered with the Australian attenuated strains KM 13 and Uriarra are the same whether groups of rabbits are infected with high dilutions of material from a rabbit lesion (parent stock) or with pure clones derived from single pocks. The basis for the variable symptomatology must therefore be variation in the innate resistance of the rabbits, and there is no evidence from these experiments, or from our other extensive experience with these strains, that either of them is a mixture of a highly virulent and an attenuated strain.

The Loiret 55 strain showed essentially the same type of behaviour as the two Australian attenuated strains (analysis of variance, $0{\cdot}10 < P < 0{\cdot}20$), but the results obtained with the Nottingham 55 strain differed greatly. The original material received from England, which had been passed once in laboratory rabbits before being freeze-dried, produced two sharply distinct clinical syndromes when inoculated in small doses into laboratory rabbits. Three of ten rabbits behaved in a manner indistinguishable from animals infected with the fully virulent European strains of virus, five suffered a mild disease without closure of the eyes, but with the development of very protuberant nodules in the later stages of the disease (see Pl. 3, fig. 7) and two suffered from a severe slowly progressive infection.

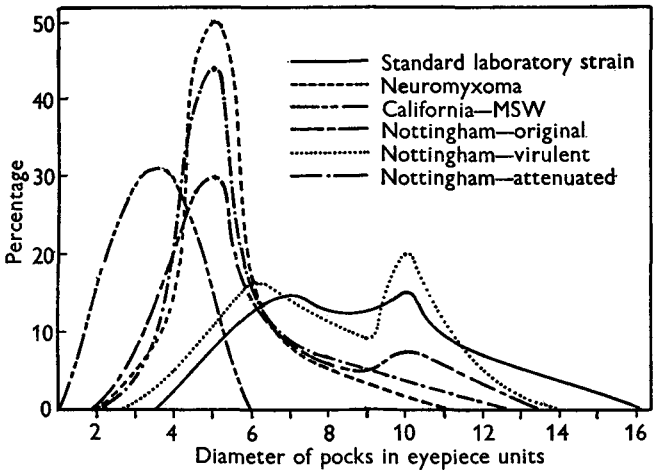
Material from tissue slices taken on the twelfth day from a rabbit which died on the thirteenth day were inoculated intradermally in small doses (about five rabbit-infectious doses) in four rabbits, and all died within 14 days with classical symptoms of the virulent Lausanne strain of the disease. Four rabbits were similarly inoculated with material from a nodule from the ear of a mild case, taken 12 days after infection. Three of these suffered the mild disease with late production of prominent skin nodules, and the other one showed slowly progressive symptoms which resulted in death on the thirtieth day. These preliminary experiments were consistent with the view that the original material contained at least two strains of virus in approximately equal proportions: a highly virulent strain of European type and an attenuated strain which was associated with the development of very prominent skin nodules. The slowly progressive nature of some relatively severe cases could be the result of either (*a*) high host susceptibility to the attenuated strain, (*b*) infection with a balanced mixture of the two strains, or (*c*) infection with a pure strain of intermediate virulence.

Three single pock isolates of the Nottingham 55 material were tested. Two produced the mild form of the disease, and all the rabbits infected with the other strain died within 13 days with classical symptoms of infection with virulent European type virus.

In the early stages of the investigation of the Nottingham 55 strain, before its mixed nature was suspected, it was noticed that there was much greater variability in the size of pocks produced on the chorioallantois than usually found with myxoma virus. Subsequently measurements of pocks produced by virus obtained

from skin lesions of rabbits infected with pure clones showed that there were two populations of pock sizes. The relevant figures are shown in Table 7 and Text-fig. 4.

The curves illustrated in Text-fig. 4 suggest that the original Nottingham material could be differentiated into two derivatives which differed in the size of the pocks they produced on the chorioallantoic membrane as well as their virulence for rabbits, and this view is supported by the fact that the pure clone which produced severe infections (no. 3 of Table 9) was derived from a large pock, whereas the other two were derived from very small pocks.



Text-fig. 4. Distributions of the diameters of pocks caused by the growth of a variety of strains of myxoma virus on the chorioallantoic membrane. All strains inoculated on 12-day-old eggs which were then incubated for 3 days at 35° C.

Hudson & Mansi (1955) found that subinoculation from cases infected with the England/Nottingham/4–55/1 strain produced cases of varying severity depending upon the stage of the disease in the donor rabbit. This point was investigated with our own material. Tissue slices were taken from the primary lesions of two rabbits infected with the parent strain at various intervals after inoculation. After titration, material taken from different animals on different days was inoculated in the standard manner in groups of five rabbits, with the results shown in Table 10. A similar experiment was carried out with material taken from rabbits infected with the KM 13 strain.

The transformed survival times were subjected to an analysis of variance, assuming as before that survivors lived for 60 days. With the KM 13 strain there was no significant difference between the survival times of any of the groups due to the time of reaping the virus ($0{\cdot}10 < P < 0{\cdot}20$). From these experiments, as from the single pock experiments, there is no evidence that KM 13 is a 'mixed' strain.

The results obtained with the strain England/Nottingham/4–55/1 were not amenable to statistical analysis based on survival time. As in previous experiments two clearly differentiated groups could be distinguished. The material taken from R 637 (a mild case) produced a majority of cases which were indistinguishable from those caused by the Nottingham attenuated strain. Only two of the ten animals

died and neither showed the symptomatology associated with the virulent European type of virus. Three of the 15 rabbits inoculated with material from R 636 died. The animal which died after 17 days (tissue slice taken on the fifth day) had not developed the lesions characteristic of the highly virulent European strains at the time of death, but the other two fatal cases, which occurred in rabbits inoculated with material reaped on the twentieth day, died with symptoms of the severe European type of disease. These results do not show the progressive diminution in the severity of cases with the passage of the time of reaping demonstrated by Hudson & Mansi (1955), but they provide further evidence that the original Nottingham strain was a mixture.

Table 10. *Infection of laboratory rabbits by the intradermal inoculation of about 5 rabbit-infectious doses of myxoma virus derived from tissue slices taken from the primary lesions of rabbits infected with Aust/Corowa/12–52/2 (KM 13) or England/Nottingham/4–55/1 strains, at various intervals after initial infection*

Virus strain	Aust/Corowa/12–52/2 (KM 13) (no. 30 of Table 1)		
Donor rabbit	R 546 (died 21 days after infection with no. 3 single pock derivative)		
Time of reaping tissue slice (days)	6	14	21
Survival times of rabbits infected with tissue slice material	12, 18, 19, 19, 24	18, 18, 28, 36, S	14, 18, 19, 20, 34
Means of transformed survival times ± S.E.	0·98 ± 0·10	1·27 ± 0·12	1·06 ± 0·10
Mean survival time and its 95 % fiducial range	11·2 **17·5** 26·9	18·7 **26·6** 40·4	12·1 **19·5** 41·0
Virus strain	Aust/Corowa/12–52/2 (KM 13) (no. 30 of Table 1)		
Donor rabbit	R 560 (died 22 days after infection with no. 5 single pock derivative)		
Time of reaping tissue slice (days)	6	14	21
Survival times of rabbits infected with tissue slice material	20, 21, 22, 25, S	17, 23, 25, 26, 32, 42, S, S, S	20, 22, 23, 28, S
Means of transformed survival times ± S.E.	1·18 ± 0·04	1·34 ± 0·08	1·18 ± 0·03
Mean survival time and its 95 % fiducial range	20·5 **23·0** 26·0	23·8 **30·4** 39·6	20·9 **23·1** 25·6

Virus strain	England/Nottingham/4–55/1 (no. 86 of Table 1)				
Donor rabbit	R 637 (recovered from mild disease after infection with parent strain		R 636 (recovered from moderately severe disease after infection with parent strain)		
Time of reaping tissue slice (days)	7	20	5	10	20
Survival times of rabbits infected with tissue slice material	S, S, S, S, S	14, 44, S, S, S	17, S, S, S, S	S, S, S, S, S	12, 12, S, S, S

S = Rabbit recovered from infection.

Comparison of the virulence of Australian field strains

The Australian epizootics were initiated by the introduction of the standard laboratory strain of virus into the rabbit population in 1950 (Ratcliffe, Myers, Fennessy & Calaby, 1952). Each spring and summer since then there have been large-scale inoculation campaigns, predominantly with the standard laboratory

strain (strains 1 and 2 of Table 1). In New South Wales an early field isolate (strain 5 of Table 1) and in South Australia another line of the standard laboratory strain (strain 3 of Table 1) were used, but these were indistinguishable in their virulence from the standard laboratory strain.

Field strains have been collected from various parts of Australia, principally in New South Wales and Victoria, ever since 1951, but collections were not made on a large scale until 1952–53. When subjected to the standard test for virulence described earlier in this paper, it was found that most field strains were attenuated, compared with the standard laboratory strain.

For convenience of discussion all field strains have been classified into five groups according to the mean survival times of rabbits inoculated in the standard manner, their virulence being described as grades I, II, III, IV or V (Table 11). Consideration of the differences in mean survival time between groups of rabbits inoculated with pure clone derivatives of the attenuated Australian strains KM 13 and Uriarra indicates that with attenuated strains it is unwise to try to make very precise distinctions between the virulence of such strains. The rarity of survival times of more than 13 days in laboratory rabbits infected with the standard laboratory strain (1 out of 43, Table 5) makes it possible to distinguish strains with mean survival times in laboratory rabbits of 13–15 days as very slightly attenuated variants (severity, grade II). The other three groups we have distinguished amongst the Australian field strains are those with mean survival times between 16 and 28 days, typified by the KM 13 strain (severity, grade III), those with mean survival times between 28 and 50 days, typified by the Uriarra strain (severity, grade IV) and very attenuated strains which cause death in less than 30 % of infected animals (severity, grade V). The distribution of strains into these five categories according to the time of collection and place of origin is shown in Table 11. In spite of the large-scale annual inoculation campaigns with highly virulent standard strains of virus the majority of strains recovered from the field in Australia have been attenuated, but there has been no evidence of a secular progression in the degree of attenuation.

Examination of the interval elapsing between the last artificial introduction of the virus and recovery of specimens showed that strains of high virulence (grades I and II) were usually recovered within 6 months of the last artificial introduction of virus (12 out of 13 cases), and were recovered after both the initial and later inoculation campaigns. Strains of moderate virulence (grade III) were much more commonly obtained after the second or later inoculation campaign, and probably, therefore, represented virus which had persisted naturally in the area, or had been naturally introduced into it from a neighbouring area. As the experiments with the French type of virus showed (Fenner *et al.*, 1957), it is possible for a recently introduced virulent strain to co-exist with a naturally occurring attenuated strain during an acute epizootic (nos. 27 and 28 of Table 1 at Urana; nos. 40 and 41 of Table 1 at Merricumbene). This situation was also detected at Werrimal, where the virulent strain was the standard laboratory strain (nos. 52 and 53 of Table 1). Failure to detect it more widely can be ascribed to the few samples obtained from most areas. The over-all trend towards moderate virulence (grade III), seen in

Table 11. *The virulence of strains of myxoma virus recovered from wild rabbits from Australia between* 1951 *and* 1955, *and from Europe between* 1953 *and* 1955, *grouped according to the year of recovery and state of country or origin*

Grade of severity ...	I	II	III	IV	V
Degree of virulence ...	Very high	High	Moderate	Low	Very low
Case-mortality rate* ...	99·5 %	99 %	90 %	60–70 %	0–30 %
Prototype strain ...	Standard laboratory (Aust)	—	KM 13 (Aust)	Uriarra (Aust)	Neuromyxoma (Aust)
	Lausanne (Europe)	—	—	Loiret 55 (Europe)	Nottingham attenuated (Europe)
Mean survival time (days)*	⩽13	>13⩽16	>16⩽28	>28⩽50	—
			Australia		
Year of recovery†					
1950–51	1	0	0	0	0
1951–52	2	3	1	0	0
1952–53	1	1	8 (19)‡	2	0
1953–54	1	4	5 (6)	1	0
1954–55	3	2 (3)	7 (8)	4 (5)	0
Place of origin					
Queensland	1	1	1 (4)	0	0
New South Wales	4	1	11 (20)	2 (3)	0
Australian Capital Territory	0	1 (2)	1	2	0
Victoria	3	3	5 (6)	2	0
South Australia	0	2	1	1	0
Western Australia	0	1	0	0	0
Tasmania	0	1	2	0	0
Totals	8	10 (11)	21 (34)	7 (8)	0
			Europe		
Year of recovery					
1953	3	0	0	0	0
1954	7	0	1 (2)	0	0
1955	3	0	0	2	1
Place of origin					
France	6	0	0	2	0
United Kingdom	7	0	1 (2)	0	1
Totals	13	0	1 (2)	2	1

* Case-mortality rates and mean survival times are approximate figures for laboratory rabbits unselected for resistance to myxomatosis.

† Since in Australia epizootics almost always occurred in the summer months the reference year is given as the appropriate warm season.

‡ 8 etc., indicates the number of separate outbreaks from which strains of the virulence indicated were recovered. (19) etc., indicates the total number of strains of the virulence indicated, several being derived from one outbreak.

Table 11, can be explained by the selective advantage for mosquito transmission of strains which cause extensive and long-persisting infectious skin lesions in rabbits (Fenner *et al.*, 1956).

The shortest interval recorded between first entry of the virulent virus into an area and the recovery of an attenuated strain was 5 months at Colo Vale (no. 35 of Table 1), 6 months at Dunroy (no. 15 of Table 1), and the longest recorded persistence after the last known introduction into the area of a virulent strain

https://doi.org/10.1017/S0022172400037098 Published online by Cambridge University Press

(grade I or II) were 12 months (no. 66) and 26 months (no. 65 of Table 1). In assessing this kind of data it must not be forgotten that the standard laboratory strain of virus spread over the continent in a remarkable manner in 1950–51 (Ratcliffe *et al.*, 1952), and it is likely that variants of moderate virulence are at least as mobile. Perhaps the best evidence of the rate of emergence of attenuated strains comes from Lake Urana, where larger numbers of virus strains were collected (Myers *et al.*, 1954). The standard laboratory strain was introduced in October, 1952 and caused an outbreak with a case-mortality rate of over 99·5 %. The epizootic which occurred spontaneously 11 months after the end of the first outbreak was entirely due to attenuated strains of grade III severity. Emergence of the attenuated strain had presumably occurred during the overwintering period.

The situation in areas in which several specimens have been collected over a period of time are set out in Table 12. In all instances in which no change in virulence was observed the strain first tested was already attenuated to the grade III stage (or in the case of Merricumbene to the grade IV stage). Only two instances were observed of an enhancement of virulence, namely, at Uriarra and at Gunbower. At Uriarra the more attenuated grade IV strain first recovered was later replaced by a less attenuated strain of the grade III type. No artificial introductions of virus have been made in this locality since 1953, and either the more highly attenuated strain (grade IV) was replaced by a more virulent, but still attenuated, strain (grade III); or else the original recovery was not representative of the strains present in the area, and the general level of attenuation has changed little during the 2 years.

At Gunbower the strain isolated in March 1952 was of high virulence (grade II), presumably because of the recent introduction of the standard laboratory strain into the area. A year later the only strain tested was of moderate virulence (grade III), but the following year a strain of high virulence (grade II) was again recovered. This almost certainly followed the reintroduction of the standard laboratory strain 3 months earlier. At Lake Urana and Merricumbene, where the French strain was introduced by inoculation in November–December, 1954, some of the large number of strains recovered from the summer epizootic were of the highly virulent French type, but all cases obtained late in the epizootic were of grade III virulence and of the attenuated Australian type (Fenner *et al.*, 1957).

The general position in Australia may be summarized as follows. At the present stage of evolution of the disease strains of virus of slightly reduced virulence (grade III) are better adapted for survival than either highly virulent strains like the standard laboratory strain (grades I and II) or more attenuated strains like Uriarra (grade IV). Mutations of the introduced virulent strains to this state of reduced virulence must occur frequently, and have occurred all over Australia (Table 11). When they do occur the slightly attenuated mutant strain rapidly becomes dominant, probably due to the long survival of rabbits in a condition highly infectious for mosquitoes (Fenner *et al.*, 1956).

Table 12. *The relative virulence of virus strains collected at certain sites at different times: results of analyses of variance of data from Table* 1

Source	No. of strains tested	Strain nos. (Table 1)	Period during which strains were collected	Mean survival time (days) of first strain isolated	Significance of difference in mean survival times	Change in virulence with time
Texas, Queensland	6	8 to 13	May, 1952–Apr., 1953	14·8	$0{\cdot}001 < P < 0{\cdot}01$	Attenuation (grade II to grade III)
Dunroy, New South Wales	2	15, 16	May–Dec., 1952	21·0	$0{\cdot}5 < P$	None (grade III)
Lake Urana, New South Wales*	11	17 to 28 (excluding 27)	Nov., 1952–Jan., 1955	18·2	$0{\cdot}20 < P < 0{\cdot}50$	None (grade III)
Corowa, New South Wales	6	29 to 34	Dec., 1952–Mar., 1955	17·4	$0{\cdot}05 < P < 0{\cdot}10$	None (grade III)
Colo Vale, New South Wales	3	35 to 37	Mar., 1953–Apr., 1955	19·2	$0{\cdot}01 < P < 0{\cdot}05$	Attenuation (grade III to grade IV)
Merricumbene, New South Wales*	2	39, 40	Nov., 1954–Mar., 1955	28·3	$0{\cdot}5 < P$	None (grade IV)
Uriarra, Australian Capital Territory	3	42, 44	Feb., 1953–Jan., 1955	28·4	$0{\cdot}01 < P < 0{\cdot}05$	Enhancement (grade IV to grade III)
Bacchus Marsh, Victoria	2	55, 56	Aug., 1954–Jan., 1955	12·8	$0{\cdot}001 < P < 0{\cdot}01$	Attenuation (grade I to grade IV)
Gunbower, Victoria	3	57 to 59	Mar., 1952–Mar., 1954	14·4	$0{\cdot}01 < P < 0{\cdot}05$	Attenuation and then enhancement (grade II, grade III, grade II)
Loiret, France	2	76, 77	Apr., 1954–Apr., 1955	11·9	$P < 0{\cdot}001$	Attenuation (grade I to grade IV)

* Strains of virus of French type (nos. 27, 41), recovered during experimental release of this strain of virus (Fenner *et al.*, 1957) have been excluded.

Comparison of the virulence of field strains from Europe

The Lausanne strain used to initiate the European epidemics had a quite different history from the standard laboratory strain used in Australia (Bouvier, 1954, and personal communication). Unlike the latter, it was only a few passages removed from the natural Brazilian host, *Sylvilagus braziliensis*, and as described earlier in this paper the symptomatology of infections due to this strain is more florid than that caused by the standard laboratory strain, and the skin lesions much more protuberant. For two years after its release in Europe the virus remained remarkably stable in its virulence (Fenner & Marshall, 1955). Attenuated strains were recovered in France and England at about the same time, in April, 1955 (Jacotot *et al.*, 1955*b*; Hudson *et al.*, 1955). Subsequent tests have revealed two attenuated strains obtained from Sussex, England, in September and October, 1954 (nos. 80 and 81 of Table 1), and since recovery of the attenuated strains from France in April, 1955, another strain of about the same virulence has been recovered from Sologne (no. 77 of Table 1). The European outbreak of myxomatosis must be considered as one episode, initiated by a single introduction of virus in France in June, 1952. The minimum interval between this introduction and the recovery of an attenuated strain (no. 80) was therefore 27 months.

The symptomatology of these attenuated European strains has been described in a previous section of this paper. It resembled that of the parent Lausanne strain in the proliferative nature of the skin lesions, but the progress of the disease was much slower, and the skin lesions became prominent only in the later stages of the disease. The lesions produced by the one much more attenuated strain—Nottingham attenuated—were individually rather like the lesions produced by the inoculation of fibroma virus, but they were generalized to a much greater extent than occurs in fibromatosis of adult rabbits.

The situation in the Sherwood Forest area (Nottinghamshire) is unique as far as our experience goes, for it appears that a mixture of highly virulent and attenuated strains has persisted for some time under conditions of natural transmission (Hudson *et al.*, 1955). A detailed analysis by the pure clone method of material obtained from this area over a period of time would be of great interest.

The virulence of strains of myxoma virus from America

Three strains of myxoma virus were obtained from South America and two from California. The strain recovered in Uruguay in 1947 (no. 88 of Table 1), and those obtained from Campo Grande and Jacarépaguá in Brazil were all highly virulent and produced the same type of florid skin lesions already described as characteristic of infections with the Lausanne strain of virus. It will be recalled that the Lausanne strain was also only a few domestic rabbit passages from its natural host *Sylvilagus braziliensis*. This symptomatology in *Oryctolagus* may be characteristic of the virus as it occurs naturally in *Sylvilagus* in Brazil. It is possible that the picture seen in infections with the standard laboratory strain and its derivatives has been modified by continued passage in *Oryctolagus* rabbits, or it may represent infection due to a strain intrinsically different from other South American strains.

The representative of the standard laboratory strain which was maintained in the Rockefeller Institute in New York until 1951 (no. 4 of Table 1) was as virulent as the Australian standard laboratory strain, but it produced dark purple skin lesions intermediate in their protuberance between those associated with the Australian standard laboratory strain and those caused by the Lausanne strain. This fact suggests that the strain used in Australia may have departed still further from an original which may have been like the Lausanne strain.

The two Californian strains differed greatly in their properties from the South American strains. The symptomatology of the disease was much less florid than in infections with either South American or Australian strains, yet all rabbits had died by the fourteenth day, usually before pronounced symptoms characteristic of myxomatosis were in evidence. The symptomatology observed in rabbits infected with these two strains differs appreciably from that illustrated in the publication of Kessel, Fisk & Prouty (1934). Their illustrations show a disease which bears a much closer resemblance to that caused by other virulent strains of myxoma virus. However, our findings are in agreement with those of McKercher with the same strains (personal communication, 1956).

Cross-immunity between strains of myxoma virus from different parts of the world

Numerous experiences in the field have shown that rabbits which have recovered from infections with the Australian field strains of virus are immune to re-infection with either the standard laboratory strain of virus or the French strain (no. 69 of Table 1; see also Fenner *et al.*, 1957). From time to time rabbits which had recovered from infection with various known strains of myxoma virus, or with fibroma virus, were challenged with other strains under laboratory conditions. The results of such heterologous challenge infections are set out in Table 13. They show that there is a high degree of cross protection between strains of myxoma virus originating from different parts of the world. Neutralization tests on the chorioallantoic membrane with sera from rabbits which had recovered from infection with various strains of virus showed that such sera neutralized strains of virus originating from all parts of the world. With unabsorbed sera the only possible antigenic difference which has been detected between any of the strains of myxoma virus tested is the less effective neutralization of the Nottingham attenuated strain of virus (Table 8).

DISCUSSION

The genetic basis for the virulence of myxoma virus for *Oryctolagus cuniculus* is an intriguing problem which is beyond the scope of the present paper. Here we have been concerned with the development of a laboratory method for studying virulence which will provide an accurate measure for field events; and with the description of the variations in virulence and symptomatology which have occurred during natural transmission of the disease in Australia, Europe and America.

We owe our present concept of the origin of myxomatosis to Aragão, who showed in 1942 that the tapeti of South America, *Sylvilagus braziliensis*, was a natural host of the virus. In this species the disease is benign, rarely causing death

Table 13. *Cross-immunity tests between strains of myxoma virus from different parts of the world*

Strain of virus causing initial infection	Strain with which recovered rabbits were challenged	No. of rabbits	Response			
				Nodule at inoculation site		
			Nil	Small	Large	Generalized symptoms
Aust/Corowa/12–52/2 (KM13), no. 30*	Standard laboratory strain, no. 1	6	0	6	0	0
	Brazil/Campo Grande/12–53/1, no. 89	6	3	3	0	0
	U.S.A./San Francisco/1950/1 (MSW), no. 91	5	5	0	0	0
	Brazil/Campinas/1949/1 (Lausanne), no. 7	6	1	4	1	0
Aust/Uriarra/2–53/1 (Uriarra), no. 42	Standard laboratory strain, no. 1	7	7	0	0	0
Australian field strains (captured immune wild rabbits)	Brazil/Campinas/1949/1 (Lausanne), no. 7	5	2	1	2	0
	France/Eure/4–54/1, no. 73	1	0	0	1	Slight
Neuromyxoma, no. 6	Standard laboratory strain, no. 1	2	0	2	0	0
	Brazil/Campinas/1949/1 (Lausanne), no. 7	2	0	2	0	0
	U.S.A./San Francisco/1950/1 (MSW), no. 91	2	0	2	0	0
England/Nottingham/4–55/1, attenuated, no. 86	Standard laboratory strain, no. 1	5	0	5	0	0
	U.S.A./San Francisco/1950/1 (MSW), no. 91	5	1	4	0	0

* Number of strain refers to numbers given in Table 1.

except in young animals inoculated with a large dose of virus (Aragão, personal communication, 1955), and the principal clinical features are the single or few tumours, 0·2–1 cm. in diameter, which appear on any part of the body after an incubation period of 6–12 days, and regress after a period of 2–6 weeks. Occasionally a larger flat skin tumour develops. These lesions are infectious for mosquitoes.

We have been able to examine three strains of myxoma virus from Brazil which have been passed only a few times in laboratory rabbits (*Oryctolagus cuniculus*), and may therefore be regarded as representing the virus as it circulates in tapeti. They are strains 7, 89 and 90 of Table 1, Brazil/Campinas/1949/1 (Lausanne), Brazil/Campo Grande/12–53/1 and Brazil/Jacarépaguá/4–54/1. All three are highly virulent for the European rabbit (mean survival times 12·9, 10·0 and 12·5 days) and the clinical symptoms of infections of *Oryctolagus* are indistinguishable, skin lesions being very large and protuberant, and deep purple in colour (Pl. 3, fig. 5). Other reports of outbreaks of myxomatosis in European rabbits in South America (Sanarelli, 1898; Aragão, 1927, 1943) suggest that this extreme virulence and florid symptomatology are characteristic of the disease produced by virus as it comes from *Sylvilagus* rabbits. For instance, Aragão (1943) illustrates the 'very characteristic high tumour' produced by mosquito-bite infection of a domestic rabbit, and illustrations in Aragão (1927) show the same feature.

The strains of virus obtained from the field in Europe are derived by natural *Oryctolagus-Oryctolagus* passage of one of these South American strains (Brazil/Campinas/1949/1 (Lausanne)). Virulence and symptomatology were unchanged for many months, in spite of the spread of the infection over a large part of Europe and the occurrence of millions of cases of the disease. Eventually, however, some attenuated strains were recovered. Strain no. 76 of Table 1, France/Loiret/4–55/1, is associated with a mean survival time of 33·1 days, and infected rabbits develop fantastically large and numerous skin tumours (Pl. 3, fig. 6). The England/Sussex/9–54/1 strain (no. 80 of Table 1) is slightly attenuated (mean survival time 21·5 days) and the skin lesions develop very slowly and become raised and nodular only after the twelfth day of the disease. The France/Sologne/8–55/1 (no. 77 of Table 1) is similar. The attenuated component of the Sherwood Forest complex (England/Nottingham/4–55/1, attenuated; no. 86 of Table 1) is much less virulent (mortality rate 23 %), but is frequently associated with the development of very protuberant nodular tumours. The pocks produced by this strain on the chorioallantoic membrane are also smaller than those of most other strains (Pl. 1, fig. 3). Thus within the last year, and by a superficial sampling, several different attenuated strains have been recovered, and they are now widespread in France (Jacotot *et al.*, 1956) although still rare in England (Mansi, personal communication, 1956). All that we have examined, with the possible exception of the Sussex strain, show the same florid proliferative skin lesions which we regard as characteristic of the parent Lausanne strain. Our experience has confirmed the observation of Andrewes *et al.* (1956) that the skin lesions produced by the strain England/Sussex/10–54/1 (no. 81 of Table 1) are relatively flat. They do not differ greatly from those found with some attenuated Australian strains.

Little information is available on myxomatosis in California. It has been said that the virus was introduced with a shipment of rabbits from Mexico in about 1930 (Vail & McKenny, 1943), and outbreaks have occurred from time to time in domestic rabbitries in all the West Coast states. The situation suggests strongly that the virus has found a reservoir host among the local native rabbits of the area, so that an enzootic situation has now developed, not unlike that in Brazil. As far as one can judge from the illustrations published by Kessel *et al.*, (1934), the disease produced in European rabbits differed little from that found with the South American strains. Viruses obtained in 1949 and 1950 in San Diego and San Francisco (California U.S.A./San Francisco/1950/1 (MSW) and California U.S.A./San Diego/1949/1 (MSD)) produce a highly lethal disease with slight general symptoms of myxomatosis, and the pocks which develop on the chorioallantois are minute (Pl. 1, fig. 4). It is possible that under the influence of natural selection in the unknown reservoir host in California the virus has deviated from its original character.

The greatest known epizootics of myxomatosis in *O. cuniculus* are those which have occurred annually in Australia since the summer of 1950–51. They were initiated by a strain of virus which had been passed in laboratory rabbits (usually in massive doses) for nearly 40 years before it became established in the Australian wild rabbit population. Two sub-lines of this strain have been compared, the Australian standard laboratory strain (no. 1 of Table 1) and the line which has been maintained since 1924 in the Rockefeller Institute for Medical Research in New York (no. 4 of Table 1). Both are highly lethal (mean survival times of 10·8 and 10·5 days respectively). The skin lesions associated with infection by these strains are much flatter and less protuberant than those characteristic of the Brazilian strains. The surface of the primary lesion produced by the Rockefeller Institute sub-line is characteristically deep purple in colour, and this feature is only occasionally seen with the standard laboratory strain. Among the one laboratory and 65 field derivatives of the standard laboratory strain which have been studied (Table 1), five levels of virulence have been recognized. One group of field strains differed only slightly in virulence and in symptomatology from the standard laboratory strain (grade II of Table 11), but the others, of which KM 13 (grade III virulence) and Uriarra (grade IV virulence) are prototypes, were distinctly less virulent. The laboratory variant, neuromyxoma, is so attenuated that it does not kill rabbits or even make them sick when a small dose is inoculated into one skin site. Up to November 1955 no field strain had been recovered in Australia which killed less than 30% of rabbits inoculated with a small dose of virus. All derivatives of the standard laboratory strain resembled it, and differed from the Brazilian strain and its derivatives, in the flat nature of the skin lesions (Pls. 2 and 3).

The very uniform nature of the response of wild and laboratory rabbits to the highly virulent standard laboratory strain of virus is attested both by the times of appearance of different signs (generalization, closure of the eyes, etc.) and by the narrow range of the survival times (mean 10·8 days, 95% range 9·3–14·1 days). If large doses are used to initiate infection the uniformity is still more pronounced.

This uniformity of response led to the view that the innate resistance of *O. cuniculus* to myxomatosis was uniformly very low, and in the early studies of moderately attenuated field strains (Mykytowycz, 1953; Jacotot *et al.*, 1955*a*, *b*) the variability of the clinical picture in different rabbits was interpreted as evidence that the infections were due to a mixture of a highly virulent and an attenuated strain, the symptomatology in different rabbits depending upon the dominating virus in the inoculum. The pure clone studies with Uriarra, KM13 and Loiret 55 strains described here show that the variability in the rate of progression of symptoms, and in survival times, is the same in infections with several pure clones of each strain as it is in infections with the parent material. Our interpretation, therefore, is that there are considerable variations in the innate resistance to myxomatosis of unselected populations of *Oryctolagus*, both laboratory and wild, but the expression of these differences is obvious only when the virulence of the virus is somewhat reduced. Studies in progress in our laboratories, using one strain of virus (KM13), and the progeny of wild rabbits which have been selected to a varying degree by exposure to the disease, show that the variation in symptomatology increases with the increasing intensity of the selection for resistance, and it is possible that highly selected wild rabbit populations may now exhibit a wider range of symptomatology and survival time when challenged with the standard laboratory strain.

The suitability of the pure clone method for demonstrating the existence of a mixed infection with a highly virulent and an attenuated strain is demonstrated with our results with the strain England/Nottingham/4–55/1, which was sent to us as freeze-dried virus from the first laboratory passage of material taken in the field. The reconstituted dried virus, inoculated in very small doses in laboratory rabbits, produced three types of response. Some rabbits died with symptoms indistinguishable from those associated with the highly virulent European strains (prototype Lausanne), some suffered a mild disease characterized by the development of scattered nodular tumours, and some suffered a more severe, but modified disease. Passage at high dilution from a rabbit showing the severe symptoms bred true, and passage from a nodule of the mild type of the disease produced the mild or the modified severe type of disease. The interpretation that the original material contained approximately equal amounts of the highly virulent and an attenuated strain was borne out by the results of the inoculation of rabbits with pure clone material. Furthermore, the clone selected on the basis of a relatively large single pock produced the classical severe disease, whereas two clones derived from small pocks produced the nodular type of disease. It will be recalled that the mean pock size of the Nottingham attenuated strain is distinctly smaller than that of the virulent European strains. The relatively severe but modified infections are probably due to infection of an innately highly susceptible rabbit with the attenuated strain. The epidemiological situation which permits the natural passage and maintenance of a mixture of strains of differing virulence calls for detailed study, and the pure clone method described here, which utilizes differences in pock morphology, should be useful in such investigations.

The basic differences in the symptomatology of rabbits infected with the highly virulent and attenuated strains are the more rapid progression of all symptoms in

infections with the virulent strains, and the widespread oedema around all skin lesions, so that the tumour due to cell proliferation shades imperceptibly into the surrounding normal tissue. Symptoms similar to those produced by attenuated strains in normal rabbits are produced by infection of passively immunized rabbits with the fully virulent strain (Fenner & Marshall, 1954), or rabbits which had been immunized some months earlier with the OA strain of fibroma virus (Fenner & Woodroofe, 1954).

In Australia it appears that a disease with about a 90% mortality rate, and characterized by numerous skin lesions containing large amounts of virus, became dominant quite early, and has maintained its dominance in spite of the occurrence of occasional more attenuated strains, and of the annual large-scale reintroduction of fully virulent virus. If the genetic resistance of the wild rabbit population were to rise appreciably this disease syndrome might be produced by the fully virulent virus, but there is as yet no field evidence of this.

There are several possible explanations for the earlier and more frequent appearance of attenuated variants in Australia compared with Europe. First, the strains of virus originally introduced were different, and they may well vary in their genetic stability as far as virulence is concerned. Certainly almost all attenuated variants recovered from Europe retain the high raised skin lesions characteristic of virulent European strains, whereas Australian attenuated strains produced flat skin lesions. Secondly, only one small-scale purposeful introduction of the virulent virus was made in Europe, whereas in Australia the standard laboratory strain is introduced annually on a large scale and over the greater part of the rabbit-infested country in the Commonwealth. Thirdly, the important mode of transmission in Australia is by mosquito bite, whereas in England at least the rabbit flea, *Spilopsylla cuniculi*, is said to be the principal vector (Lockley, 1954; Armour & Thompson, 1955). It seems to us likely that mosquitoes do play a role in transmission in continental Europe. Differences in the mode of transmission could influence the type of disease favoured by selection. Mosquito transmission would be most effective with a disease which combined numerous persistent skin lesions containing high concentrations of virus with reasonably long survival time, i.e., moderately attenuated virus strains (Fenner *et al.*, 1956). More attenuated strains, associated with rapid healing of skin lesions, would fail to spread in competition with the moderately attenuated strains, just as the highly virulent strains would remove infectious rabbits too rapidly by death. If fleas rarely migrate except from a dead host, flea transmission might be expected to favour the spread of lethal infections, i.e., of virulent strains. It is also possible that some environmental difference may render the establishment of attenuated strains in competition with virulent ones more likely in Australia than Europe. Whatever the reason for the delayed appearance of attenuated strains in Europe they are now relatively common and widespread (Jacotot *et al.*, 1956).

During 1953 susceptible wild rabbits captured near Canberra were used for virulence tests, owing to a shortage of laboratory rabbits at that time. The information presented in Table 1 and Text-fig. 1 shows that the differentiation of virulence established with laboratory rabbits was paralleled in tests on wild rabbits, although

the latter lived slightly longer. Experiments to be reported elsewhere show that wild rabbits from some parts of Australia which have experienced several annual outbreaks of myxomatosis are considerably more resistant than laboratory rabbits, and in Australia all future tests on virus virulence will have to be carried out with laboratory rabbits.

SUMMARY

Strains of myxoma virus associated with a case-mortality rate in wild rabbits of 90–95 % can be distinguished from those associated with a case-mortality rate of more than 99 % by the use of a standardized virulence test carried out in groups of five laboratory rabbits, and tests carried out on laboratory rabbits are closely correlated with the results obtained with Australian wild rabbits. The test, which attempts to mimic natural mosquito transmission, consists of the intradermal inoculation in one site of about 5 rabbit-infectious doses of the virus, and the subsequent observation of the rate of progress of clinical symptoms and ultimate fate of the rabbit. The mean survival time provides a figure which allows satisfactory classification of all known myxoma virus strains into one of four grades of virulence, very attenuated strains being recognized by their low case-mortality rates.

Using this test, 92 strains of myxoma virus have been compared. These comprised 6 laboratory strains, 62 strains recovered from Australian wild rabbits or from wild caught mosquitoes, between February, 1951 and March, 1955, 19 strains recovered from naturally infected rabbits in Europe between October, 1953 and August, 1955, 3 strains from South America, and 2 from California. Eight types have been described and illustrated, differentiation being based on their virulence and the clinical picture of the disease they cause in laboratory rabbits. The differences are summarized in Text-fig. 2. Four of the virus types produce recognizably different pocks on the chorioallantois of developing chick embryos.

In Australia the repeatedly introduced standard laboratory strain of virus has been replaced by a moderately virulent strain, which appears to be better adapted for survival than either the highly virulent standard laboratory strain, or less virulent variants of which only a few had been recovered up to 1955. Strains of the type dominant in 1953–55 have appeared independently in many widely separated parts of Australia. Examination of these attenuated variants by pure clone techniques showed that the variability in symptomatology associated with them is not due to a mixture of virus strains, but to the expression of differences in innate resistance of the host animals which are obscured by the overwhelming virulence of the highly virulent strains.

Only one introduction of a different strain of virus was made in Europe. All field strains recovered during the first two years after the introduction were of high virulence, but a few attenuated strains appeared in 1955, and they have since become widespread. One of them appears to be a relatively stable mixture of a highly virulent and a greatly attenuated strain.

The collection of the large numbers of strains of virus described in this paper has been made possible only by the generous collaboration of many people. In

Australia we would like to acknowledge the great help afforded us by Mr F. N. Ratcliffe, officer-in-charge of the Wildlife Survey Section, Commonwealth Scientific and Industrial Research Organization and his officers, especially Messrs J. H. Calaby, G. Douglas, A. L. Dyce, B. V. Fennessy, E. W. Lines, K. Myers, R. Mykytowycz, W. E. Poole and E. Waterhouse. Messrs B. D. Robinson and T. Pearce of the Lands Department of Victoria have also provided us with specimens, as have Mr G. Edgar, Director of the Veterinary Research Station, Glenfield, New South Wales, Dr D. Surrey Dane of the Institute of Medical and Veterinary Science, Adelaide, Mr D. Gooding, Department of Agriculture, Western Australia, and Messrs T. B. Alexander and A. F. Ryan, Department of Agriculture, Tasmania.

We are indebted to Dr H. de B. Aragão of the Instituto Oswaldo Cruz, Rio de Janeiro, and Dr B. Szyfres, of the Laboratorio de Biologia Animal, Uruguay, for specimens from South America; Dr D. G. McKercher of the University of California School of Veterinary Medicine, Davis, California, for the Californian strains; Drs P. Lépine and H. Jacotot, of the Institut Pasteur, Paris, for strains from France; and Mr J. R. Hudson, of the Veterinary Laboratory, Weybridge, and Dr C. H. Andrewes of the National Institute for Medical Research, Mill Hill, for material from England.

We are grateful to Dr S. Fazekas de St Groth and Dr G. S. Watson of this University for advice on the statistical treatment of the data.

Mr V. Paral took the photographs.

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EXPLANATION OF PLATES 1–3

PLATE 1

Appearance of pocks produced by the growth of different strains of myxoma virus on the chorioallantoic membrane. Twelve-day-old eggs incubated at 35° C. for 3 days after inoculation. The density of pocks on all the membranes illustrated (except standard laboratory strain) is considerably greater than usually used for pock-counting, or for the measurement of pock size.

Fig. 1. Standard laboratory strain, mean diameter 0·40 mm.
Fig. 2. Neuromyxoma strain, mean diameter 0·24 mm.
Fig. 3. England/Nottingham/4–55/1, attenuated, mean diameter 0·26 mm.
Fig. 4. U.S.A./San Francisco/1950/1 (MSW), mean diameter 0·16 mm.

PLATES 2 and 3. Appearance of laboratory rabbits infected by the standard method with each of the eight prototype strains of myxoma virus. The time selected for photography in each case was the time of maximum development of the lesions characteristic of each strain.

PLATE 2

Fig. 1. Standard laboratory strain, 10 days after inoculation. Rabbit died later the same day. Note the completely closed eyes and profuse conjunctival discharge, general oedema of the head, and lack of clear definition of the edges of the primary lesion.
Fig. 2. Aust/Corowa/12–52/2 (KM13), 21 days after inoculation. Rabbit died on the twenty-eighth day. Note that the eyes are not quite closed though there is considerable oedematous swelling of the head, and that the primary lesion and nearby secondary skin lesions are flat and clearly demarcated from the adjacent normal skin.
Fig. 3. Aust/Uriarra/2–53/1 (Uriarra), 24 days after inoculation. Rabbit recovered. The eyes are not closed although the lids are deformed with nodules, and there is little general oedema of the head. Secondary lesions are numerous and they and the primary lesions are relatively flat and clearly demarcated from the adjacent normal skin.
Fig. 4. Neuromyxoma, 10 days after inoculation. The rabbit recovered and was never sick at any stage. The primary lesion is clearly demarcated from the adjacent normal skin, and is already regressing, the centre being depressed and dark purple in colour. There are very few secondary lesions in the skin, and the only abnormality on the face is one small nodule on the right upper eyelid.

PLATE 3

Fig. 5. Brazil/Campinas/1949/1 (Lausanne), 10 days after inoculation. The rabbit died on the twelfth day. The primary lesion is very large, hard, protuberant and deep purple in colour. The head is very oedematous and the eyes are completely closed with a profuse conjunctival discharge. Secondary lesions are raised and purple in colour and are not demarcated from the surrounding skin.

Fig. 6. France/Loiret/4–55/1 (Loiret 55), 25 days after inoculation. The rabbit died on the twenty-sixth day. The primary lesion is very large and exudes serum. Secondary lesions are very numerous and occur all over the body. The eyes are completely closed by deformed nodular eyelids and the ears hang down due to the numerous nodules on the pinnae.

Fig. 7. England/Nottingham/4–55/1, attenuated, 23 days after inoculation. The rabbit survived. The primary lesion is very large and is clearly demarcated from the adjacent normal skin. There is a moderate number of secondary lesions which resemble the primary lesion in their protuberance and the clear demarcation from the surrounding skin. In spite of the numerous nodules on the eyelids the eyes were never closed and there was no general oedema of the head.

Fig. 8. U.S.A./San Francisco/1950/1 (MSW), 7 days after inoculation. Rabbit died on the eighth day. The primary lesion is small, with indefinite edges. There are a few small secondary lesions in the skin and the eyelids are slightly swollen, with a thin conjunctival discharge. External lesions were rarely more severe than shown in the photograph, but the disease was invariably rapidly fatal.

(*MS. received for publication* 30. VII. 56.)