STUDIES IN THE EPIDEMIOLOGY OF INFECTIOUS MYXOMATOSIS OF RABBITS*

V. CHANGES IN THE INNATE RESISTANCE OF AUSTRALIAN WILD RABBITS EXPOSED TO MYXOMATOSIS

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(With 1 Figure in the Text)

The host-parasite balance in myxomatosis of *Oryctolagus cuniculus* may be influenced by several factors—changes in the virulence of the virus, alterations in the innate resistance of the host, actively or passively acquired immunity, or certain environmental factors (Fenner, 1953). Attenuation of the virus has already been demonstrated to have occurred in most parts of Australia, and more recently in Europe also (Fenner & Marshall, 1957). The epidemiological consequences of antibody transferred from immune mothers to their young have been discussed elsewhere (Fenner & Marshall, 1954). This paper records experiments designed to measure variations in the innate resistance of rabbits whose forbears had been exposed to myxomatosis for different numbers of generations.

MATERIALS

Myxoma virus

Two strains of myxoma virus were used in the laboratory experiments, the highly virulent standard laboratory strain and the slightly attenuated strain Aust/Corowa/12–52/2 (KM 13) (Fenner & Marshall, 1957).

Many ampoules of one preparation of each strain of virus were stored in a dry-ice cabinet throughout the experiments, one ampoule being used for each batch of rabbits tested.

Rabbits

Laboratory rabbits, bred in the University Animal Breeding Establishment were used when 4 months old. These animals were from stock which had never been exposed to myxomatosis. They had been shown earlier to react in very nearly the same fashion to both virulent and attenuated strains of myxoma virus as wild rabbits which had never been exposed to the disease (Fenner & Marshall, 1957).

Australian wild rabbits were caught from each of the several different parts of Australia described below, and from Macquarie Island, weanling kittens being taken where possible. In 1953 samples were usually collected by digging out rabbit

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warrens, and this probably introduced some bias due to the inevitable collection of siblings. At Lake Urana in 1954 and 1955 rabbits were captured by netting at night while they were immobilized in a spotlight beam. As the vehicles used for this purpose were able to traverse the whole experimental site these samples could be regarded as random. The young rabbits were transported to the laboratories in Canberra. Here they were numbered and tagged, bled, and the serum tested for myxoma antibodies. They were reared until they were about 4 months old and had attained a live weight of at least 1·2 kg. Bleeding was deferred until just before the challenge inoculation in 1954 and 1956 (Lake Urana and Maryvale Station), and in 1955 the rabbits from Lake Urana were not bled.

It has not yet been possible to construct life-tables for Australian wild rabbits, but the field data available suggest that very few animals survive through a second breeding season (Ratcliffe, personal communication). Practically all the kittens captured each spring are thus the progeny of rabbits which have survived the epizootic of the previous summer, but have not been exposed to earlier outbreaks of myxomatosis.

It is quite difficult to maintain wild rabbits in the laboratory and about half the captured rabbits died during the first few weeks of adjustment to captivity. In 1953 four cases of myxomatosis occurred within a week of arrival of the different batches of rabbits, but their immediate removal at the earliest sign prevented secondary cases. One rabbit in the group from Lake Urana in 1956 developed myxomatosis shortly after arrival at the laboratory. Although it was removed when first noticed there were four more isolated cases over a period of about 6 weeks.

Several observers (Mykytowycz, 1956; Sobey, personal communication) have commented on the adverse effect of cold upon rabbits suffering from myxomatosis. All the experiments described in this paper were conducted during the summer months, and no unusually high or unusually low temperatures were recorded. The rabbits were fed on a dry pellet diet, supplemented with unlimited water and ample fresh green feed, although supplies of the latter were limited during the early months of 1954. No rabbit was inoculated with virus unless it appeared to be in good physical condition, for earlier experience had confirmed Houlihan & Derrick's (1945) observation that thin or sickly rabbits reacted abnormally to the infection.

METHODS

Virus, antibody and antigen titration

Virus was titrated on the chorioallantoic membrane of developing chick embryos, as described previously (Fenner, Marshall & Woodroofe, 1953).

Sera were tested for the presence of antibody by complement-fixation, and by neutralization on the chorioallantoic membrane (Fenner *et al.* 1953), and for complement-fixing antigen as described by Fenner & Woodroofe (1953).

Preparation of rabbits for challenge inoculation

Rabbits were not challenged until they were 4 months old and weighed at least 1·2 kg. This course of action was taken for several reasons. First, at that age normal susceptible rabbits react like adult animals, and lose the greater susceptibility encountered in younger animals (Fenner & Marshall, 1954). Secondly, passively acquired antibodies, if present initially, would have disappeared by the end of the fourth month (Fenner & Marshall, 1954). Thirdly, it took a considerable time for wild rabbits to become accustomed to feeding and drinking under laboratory conditions, but by the end of 2 or 3 months in captivity animals which had failed to accommodate themselves had died.

Determination of the specific nature of deaths in inoculated rabbits

In experiments carried out with captive wild rabbits difficulties sometimes arose in the assessment of the significance of deaths occurring towards the end of the second week after inoculation. Deaths before the twelfth day were invariably non-specific. In fatal cases occurring between the thirteenth and the eighteenth day the serum was tested for the presence of complement-fixing antigen, for it was found earlier (Fenner & Woodroofe, 1953) that in myxomatosis, as in smallpox, (Downie, McCarthy, Macdonald, MacCallum & Macrae, 1953), soluble antigen is present in the serum of acutely fatal cases. In cases in which the course was more prolonged the clinical course and autopsy findings provided reasonably good evidence of the specific cause of death.

Sources of rabbits

'Normal' wild rabbits

The universal distribution of myxomatosis throughout the rabbit-infested parts of Australia at the time these investigations commenced made it impossible to collect young wild rabbits with any assurance that none of their forbears had recovered from the disease. To obtain 'normal' wild rabbits recourse was had to animals bred in the Animal Breeding Establishment of the Australian National University from a parent stock of two bucks and three does obtained from the Institute of Anatomy, Canberra. These rabbits, and as far as could be ascertained their progenitors, had never been infected with myxomatosis. This narrow parental range, which has necessitated a high degree of inbreeding, makes it uncertain how accurately these rabbits may reflect the pre-1950 genetic resistance of the Australian wild rabbit to myxomatosis.

To obtain further information of the innate resistance of rabbits from a population which had never been exposed to myxomatosis, we arranged for a small consignment from the colony on Macquarie Island (latitude 54° S., longitude 159° E.). Scott visited the island in 1880 and recorded that the colony was derived from a few tame parti-coloured rabbits released on the island 'a few years ago'. (Scott, 1882). Twelve rabbits were captured by Mr K. Keith of the Wildlife Survey Section of the Commonwealth Scientific and Industrial Research Organization, and were shipped to Canberra in December 1956 by the courtesy of the Antarctic Division of the Commonwealth Department of External Affairs.

Yarram

October 1953 *collection.* Forty-four kittens were collected from foothill country near the coast of southern Victoria by Mr G. Douglas of the Victorian Lands Department. Myxomatosis appeared in the area for the first time during the summer of 1952–53 after an inoculation campaign in which the standard laboratory strain of virus was used. Few rabbits remained after the conclusion of the epizootic, but only about 30 % of the surviving adult population sampled in August 1953 were immune. The situation was not unlike that at Lake Urana after the first epizootic (Myers, Marshall & Fenner, 1954). The majority of the kittens collected were therefore the progeny of normal parents which had not been subjected to selection for resistance to myxomatosis.

Lake Urana

The history of myxomatosis in the area has been described in detail elsewhere (Myers *et al.* 1954; Fenner, Poole, Marshall & Dyce, 1957). Briefly, myxomatosis did not occur at Lake Urana in the first season (1950–51) but there were severe outbreaks each succeeding summer. Serological surveys of the surviving adult population each autumn showed that 22 % of the survivors of the first epizootic were immune, and practically all animals tested after the later epizootics had recovered from myxomatosis. The 1951–52 outbreak was initiated by the inoculation of local rabbits with the standard laboratory strain, but all virus strains recovered from the area since November 1952 have been found to be attenuated except for strains of the virulent French type recovered after the experimental release of this virus in November 1954 (Fenner *et al.* 1957). The results of standard virulence tests on all strains recovered from Lake Urana during the second, third, fourth and fifth epizootics are shown in Table 1.

Table 1. *The virulence of virus strains causing epizootics of myxomatosis in areas from which young rabbits were obtained for challenge inoculation*

Locality	Progeny group (Table 2)	Epizootic number	% of selected (immune) parents	Grades* of virulence of virus strains recovered during epizootic				
				I (99·5 %)†	II (99 %)	III (90 %)	IV (60 %–70 %)	V (0–30 %)
Yarram	C	1	30	1	—	—	—	—
Urana	—	1	22	1	—	—	—	—
Urana	H	2	100	—	2	6	—	—
Urana	I	3	100	—	—	1	—	—
Urana	J	4	98	70 % of cases‡	—	30 % of cases‡	—	—
Urana	K	5	95	—	2	5	5	4
Maryvale	G	5	62	—	—	2	—	—

* Grades of virulence as in Fenner & Marshall (1957), Table 2.

† Approximate case-mortality rates associated with the infection of normal laboratory rabbits with trains belonging to the virulence grade indicated.

‡ See Fenner, Poole, Marshall & Dyce (1957).

December 1953 *collection.* Seventy kittens were collected by digging out warrens in sandhills adjacent to the Lake. These were the progeny of immune parents, and about one-quarter of their grandparents were immune.

September 1954 *collection.* 350 rabbits were collected by netting animals immobilized at night in the beam of spotlights. 106 of these, judged to be adults, were challenged by the inoculation of a moderate dose of the French strain of virus, and 101 of these proved to be immune (Fenner *et al.* 1957). This figure confirmed the March 1954 serological survey which showed that practically all of the survivors of the 1953–54 epizootic were immune. 244 young animals were taken to Canberra and reared as described earlier, and 116 of these were available for the final tests.

September 1955 *collection.* There were many fewer rabbits in the area than in 1954, due to the high mortality rate associated with the widespread infection with the French type of virus the previous summer. Sixty-seven young rabbits were collected by netting animals immobilized in the beam of spotlights. Thirty-one of these survived for challenge infection in January 1956. The serological survey carried out late in February 1955 showed that virtually all animals then surviving, the parents of the young captured in September, had recovered from myxomatosis.

October 1956 *collection.* The rabbit population remained very small throughout 1956; the abnormally wet conditions presumably hampered breeding. The wet conditions also precluded the use of the night-netting technique over much of the area and nearly all of the rabbits obtained were dug out of warrens. Seventy young rabbits were collected, but fourteen of these were immune. Thirty-five rabbits survived for challenge infection. The serological survey in 1956 again showed that practically all the parents of this group had recovered from myxomatosis.

Mr W. E. Poole of the Wildlife Survey Section of C.S.I.R.O. was responsible for collection of the kittens on all occasions, assisted in 1954, 1955 and 1956 by a team from the Australian National University.

Albury

July 1953 *collection.* Sixty-seven kittens were collected by Mr K. Myers of Wildlife Survey Section of C.S.I.R.O. in foothill country close to the Murray River and about 20 miles downstream from Albury. Occasional sick rabbits were seen in the area in 1950–51, and major epizootics occurred in the two succeeding summers. Serological surveys showed that 20 % of the survivors of the 1951–52 and 94 % of survivors of the 1952–53 epizootic were immune. Thus, practically all the parents and 20 % of the grandparents of the kittens obtained in July 1953 had recovered from myxomatosis.

No virus samples were obtained from the area.

Barrenbox swamp

August–November 1953 *collection.* Sixty-one kittens were collected from swamp country on the Murrumbidgee Flats near Griffith, New South Wales, by Mr H. Frith of the Wildlife Survey Section of C.S.I.R.O. Three major summer epizootics had occurred in the area, and observations made in the last outbreak suggested to local

observers that a change in the host-parasite balance had occurred. A serum survey in March 1953, showed that practically all survivors of the 1952–53 outbreak were immune. No virus samples were obtained from the area.

Noorong station

September 1953 *collection.* Seventy-five kittens were collected by Mr B. V. Fennessy of the Wildlife Survey Section of C.S.I.R.O., from the western Riverina Plain country, 36 miles north-west from Barham, New South Wales. Violent outbreaks of myxomatosis occurred in the summer of 1950–51 and 1951–52 and greatly reduced the number of the rabbits. The rabbit population was built up somewhat by winter breeding in 1952, and another epizootic began in November, 1952. Just before Christmas the great majority of rabbits seen were obviously suffering from myxomatosis, but many of these rabbits recovered from the infection. A serum survey in January 1953 showed that all rabbits tested were immune. No virus samples were obtained from the area.

Maryvale station

September–October 1956 *collection.* Over 300 kittens were collected from low rolling hill country near Goroke in the northern Wimmera district of western Victoria. The first batch of over 200 kittens was collected by Mr T. Pearce of the Victorian Lands Department in September, but partly because of a very heavy infestation of helminths only twenty-eight survived the first few weeks of captivity. A second batch of ninety-eight kittens was dug out in October by a team from the Australian National University, and transported to the laboratory within 24 hr. They were immediately dosed with phenothiazine for helminth control, and subsequently with 'Nefco'* in an attempt to control an outbreak of coccidiosis. Seventy-six rabbits from this area were eventually included in the experiment.

Rabbits were in plague proportions in the area in August 1951 when inoculation of rabbits with the standard laboratory strain of myxoma virus was commenced; 600 rabbits were inoculated by December 1951 when an explosive epizootic developed which drastically reduced the rabbit population. Since then there have been annual inoculations of rabbits with the standard laboratory strain, although usually on a very small scale. There have been annual epizootics at the beginning of each summer and although these have controlled the rabbit population adequately, there are still foci of dense rabbit infestation associated with the sand drifts which are scattered throughout the district.

In April 1955 a serological survey indicated that 79 % of the rabbits in the area were immune and in July 1956, 62 % were immune. Two virus strains were obtained during the 1955–56 outbreak and the results of virulence tests on these are included in Table 1.

Statistical treatment of results

The percentage mortality in each group of rabbits has been used as a measure of the innate resistance of that group. In the graph relating this to the number of

* Brand of Nitrofurazone manufactured by Messrs A. and G. Nicholas Ltd.

epizootics the 95 % confidence intervals have been taken from the table of Clopper & Pearson (1934).

The virulence of strains of myxoma virus was assessed by comparing the mean *survival times of groups of 'normal' rabbits challenged with small doses of the* virus, (Fenner & Marshall, 1957). The metameter $y = \log_{10}$ (survival time in days $- 8$) has been used to render variances homogeneous (Kapteyn, 1903).

EXPERIMENTAL RESULTS

Evidence of passive or active immunity in test rabbits

Passively acquired antibody to myxoma virus was detected in forty of the 312 sera obtained from weanlings collected during 1953 and 1954. The positive sera came from the youngest age groups and the titre of complement-fixing antibody was never high (usually 1/5–1/10). Only fourteen of these forty kittens were successfully reared to the age of 4 months and these reacted normally to challenge infection with the usual small dose of myxoma virus. Only one recovered. This result is consistent with the laboratory observation that passively acquired maternal antibodies are capable of modifying the disease syndrome for only a few weeks after birth (Fenner & Marshall, 1954).

The rabbits of the 1955 collection from Lake Urana (group J of Table 2) were not bled before challenge. Three which failed to react to the initial inoculation were bled 6 days later. Two showed complement-fixing antibodies at titres (1/40, 1/80) consistent with experience of a mild disease some months earlier and it is possible that they were infected in the field whilst partially protected by maternal antibodies. They were immune to subsequent challenge and were excluded from the group. The serum of the third rabbit did not contain antibodies, and the animal reacted normally when challenged a second time with the same estimated small dose of virus; it subsequently recovered after experiencing a severe form of the disease. There is no evidence that passive or active immunity played any part in determining the response of rabbits included in the nine field groups tested.

Choice of virus strain

It was necessary to select an appropriate strain of virus for the challenge inoculations which would make it possible to detect small differences in the average innate resistance of different groups of rabbits. Too virulent a strain, like the standard laboratory strain or Brazil/Campinas/1949/1 (Lausanne) strain, would obscure small differences in innate resistance, whereas too attenuated a strain would make it impossible to assess host resistance by case mortality rates.

We therefore chose a strain of grade III virulence (about 90 % case mortality rate in laboratory rabbits)—the strain Aust/Corowa/12–52/2 (KM 13), which we had shown by pure clone methods (Fenner & Marshall, 1957) was not a mixed strain, as some other workers had suggested.

When they were at least 4 months old, and in good physical condition, the wild rabbits were challenged by the intradermal inoculation of about five rabbit-infectious doses of the KM 13 strain of myxoma virus in a shaved area of the right flank. The results of tests are summarized in Table 2.

Table 2. *The mortality rates of groups of Australian wild rabbits and laboratory rabbits inoculated intradermally with about five rabbit-infectious doses of myxoma virus strain* Aust/Corowa/12–52/2 (KM13)

	Locality of origin	Time of collection	No. of epizootics in locality prior to collection of rabbits	Immune rate* after epizootic number					No. of rabbits		% mortality
				1	2	3	4	5	Captured	Tested	
				(1) Normal controls							
A	Wild Rabbits	—	—	—	—	—	—	—	—	58	88
B	Laboratory Rabbits	—	—	—	—	—	—	—	—	93	89
				(2) Field collections							
C	Yarram	Oct. 1953	1	32/108	—	—	—	—	44	24	96
D	Albury	July 1953	2	9/46	44/47	—	—	—	48	19	74
E	Noorong	Sept. 1953	3	(35%)†	(90%)	25/25	—	—	75	37	81
F	Barrenbox	Aug.–Nov. 1953	3	(35%)	(90%)	101/102	—	—	61	38	79
G	Maryvale	Sept.–Oct. 1956	5	(35%)	(90%)	(100%)	41/52	36/58	308	76	70
H	Urana	Dec. 1953	2	25/114	61/61	—	—	—	70	41	88
I	Urana	Sept. 1954	3	25/114	61/61	201/201	—	—	244	116	80
J	Urana	Sept. 1955	4	25/114	61/61	201/201	82/84	—	67	31	45
K	Urana	Oct. 1956	5	25/114	61/61	201/201	82/84	40/42	70	35	54

* Numerator = number of sera containing complement-fixing or neutralizing antibody; denominator = number of sera tested.

† Estimated percentage immunity, based on Table 4.

The same virus preparation was used throughout the tests, but the percentage mortality in the groups of rabbits challenged during 1956 and 1957 (group G, Maryvale Station, groups J and K, Lake Urana) was considerably lower than in the groups challenged in 1954 and 1955. Conclusive evidence that this alteration in mortality rate was not due to a change in the virus used for inoculation is summarized in Table 3, which shows the response of the 'normal' rabbits included as controls with each test batch. Mortality rates, survival times, and symptomatology in these control animals were uniform throughout.

Table 3. *Response of 'normal' rabbits to challenge with about five rabbit-infectious doses of myxoma virus strain* Aust/Corowa/12–52/2 (KM 13)

Year of inoculation	Controls for groups*	No. of rabbits	Type of rabbit	% mortality	Mean survival time (in days) of fatal cases and its 95 % fiducial range		
1954–55	C, D, E, F, H	60	Laboratory	87	19·5	*21·1*	23·0
		58	'Normal' wild	88	21·7	*23·5*	25·5
1956	I	15	Laboratory	93	13·0	*22·3*	48·5
1957	J	18	Laboratory	94	12·9	*20·6*	40·5
		7	Macquarie Island	86	17·1	*20·3*	24·5

* Group lettering from Table 2.

Analysis of results

Rabbits over wide areas of Australia have been subjected to severe mortuary selection due to annual epizootics of myxomatosis, and the experiments described were designed to find whether such selection would cause successive generations of rabbits to become more resistant to the disease.

The incidence of recovered animals amongst the survivors of the first epizootic in an area was usually much lower than after succeeding outbreaks (Table 4) and since our interest is in selection pressure and the resulting change in the progeny we have taken outbreaks with a 30 %, etc., immune rate as 0·3, etc., of an epizootic. No information was available on the immune rate at Noorong (E), Barrenbox (F) and Maryvale (G) for the early epizootics, but on the basis of the information presented in Table 4 it has been assumed that there was a 35 % immune rate after the first epizootic, a 90 % immune rate after the second and a 100 % immune rate after the third. The mortality rates were plotted against these adjusted figures for the number of epizootics (selection pressure) as shown in Fig. 1.

The data presented in Fig. 1 were examined for correlation by a regression method using angle transformation, (Claringbold, Biggers & Emmens, 1953). A negative correlation between the intensity of selection and mortality rates was found to be highly significant, ($\chi^2 = 50{\cdot}2$, D.F. $= 10$, $P < 0{\cdot}001$). Since active and passive immunity of the tested rabbits has been excluded, environmental conditions were constant, and aliquots of the same preparation of virus were used for all tests, these results provide unequivocal evidence of an increase in the genetic resistance of the rabbits to infection with the slightly attenuated KM 13 strain of virus. The evidence derived from a consideration of the mortality rates is supported

Table 4. *Percentage immunity of survivors remaining after each of five epizootics in areas in which there was a grade I kill on each occasion*

	Epizootic no.									
	1		2		3		4		5	
Locality	No. tested	% immune	No. tested	% immune	No. tested	% immune	No. tested	% immune	No. tested	% immune
Dunroy*	44	23	—	—	—	—	—	—	—	—
Hiawatha†	49	22	—	—	—	—	—	—	—	—
Giffard†	66	59	—	—	—	—	—	—	—	—
Yarram‡	108	30	—	—	—	—	—	—	—	—
Rutherglen§	—	—	57	88	—	—	—	—	—	—
Deniliquin‖	—	—	18	72	—	—	—	—	—	—
Deniliquin‖	—	—	30	77	—	—	—	—	—	—
Kerang‖	—	—	69	87	—	—	—	—	—	—
Swan Hill‖	—	—	18	78	—	—	—	—	—	—
Noorong‡	—	—	—	—	25	100	—	—	—	—
Barrenbox‡	—	—	—	—	102	99	—	—	—	—
Albury‡	46	20	47	94	—	—	—	—	—	—
Bacchus Marsh†	42	95	121	90	—	—	25	92	—	—
Lake Urana‡	114	22	61	100	201	100	84	98	42	95
Weighted Average	35%		90%		100%					

* Marshall, Dyce, Poole & Fenner, (1955). † Marshall (unpublished). ‡ Table 2 of this paper. § Myers *et al.* (1954). ‖ Fenner *et al.* (1953).

by the observation that the number of moderate and mild cases of myxomatosis increased greatly in the Maryvale group of rabbits (group G) and the last two groups of rabbits from Lake Urana (groups J and K) as shown in Table 5.

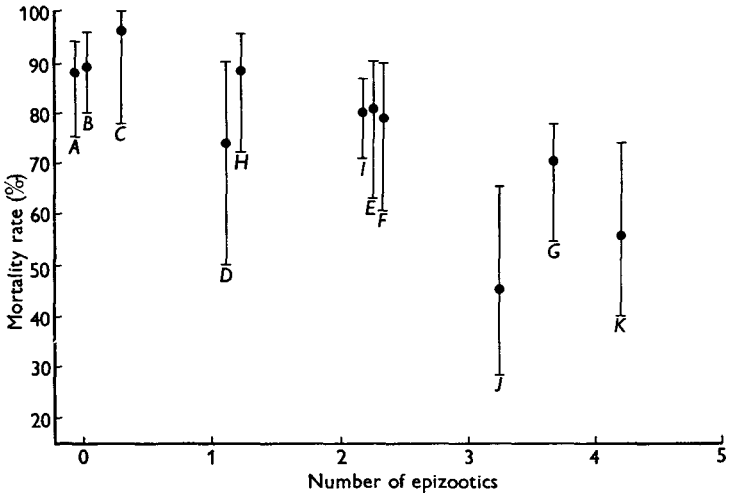

Fig. 1. The relation between mortality rates of Australian wild rabbits after challenge infection with small doses of the KM 13 strain of myxoma virus, and the selection pressure for increased genetic resistance exerted by exposure of the antecedents of the tested rabbits to severe epizootics of myxomatosis. Data from Table 2. Mortality rates and 95 % confidence limits shown. Ordinates—mortality rates (%). Abscissae—numbers of annual epizootics which occurred in areas from which rabbits were obtained, corrected for immune rates in survivors of each epizootic (see text). Group lettering as in Table 2.

Table 5. *The severity of myxomatosis in groups of wild rabbits inoculated with five rabbit-infectious doses of strain* Aust/Corowa/12–52/2 (KM 13)

	Symptomatology		
Group	Severe (including fatal)	Moderate	Mild
A Normal wild rabbits	93	5	2
G Maryvale, 1956	83	9	8
H Urana, 1953	95	5	0
I Urana, 1954	93	5	2
J Urana, 1955	61	26	13
K Urana, 1956	75	14	11

The complexity of factors operating in the behaviour of myxomatosis in these wild rabbit populations makes it inadvisable to attempt to calculate a figure for the heritability of resistance to myxomatosis.

DISCUSSION

There has been a good deal of experimental investigation of the inheritance of resistance to bacterial and virus diseases (reviews by Gowen, 1948, 1951), but very little precise information is available concerning changes in innate resistance to infectious diseases under natural conditions.

One of the few recorded examples of differences in the innate resistance of different populations of a single species of a wild mammal to a natural infection is plague in rats (Anon, 1912; Sokhey & Chitre, 1937). Wild rats were captured from many cities in India in which the recent experience of plague differed greatly. After maintenance for 2 weeks in the laboratory they were inoculated with a standard dose of plague bacilli. The mortality rates were inversely proportional to the recent experience of plague, and varied from 91 % for rats from cities with no plague for the previous thirty years, to 10 % for rats from cities with severe plague up to 2 years before the capture of the rats. The design of the later experiments (Sokhey & Chitre, 1937) appeared to exclude active immunization, and it seems likely that continued exposure to plague does exert a powerful selective effect on rat populations, although no information is available upon the time required to effect an appreciable change in resistance.

Intermittent epizootics of plague appear to have had no effect on the innate resistance of gerbils (*Tatera brantsi*), as far as can be recognized by observations on mortality rates in the field. Plague has been enzootic in the gerbils of northern Orange Free State, with epizootic episodes, since 1922. The observations of Davis (1953) showed that the gerbil population was still extremely susceptible to plague in 1940, and the same author noted that reports by officers of the anti-plague staff of the Union Health Department showed that the same pattern of temporary increase followed by population decline occurred throughout the next decade.

The occurrence in Australia of major epizootics of myxomatosis in *Oryctolagus cuniculus* has provided an unique opportunity to study, under natural conditions, the rapidity of genetic change in resistance to an infectious disease. The situation is particularly favourable because the strain of virus responsible for the initiation of the disease in Australia is available, as are many field isolates obtained since the initiation of the epizootics. The viruses can be preserved in an unchanged state for many years by storage at $-70°$ C. As far as the mammalian host is concerned the laboratory rabbit reacts to infection with virulent and attenuated strains of myxoma virus in almost the same way as Australian wild rabbits which have not been exposed to the disease (Fenner & Marshall, 1957). Progressive changes in the host and in the virus under field conditions can, therefore, be checked against control host animals and virus preparations.

Barber (1954) reported the existence of considerable local differences in fur colour in wild rabbits in different parts of Tasmania. He suggested that such rabbit populations may possess an amount of genetic variability affecting other characteristics which would allow them to adapt themselves quickly to myxomatosis, but no information is available, and none can now be obtained, concerning possible geographical differences in the initial innate resistance of wild rabbits to myxomatosis.

The results summarized in Fig. 1 and Tables 2 and 5 show that after four selective episodes (epizootics of myxomatosis) and four generations of breeding from selected parents considerable differences in innate resistance became apparent.

The most valuable data, on this as on many other aspects of myxomatosis, are derived from Lake Urana, due in large part to the intensive observation carried out

there over several years by the Wildlife Survey Section of the Commonwealth Scientific and Industrial Research Organization. The results of virulence tests of virus strains recovered from that area (Table 1) show that during the second and third epizootics there was practically universal infection with slightly attenuated strains of virus. The situation was complicated in 1954–55 by the large-scale introduction of the virulent French strain of virus (Fenner, Poole, Marshall & Dyce, 1957), and large-scale sampling of virus during the next summer (1955–56) showed a great range in virulence, with a number of highly attenuated strains.

The early predominance of attenuated strains was of great importance, for if the virulent standard laboratory strain were the only strain in the field the development of genetic resistance would be very slow, since very few animals would be left after an epizootic and many of the surviving bucks would be sterile (Sobey & Turnbull, 1956). The next generation would be sired largely by normal bucks which escaped infection and these progeny in turn would be practically wiped out in the succeeding epizootic, if this were due to a highly virulent strain of virus.

Slightly attenuated strains of virus have had a survival advantage over the more virulent strains, due to their better transmission by mosquitoes (Fenner, Day & Woodroofe, 1956). Significant numbers of rabbits remain after epizootics due to attenuated strains of virus, and as a number of the bucks have sustained a milder form of the disease they, as well as the recovered females, contribute to the build-up of genetic resistance in their progeny.

The experiments described show that in an area (Lake Urana) where there have been annual severe outbreaks of myxomatosis, the genetic resistance of the wild rabbits has increased after four epizootics to such a level that the mortality rate of rabbits infected with small doses of a slightly attenuated strain of virus has fallen from just under 90 % to about 50 %.

The rabbits collected from Lake Urana in September 1955 (group J) showed an unexpectedly low mortality rate and an unexpectedly high proportion of milder cases (Fig. 1 and Table 5). These animals were the progeny of the survivors of the 1954–55 outbreak, in which it was estimated that 70 % of the rabbits in the area were infected with the highly virulent French strain of virus (Fenner *et al.* 1957). It is possible that this more stringent selection left a greater proportion of highly resistant rabbits in the area than would have been the case if the French strain of virus had not been introduced on a large scale. Most of the strains of virus recovered from the 1955–56 outbreak at Lake Urana were of low virulence (Table 1) so that a much less stringent selection was exerted on the parents of group K.

There has as yet been no convincing evidence from the field to suggest that the increased genetic resistance is leading to increasing rabbit populations, but several factors combine to make early detection of such changes in the field difficult. First, observation except at a very few selected areas is casual in nature, and would only reveal large changes in population. Without detailed studies, including a serological survey and the examination of the strains of virus responsible for the epizootics, it is not possible to say whether a large rabbit population is due to increased innate resistance in the host, failure of the disease to spread effectively, or a high degree of attenuation of the virus. Secondly, most parts of the rabbit-infested country in

Australia have not been exposed to annual severe outbreaks of myxomatosis and selection is directly related to the infection rate in the survivors of each epizootic and to the number of epizootics. Thirdly, in order to standardize conditions for the challenge infections so that comparable results may be obtained in experiments made at yearly intervals, rabbits are maintained under the best attainable conditions and some animals survive which in the field would die or be killed.

Nevertheless, it is clear that genetic resistance to myxomatosis is being built up by the Australian wild rabbit population at a relatively rapid rate, and must soon reach a level in some areas at least which will result in a significantly diminished mortality rate in the field if attenuated strains of virus like KM 13 remain dominant.

As pointed out elsewhere (Fenner, 1956) it is possible that the increased resistance of the rabbits may result in the displacement of the present dominant strain (KM 13) by one of greater virulence, similar to the standard laboratory strain, and consequent temporary arrest of the declining case mortality rate. Such an occurrence, which is by no means certain, would only have a temporary effect for the same selective process would lead in time to a greater resistance to the virulent strain of virus also. That increased genetic resistance to the highly virulent strain accompanies increased resistance to KM 13 is indicated by preliminary experiments on the progeny of wild rabbits which recovered after mild infections with strain KM 13. Several such rabbits were mated, and ten out of twenty-one progeny recovered from infection with the highly virulent standard laboratory strain of virus.

Because of the increasing complexity of the factors affecting the case-mortality rate (the existence of several enzootic strains of myxoma virus of differing virulence, the variable incidence and severity of epizootics of myxomatosis, the possible change in the dominant virus due to transmissibility factors, and the increasing breeding potential of recovered bucks due to the milder infections suffered) it is probably unwise to extrapolate from the information summarized in Fig. 1. From the practical point of view there is unequivocal evidence of a rapid increase in the genetic resistance of Australian wild rabbits. The importance of this fact for those concerned with the problem of rabbit control in Australia need not be emphasized.

SUMMARY

Annually for 4 years groups of young Australian wild rabbits have been captured during non-epizootic periods from areas in which myxomatosis has occurred during the previous summer. The intensity of the preceding epizootic was measured by testing a sample of survivors for antibody. The captured young rabbits were raised in the laboratory until they were about 4 months old and then inoculated intradermally with small doses of the slightly attenuated myxoma virus strain Aust/Corowa/12–52/2 (KM 13), all samples of virus being derived from the same batch which has been stored at $-70°$ C.

The results show there is a significant negative correlation between the mortality rate and the degree of exposure to myxomatosis of the forbears of the tested animals. Passive and active immunization have been excluded and this result is ascribed to increased genetic resistance.

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