PASSIVE IMMUNITY IN MYXOMATOSIS OF THE EUROPEAN RABBIT (*ORYCTOLAGUS CUNICULUS*): THE PROTECTION CONFERRED ON KITTENS BORN BY IMMUNE DOES*

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(With 1 Figure in the Text)

Several explanations have been suggested for the increasing incidence of immune wild rabbits observed after successive annual epizootics of myxomatosis in Australia (Fenner, 1953). One possible mechanism is the infection and active immunization of young rabbits while they are partially protected by maternal antibody received from their immune mothers. This mechanism is of major importance in converting virulent mousepox from an epizootic disease with a high case-mortality rate to an enzootic disease causing only occasional deaths (Fenner, 1948, 1949). Although Hyde (1936) had demonstrated a slight degree of passive immunity to myxomatosis by inoculating adult rabbits on several occasions with blood from immune animals, neither Martin (1936) nor Hyde & Gardner (1939) were able to demonstrate transfer of immunity from immune mothers to their offspring. This failure may have been due to the timing of the inoculations and the dosage and virulence of the virus used.

In examining the factors which might influence the host-parasite balance in myxomatosis passive immunity could not be neglected, and the experiments reported here have been designed with particular reference to the part that maternal antibody might play in altering the case-mortality rate under field conditions.

MATERIALS AND METHODS

Virus strains

Five strains of virus were used:

(*a*) Myxoma virus, South American strain (strain B of Martin, 1936), hereafter referred to as the standard laboratory strain.

(*b*) Myxoma virus, Uriarra III strain. This was derived from a rabbit of the series described by Mykytowycz (1953), but was intermediate in its virulence between Mykytowycz's strains and the standard laboratory strain.

(*c*) Neuromyxoma virus (Hurst, 1937) kindly provided by Dr E. Weston Hurst.

(*d*) and (*e*) Fibroma virus, OA and Boerlage strains, kindly provided by Dr Richard E. Shope.

Suspensions in normal rabbit serum of ground-up skin and subcutaneous tissue lesions were stored in ampoules at $-70°$ C. for use as preparations of known

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potency. Antigen for complement-fixation tests was prepared by suspending myxoma-infected chorioallantoic membranes from 14-day-old chick embryos in calcium-magnesium saline.

Rabbits

Laboratory rabbits from various sources were used, some being provided by Glenfield Veterinary Research Station, some by the Walter and Eliza Hall Institute of Medical Research, and some by the University Breeding Establishment. Female rabbits which had recovered from neuromyxoma infections or from myxoma infections after earlier vaccination with fibroma virus were used for breeding. Potent 'fibro-myxoma' immune serum was obtained by exsanguinating animals which had recovered from myxoma after vaccination with fibroma virus.

Mosquitoes

Aedes aegypti bred in an insectary maintained at 80° F. and with a relative humidity of 60–80% were kindly provided by Dr M. F. Day of the Division of Entomology, Commonwealth Scientific and Industrial Research Organisation.

Titration of virus

Myxoma and neuromyxoma viruses were titrated on the chorioallantois of the developing chick embryo (Lush, 1939). The titre (infective units per ml.) determined by this method is one log lower than the titre (ID_{50} per ml.) obtained by the intradermal inoculation of rabbits (Fenner & Woodroofe, 1953).

The fibroma viruses were titrated intradermally in rabbits, the end-point being determined by the method of Reed & Muench (1938).

Titration of antibody

Sera were stored in the frozen state, and inactivated before the performance of the serological tests. The methods employed for testing the serum samples have been fully described elsewhere (Fenner, Marshall & Woodroofe, 1953).

EXPERIMENTAL RESULTS

Passive immunity following inoculation of immune serum

Several females amongst a batch of normal rabbits sent to our laboratory from the Glenfield Veterinary Research Station produced young before they had been used as experimental animals. Advantage was taken of this circumstance to carry out some experiments on passive immunization.

Young rabbits aged between 4 and 10 days were inoculated intraperitoneally with either normal saline, or pooled 'fibro-myxoma' immune serum, the volume of the inoculum in millilitres being 1/40 of the weight of the animals in grams. Blood was collected by heart puncture a day later, and the young rabbits challenged that day, or at intervals up to 21 days later, with small infective doses (about 10 ID_{50}) of the standard laboratory strain of myxoma virus, inoculated intradermally. Blood was always withdrawn for antibody titrations the day before the challenge inoculation, and in most litters one young rabbit was bled at weekly intervals for the first 3 weeks.

The pooled serum used for inoculation had a titre by the complement-fixation test of 1/480, and in the neutralization test it reduced the pock count by 98·5%. Little change could be detected in the neutralizing antibody titres of the young rabbits for the first 21 days after their inoculation, but there was a steady fall in the titre of complement-fixing antibody from 1/320 the day after the inoculation to 1/40 3 weeks later. This difference was undoubtedly due to the fact that neutralization tests were carried out with undiluted serum and were not sensitive enough to detect the changes in antibody titre which occurred during the first 3 weeks.

Table 1. *The results of challenge inoculation of young rabbits with a small dose (10 ID_{50}) of myxoma virus (standard laboratory strain) at various intervals after the intraperitoneal inoculation of saline or immune serum*

Age of rabbit at time of inoculation of serum or saline (days)	Interval between serum inoculation and challenge infection (days)	Result of challenge inoculation	
		Rabbits inoculated with serum	Rabbits inoculated with saline
4	1	11, 13, S*	4, 6
	7	9, 10	5, 6
	21	10	
7–8	1	11, 20	6
	7	14, S	
	21	S	
9–10	1	N.I.†, N.I.	6, 6
	21	15, 15	
	7	N.I., N.I.	6, 6
	21	6 (N.S.)‡, 11	
	14	11, 11	8, 8
	21	6 (N.S.), 10, 14	

* 11, 13, etc. = death from myxomatosis 11, 13, etc. days after inoculation. S = survivor.
† N.I. = not infected: these animals were challenged again 21 days after passive immunization.
‡ N.S. = non-specific death, not due to myxomatosis.

When the results of the challenge inoculations are considered (Table 1) several differences are apparent in the response of the passively immunized and the control rabbits.

In four cases the passively immunized rabbits were not infected when inoculated with 10 ID_{50} of myxoma virus, although all four control animals died of myxomatosis 6 days after inoculation of the same preparation of virus. Similar cases of complete protection were encountered in the mosquito-infection experiments described later in this report. The four rabbits concerned had not sustained a subclinical attack of myxomatosis, for not only were the serological tests carried out before a second challenge negative, but all four were infected and three died of myxomatosis after a second inoculation of 10^3 ID_{50} of virus 21 days after the inoculation of the immune serum. The fourth rabbit died from a non-specific cause 6 days after the challenge inoculation, but a definite local lesion had developed at the site of the myxoma virus inoculation.

The survival times of the passively immunized rabbits were always greater than those of the control animals, and in three cases they survived. Two of these animals developed only a local lesion at the inoculation site, while the third recovered after severe generalized myxomatosis. It was possible to re-inoculate two of these three rabbits. Serological data and the results of the challenge tests are shown in Table 2. The antibody titres 6 months after the first challenge inoculation were negative in the rabbit in which this inoculation had produced a local lesion only, and low but positive in the other animal, which had survived after fairly severe generalized myxomatosis. On re-inoculation with myxoma virus both animals suffered from generalized myxomatosis, which was much more severe in the animal with the lower antibody levels at the time of challenge.

Passive immunity due to maternal antibody

The foregoing experiments demonstrated unequivocally the existence of passive immunity in myxomatosis. The intraperitoneal inoculation of high titre homologous immune serum invariably prolonged the survival time of young rabbits, and some recovered from infection with the highly virulent standard laboratory strain of myxoma virus. The next experiments were concerned with passive immunity following breeding from does which had recovered from myxomatosis, either after vaccination with fibroma virus, or after infection with neuromyxoma virus. Seven does which had been infected with myxoma or neuromyxoma virus at intervals of 5 weeks to 11 months earlier, and four normal does were mated. In addition, one doe (R 418) was mated on the same day that it was used for the titration of Boerlage fibroma virus. Many skin lesions developed at the inoculation sites used for this titration.

The offspring were tested for their resistance to infection with myxoma virus at the ages of 9–11 days, 4 weeks, and 7 weeks. Just before the challenge inoculation, one rabbit in each litter was bled and the serum tested for complement-fixing and neutralizing antibodies.

One family was preserved for antibody decay observations, and at least two representatives of this family were bled every week for 7 weeks. These results are set out in Fig. 1. Complement-fixing antibodies were still just detectable after 7 weeks, but neutralizing antibodies could not be detected after the 6th week. The form of the decay curve of neutralizing antibody is due again to the difficulty of detecting small differences in antibody concentration when undiluted serum is used.

Challenge inoculations were made by allowing two *Aedes aegypti*, which had probed through advanced skin lesions of a rabbit infected with the standard laboratory strain of myxoma virus, to bite each kitten, one insect on each side. The results are presented in Table 3.

The survival times of normal rabbits after challenge with the standard laboratory strain of myxoma virus varies with the age of the animals. Adult rabbits survive for 9–11 days after the intradermal inoculation of 10 ID_{50} of virus. As shown in Table 4, the survival time diminishes with decreased age, and very young kittens die after 5–6 days with few external signs of generalized disease

Table 2. *Serological data and the results of further challenge inoculations with* 10 ID_{50} *of the standard laboratory strain of myxoma virus in two rabbits which survived the initial challenge*

		Age of rabbit in weeks							Challenge inoculations			
Rabbit no.	Serological test	1*	2*	4	9	16	31	40	Age of rabbit (weeks)	Result	Age of rabbit (weeks)	Result
247A	Complement-fixation†	—	120	—	160	30	10	80	2	Large primary lesion and scattered secondary nodules without surrounding oedema body, eyelids and anogenital region	32	Accelerated primary lesion and many scattered secondary nodules without surrounding oedema, on body, eyelids and anogenital region
	Neutralization‡	—	97 %	—	99 %	97 %	91 %	—				
248A	Complement-fixation	160	—	40	30	5	0	320	1	Large primary lesion, no evidence of generalization	32	Large primary lesion and severe generalized myxomatosis with eventual recovery
	Neutralization	95 %	—	94 %	N.S.R.	N.S.R.	N.S.R.	—				

* Sera from siblings of the experimental rabbits 247A and 248A.

† Expressed as the reciprocal of the serum dilution at which 50 % haemolysis occurred.

‡ Expressed as the percentage reduction in the pock count on the chorioallantoic membrane of the chick embryo. N.S.R. = no significant reduction.

beyond some thickening of the margins of the eyelids. Prolongation of the survival time with increasing age is apparent in both the immune and the normal kittens.

There was only one survivor in the experiment recorded in Table 3. Nevertheless, the survival times of the immune kittens were invariably prolonged, compared with normal animals of the same age (Table 4). This prolongation of life was accompanied by the development of the florid signs of advanced myxomatosis: multiple skin tumours, great swelling of the head and closure of the eyes, conjunctival discharge, snuffles, etc.

Kittens born by immune does survived longer if they became infected, but they often failed to contract myxomatosis after the introduction of doses of virus which

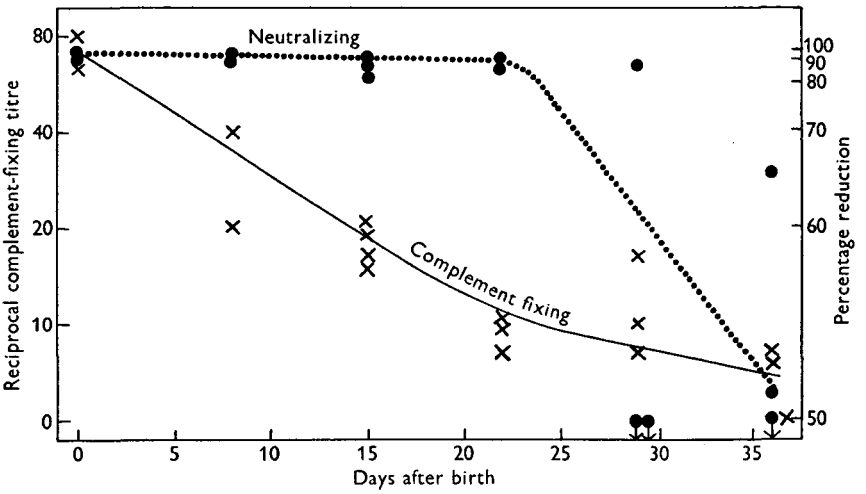

Fig. 1. The persistence of complement-fixing and neutralizing antibody in the progeny of an immune rabbit in which the titre of complement-fixing antibody was 1/80 and the percentage neutralization of myxoma virus 95% at the birth of the kittens.

were infectious for normal rabbits. The dose transferred by interrupted feeding of a mosquito which has bitten through an advanced skin lesion in the donor rabbit may vary between 1 and 100 ID_{50} (usually 5–30 ID_{50}) (Day & Woodroofe, unpublished observations). In Table 5 a comparison is made of the positive results achieved by mosquito bite inoculation of normal and immune kittens in the two larger experiments recorded in Table 3. It is immediately apparent that failures to produce infection were very rare in normal kittens (1 out of 26) but common in the passively immune animals (37 out of 68). A similar failure of doses of virus known to be infective for normal rabbits to infect fibroma-vaccinated rabbits has been recorded elsewhere (Fenner & Woodroofe, 1954). Two progeny of R415 were extraordinarily resistant to infection. Not only did they fail to develop local lesions after three successive attempts by mosquito biting, but they proved difficult to infect by intradermal inoculation. Their histories are summarized in Table 6. R415C, the animal which recovered after eventually being infected with 3000 ID_{50}

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Table 3. *The results of challenge inoculation with the standard laboratory strain of myxoma virus of the progeny of immunized and normal does, by interrupted feeding of* Aedes aegypti

Rabbit no.	Method of immunization	Interval between immunization and conception	Antibody titres of does at birth of kittens: Complement-fixation	Antibody titres of does at birth of kittens: Neutralization (%)	Age of kittens when challenged (days)	Antibody titres of kittens before challenge: Complement-fixation	Antibody titres of kittens before challenge: Neutralization (%)	Response: Reactions at sites of mosquito bites	Response: Survival time (days)
R 415	Neuromyxoma	5 weeks	120*	99*	9	30	91	0, 0, 0, 0, 1, 1†	8, 8
					14‡	—	—	0, 0, 1, 1	9, 11
					22‡	15, 10	97, 92	0, 0	—
R 356	Fibro-myxoma	8 months	160	97	8	40	96	Inoc. 10 ID_{50}	8, 8, 10
R 426	Neuromyxoma	3 weeks	60	92	10	20	90	0, 1	11
					15‡	—	—	1	10
R 353	Fibro-myxoma	11 months	15	93	11	5	91	1, 2, 1	8, 8, 12
R 418	Fibroma	0 days	0 (myxoma antigen)	89	9	0	70, 94	0, 1, 1, 1, 2, 2	8, 8, 8, 8, 9
			60 (fibroma antigen)	—	14‡	—	—	2	7
R 428	Normal	—	—	—	10–11	—	—	1, 2, 2, 2, 2, 2	5, 5, 5, 5, 5, 6
R 495	Controls	—	—	—	—	—	—	2, 2	6, 6
R 355	Fibro-myxoma	8 months	80	97·5	21	60, 15	94, 97	Inoc. 10 ID_{50}	10, 11, 13, 13
R 351	Fibro-myxoma	11 months	15	92	27	5, 0	93, 91	1, 2	12, 8
R 354	Fibro-myxoma	11 months	5	84	26	0, 0	67, 70	0, 0, 0, 0, 1, 1	9, 9
					32	—	—	Inoc. 10 ID_{50}	10, 11, 11, 11
R 355	Fibro-myxoma	11 months	15	98·5	27	0, 0	91, 87	2, 2, 1, 1, 1, 1, 1	8, 9, 9, 9, 9, 9, 10
R 497	Normal control	—	—	—	27	—	—	2, 2, 2, 2, 2, 2	5, 6, 6, 6, 6, 7
R 352	Fibro-myxoma	11 months	160	98·8	47	0	60	1, 2, 2	10, 11, 11
R 496	Normal control	—	—	—	45	—	—	1, 1, 1	7, 7, 8

* Complement-fixing antibody: titre shown as reciprocal of serum dilution giving 50% fixation with 3 M.H.D. complement, using myxoma antigen. Neutralizing antibody: titre shown as percentage reduction in pock count with myxoma virus on chorioallantois using undiluted serum.

† Each kitten was bitten at two sites. 0 = no reaction at either site; 1 = local reaction at one site; 2 = local reaction at both sites. Those kittens which showed no reaction were bitten again at intervals as shown.

‡ Rechallenges.

inoculated intradermally, may have sustained an inapparent and weakly immunizing infection during the several trials made before a local lesion was produced, although the steady fall in the titre of complement-fixing antibody would not be expected under these circumstances. Unless this occurred it is hard to understand the production of only a localized lesion after the inoculation of a relatively large dose of virus 41 days after birth.

The progeny of R418, which had been inoculated with Boerlage fibroma virus in many sites on the day of mating, survived almost as long as the progeny of rabbits immunized with myxoma virus. Experiments described elsewhere (Fenner & Woodroofe, 1954) showed that this strain of fibroma virus induces the vigorous production of antibodies which neutralize myxoma as well as fibroma virus, and the increased survival time was undoubtedly due to the passage of such antibodies to the foetuses in the late stages of pregnancy.

The experiments just described indicate that the antibody transferred from immune mothers to their kittens modified the response of the latter to challenge inoculation with the highly virulent standard laboratory strain of myxoma virus. However, the recovery rate was so low that it is doubtful whether such a mechanism would appreciably affect the case-mortality rate in epizootics of myxomatosis due to a virus with the same virulence as the standard laboratory strain. During the past year several strains of virus recovered from naturally-occurring cases of myxomatosis have been found to be somewhat less virulent than the standard laboratory strain (Fenner, 1953; Mykytowycz, 1953). It seemed not unlikely that the slight increase in host resistance conferred by maternal antibody would exert a much greater effect if the infecting virus was somewhat less virulent than the standard laboratory strain, and the next experiment was undertaken to test this possibility. The virus strain used is known as Uriarra III, and was obtained from one of the rabbits in the series described by Mykytowycz (1953). However, it appears to be somewhat more virulent than the strains maintained by contact infection by Mykytowycz, although definitely less virulent than the standard laboratory strain.

In this experiment kittens were challenged at the age of 4 weeks. This interval was chosen for two reasons. First, it was the age at which the maximum increase in survival time occurred, due to an interaction of the age effect and decay of the passively received antibody. Secondly, it is at about this age that young rabbits leave the nests built by the does and play at the entrance to the burrows, thus becoming readily accessible to mosquitoes. Although *Anopheles annulipes*, the major vector in south-eastern Australia, may feed on rabbits in the burrow (Myers, unpublished) the likelihood that these mosquitoes enter the nests of breeding females is remote.

At the age of 4 weeks eleven normal kittens and thirty-seven offspring of immune mothers were inoculated intradermally with 10 ID_{50} of Uriarra III strain of myxoma virus. Blood was withdrawn from each kitten before the challenge inoculation, and again after symptoms had subsided in those animals which recovered, and tested for complement-fixing and neutralizing antibodies. The results are set out in Table 7.

Table 4. *Increase in survival time due to passive immunity after mosquito-bite infection with the standard laboratory strain of myxoma virus*

Age at infection (days)	Survival time (days) ± standard error		
	Normal kittens	Immune kittens	Increase
9–11	5·4 ± 0·2	8·8 ± 0·3	3·4 ± 0·4
21–27	6·0 ± 0·3	10·1 ± 0·4	4·1 ± 0·4
47	7·3 ± 0·3	10·7 ± 0·3	3·4 ± 0·4

Table 5. *The resistance of progeny of immune does to infection with the standard laboratory strain of myxoma virus by interrupted mosquito biting (excluding repeated attempts at infection)*

No. of effective mosquito bites	9–11 days old progeny of		27-day-old progeny of	
	Immune does	Normal does	Immune does	Normal does
0/2	6	0	4	0
1/2	9	1	8	0
2/2	4	6	3	6
Total effective bites / Total bites	17/38	13/14	14/30	12/12

Table 6. *Histories of two young rabbits which repeatedly withstood attempts to infect them with myxomatosis*

Date	Method of inoculation	R415C			R415F		
		Antibody titres			Antibody titres		
		Complement-fixation	Neutralization (%)	Result of challenge	Complement-fixation	Neutralization (%)	Result of challenge
26. iii. 53	Mosquito bite	—	90	Negative	—	93	Negative
31. iii. 53	Mosquito bite	—	—	Negative	—	—	Negative
8. iv. 53	Mosquito bite	15	97	Negative	10	95	Negative
14. iv. 53	15 ID_{50} I.D.*	—	—	Negative	—	—	Negative
24. iv. 53	300 ID_{50} I.D.	< 5	89	Negative	< 5	87	Died of generalized myxomatosis on ninth day
5. v. 53	3000 ID_{50} I.D.	—	—	Large primary lesion, no generalized signs, recovery	—	—	—
24. vi. 53	—	120	—	—	—	—	—

* I.D. = intradermal inoculation. Symbols as in Table 2.

Table 7. *The results of challenge infection of the progeny of immune and normal does by the intradermal inoculation of small doses (10 ID_{50}) of Uriarra III strain of myxoma virus*

Immune status of mothers	Age of kittens challenged when (days)	Antibody titres of kittens before challenge		Response		Antibody titres of kittens after recovery		Second challenge infection with 200 ID_{50}, 2 months after first	
		Complement-fixation	Neutralization (%)	Symptoms	Survival time (days)	Complement-fixation	Neutralization (%)	Strain of virus	Response
Normal controls	28	0	0	Severe	11, 13, 14, 16, 16, 16, 17, 18, 20, 21, 25	—	—	—	—
Immune	28	0	90	Severe	11	—	—	—	—
	28	7	99	Severe	12	—	—	—	—
	28	15	98·8	Severe	12	—	—	—	—
	28	7	99·3	Severe	13	—	—	—	—
	28	60	99	Severe	14	—	—	—	—
	28	5	—	Severe	14	—	—	—	—
	28	7	98·6	Severe	14	—	—	—	—
	28	0	80	Severe	17	—	—	—	—
	28	40	97	Severe	17	—	—	—	
	28	0	93	Severe	17	—	—	—	—
	28	7	97·5	Accelerated severe	18	—	—	—	—
	28	15	98·4	Severe	19	—	—	—	—
	29	0	90	Severe	21	—	—	—	—
	28	0	93·5	Severe	21	—	—	—	—
	28	< 5	60	Severe	23	—	—	—	—
	29	0	95	Severe	24	—	—	—	—
	29	< 5	98	Severe	27	—	—	—	—
	28	0	95	Severe	27	—	—	—	—
	29	0	85	Severe	31	—	—	—	—
	29	< 5	70	Severe	37	—	—	—	—
	28	0	99	Severe	41	—	—	—	—
	29	0	95	Severe	Recovered	960	99	Uriarra III	None
	28	30	98·5	Severe	Recovered	2560	99·5	Standard	None
	28	30	99	Accelerated severe	Recovered	1920	98·8	Standard	None

28	5	98	Mild	Recovered	80	97	Uriarra III	Very mild, no generalization
28	7	99	Mild	Recovered	480	97	Standard	Very mild, no generalization
28	0	95	Mild	Recovered	240	99·5	Uriarra III	Very mild, no generalization
28	5	70	Very mild	Recovered	160	94	Uriarra III	Very mild, no generalization
28	7	99·4	None	—	—	—	—	—
45	0	93	Severe	16	—	—	—	—
28	5	99·3	None	—	—	—	—	—
45	5	99	Severe	45	—	—	—	—
28	7	97	None	—	—	—	—	—
45	< 5	96·5	Severe	Recovered	2560	99	Standard	None
28	15	99·6	Doubtful	—	—	—	—	—
45	5	96·5	Accelerated severe	Recovered	480	99·4	Standard	None
28	15	99·5	None	—	—	—	—	—
45	0	96	Accelerated severe	Recovered	160	98·6	Standard	None
28	15	99·6	None	—	—	—	—	—
45	5	98·4	Very mild	Recovered	320	98·2	Uriarra III	None
28	30	99·3	None	—	—	—	—	—
45	15	98·9	Accelerated mild	Recovered	160	98·5	Uriarra III	None
28	30	99·2	None	—	—	—	—	—
45	7	95·5	Mild	Recovered	60	96	Uriarra III	Very mild, no generalization
29	0	80	None	—	—	—	—	—
46	0	80	Accelerated moderate	Recovered	480	98	Standard	None

Symbols as in Table 3.

The diminished virulence of this Uriarra III strain is apparent when the survival times of the normal kittens are compared with survival times of normal kittens of the same age inoculated with the standard laboratory strain of the virus. The average figures in the current experiments were 6 days with the standard virus, and 17 days with the Uriarra III strain.

As in previous experiments some passively immune kittens (9/37) failed to become infected at the first inoculation, although all were infected when a second inoculation of the same dose of virus was made 17 days later.

Not only were the survival times of fatal cases usually considerably prolonged, but fourteen of the thirty-seven offspring of immune mothers recovered from the infections. Seven of these recoveries occurred in the nine animals which had missed infection on the first occasion. Owing to the age effect discussed earlier they are not strictly comparable with the control animals, but from our experience with this strain of virus in normal adult rabbits such a high recovery rate would be most unexpected, if it were not for the passive immunity. The other seven recoveries occurred in the twenty-eight animals infected at the age of 4 weeks, and five of the fatal cases in this group survived longer than any of the control animals, their skin lesions showing signs of healing when they died. If we consider only those animals which were infected when they were 4 weeks old it is apparent that, when the strain of virus used for challenge inoculation was somewhat attenuated, passively transferred antibody permitted the survival of 25%, compared with no survivors amongst normal rabbits of the same age. Such a result is obviously of some epidemiological significance. There did not seem to be any close correlation between the titre of antibody in the serum just before challenge inoculation and the outcome. The titre after recovery was clearly influenced by the severity of the infection suffered, very high antibody titres being recorded in animals which recovered after severe infections and low titres in those which had sustained mild attacks only.

The response of the surviving rabbits to a second challenge inoculation with either the standard laboratory strain or the Uriarra III strain of virus demonstrated the complete cross-immunity between these strains.

DISCUSSION

Brambell and his collaborators (Brambell, Hemmings & Henderson, 1951) have shown that antibody passes from a pregnant rabbit to the foetuses via the uterine lumen and yolk-sac splanchnopleur. Post-natal transfer, either by colostrum or milk, does not occur in this species. The titre of antibody found in the blood of the newborn rabbits is usually the same as that of the mother, and our data showed a similar correspondence of maternal and newborn antibody titres (mother: complement fixation 1/80, neutralization 95%; newborn young: complement fixation 1/60, 1/80, neutralization 91%, 94%).

The half-life of homologous antibody in the adult rabbit is 4–5 days (Dixon, Talmage, Maurer & Deichmiller, 1952, from data of Glenny & Hopkins, 1923). In the present experiments the decay curves for complement-fixing antibody, which could be more precisely measured than the neutralizing antibody titre, suggested

a half-life of about 7 days. In newborn humans the half-life of homologous γ-globulin is longer than in adults (Dixon, *et al.* 1952). It is possible that a similar difference may exist between young and adult rabbits.

In these experiments on passive immunization, as in experiments on active immunization with fibroma virus (Fenner & Woodroofe, 1954) there was only an approximate correspondence between antibody titre (complement-fixing or neutralizing) and the response to infection. The progress of the disease in some young animals with high antibody titres at the time of the challenge inoculation differed little from that in the control animals, especially in experiments with the somewhat attenuated Uriarra III strain of virus. Other young animals with much the same antibody levels suffered a mild infection only, with few signs of generalization.

Anderson & Hamilton (1949) found that babies failed to become infected with herpes simplex virus when living in a contaminated environment until they were about 11 months old, although neutralizing antibody (derived from the mothers) could not be detected after the 7th month. A similar persistence of some degree of protection after the disappearance of detectable antibodies was found in the three 47-day-old progeny of R 352 (Table 3), although here it was shown merely by a prolongation of the survival time.

Since the time of Jenner there have been numerous reports that newborn infants whose mothers had been recently vaccinated against smallpox were refractory to vaccination, but few experimental studies have been made of the transition from absolute protection against virus infection, through a modified response to normal susceptibility. Andersen (1937) found that the newborn offspring of rabbits which had recovered from infection with vaccinia were completely resistant to infection with vaccinia virus. Three weeks later slight reddening occurred at the scarification site, while the response at 3 months of age was normal. He did not determine whether any of the young rabbits had been actively immunized by the greatly modified infection experienced three weeks after vaccination.

In chimpanzees inoculated with poliomyelitis-immune serum and challenged with oral doses of virus, Bodian (1953) found that in a few animals the antibody titre declined at the same rate as in the controls. In the majority there was, after an initial period of declining antibody titres, a rise indicating that active immunization had occurred. This response occurred later than in normal animals. In this example, therefore, both complete protection without active immunization and a modified immune response occurred, although all animals excreted virus in the faeces during the 2nd to 4th weeks after virus feeding.

In the experiments reported in this paper complete protection against infection with myxoma virus occurred in a number of instances amongst the passively immunized kittens. It occurred against both the standard laboratory strain of virus and the Uriarra III strain, and after both intradermal inoculation and mosquito-bite infection.

From the epidemiological point of view, complete protection is of less significance than infection and recovery accompanied by active immunization. This was rare when the standard laboratory strain of myxoma virus was used (one case in

forty-one amongst the progeny of immune does, three cases in eighteen amongst kittens inoculated with immune serum), but it occurred in 25 % of the progeny of immune does challenged with a somewhat attenuated strain of virus, which nevertheless killed all the control animals.

The responses of the survivors of the first experiment (standard laboratory strain of virus) to reinfection with the same strain were of interest. The animal (247 A) which had shown mild generalized symptoms at the time of the first inoculation still had some neutralizing antibody in its serum at the time of the second challenge 7 months later, when it developed many nodules on the eyelids and skin without any surrounding oedema. The response was very similar to that recorded in some of the wild rabbits of Mykytowycz (1953) Uriarra contact series; and it is quite possible that the very mild symptoms shown by some of his animals were due to the operation of a similar mechanism, although the virus he used was undoubtedly less virulent than the standard laboratory strain. The animal (248 A) in which the first challenge produced only a localized reaction had completely lost demonstrable antibodies 7 months later. Nevertheless, it survived challenge infection with the standard laboratory strain of virus, although suffering a severe generalized disease. When shortage of laboratory rabbits forced us to use wild-caught rabbits for determining the virulence of field strains of virus we encountered a few animals which gave the same kind of response: although preliminary serological tests showed that they had no demonstrable antibody, they reacted as 'old immune' animals. In view of the long persistence of demonstrable antibodies after recovery from a severe attack of myxomatosis (Fenner *et al.* 1953) these animals also may have sustained a relatively mild attack many months earlier, possibly due to maternally transmitted antibody.

The epidemiological significance of maternal antibody in myxomatosis in Australia depends primarily on two factors, the seasonal incidence of breeding and myxomatosis, and the virulence of field strains of virus. In most parts of southern Australia breeding occurs in the late autumn, winter and spring, and myxomatosis as an epizootic disease in the summer. Most young animals are at least 3 months old before they are exposed to epizootic myxomatosis, and maternal antibody may therefore be expected to have little influence on the course of the disease.

At Lake Urana (Myers, Marshall & Fenner, 1954) kittens emerged from the burrows during August, September and October, and the peak of the epizootic occurred in November–December. Although some rabbits were only about 2 months old when infected, most were at least 3 months old. The immune rate amongst the parent rabbits was only about 30 %, and it is unlikely that maternal antibody played a great part in the change in the case-mortality rate observed at Lake Urana.

Further north, at the northern borders of New South Wales, the main breeding season again occurred during the winter and spring, but seasonal conditions permitted some breeding throughout the year. Here myxomatosis also persisted throughout the year with exacerbations in the summer months (Marshall, Dyce, Poole & Fenner, 1955) and under these conditions partial protection by maternal antibody could be an important epidemiological mechanism.

Another epidemiological consequence of passive immunity which warrants discussion is its effect on transmission. Complete protection might retard spread of the disease in the same way as the existence of a considerable proportion of actively immunized animals in the exposed population. It is also possible that virus on the mouth-parts of infected mosquitoes which bite actively or passively immune rabbits may be neutralized by the antibody which adheres to the proboscis after feeding. On the other hand, the prolonged survival time and concomitant severe external lesions of young rabbits that became infected would provide an important source of virus for mosquitoes, which non-immune kittens do not, owing to their short survival time and the absence of well-developed external lesions.

In parts of Britain, where the main breeding season extends from January to June, with some breeding throughout the year (Stephens, 1952; Brambell, 1944) summer epizootics of myxomatosis would coincide with the presence of large numbers of kittens. If myxomatosis should become established as an enzootic disease in Britain a proportion of these young animals would, within a few years, be the offspring of immune does. Under these circumstances the modifying influence of maternally transmitted antibody might be of considerable importance in reducing the case mortality rate of myxomatosis.

The control groups included in these experiments provide some information on the severity and symptomatology of myxomatosis in young rabbits. The results closely parallel those reported elsewhere with mousepox (Fenner, 1949). In young animals death is invariable and the survival time is much shorter than in adult rabbits. As the age of the rabbits increases, the survival time increases. The occurrence of the typical signs of advanced myxomatosis depends on the survival of the infected rabbits for a sufficient period, and very young animals, which may die on the 5th or 6th day after infection, usually show only a lump at the site of inoculation and perhaps a slight thickening of the margins of the eyelids. If animals of the same age survive for longer periods, due to the use of a less virulent virus strain or to passive immunization, the symptoms characteristic of severe myxomatosis in adult rabbits develop.

SUMMARY

1. The existence of passive immunity to myxomatosis was demonstrated by the inoculation of normal young rabbits with either myxoma-immune serum or saline, and their subsequent inoculation with the standard laboratory strain of myxoma virus. All the passively immunized animals lived longer than the control animals and a few survived.

2. Passive immunity could also be demonstrated in the offspring of myxoma-immune mothers. When these were challenged by mosquito bite inoculation with the standard laboratory strain of myxoma virus they either failed to become infected, or survived infection for several days longer than the progeny of normal does. When challenged by the intradermal inoculation of a slightly attenuated strain of myxoma virus 25% of the progeny of immune does survived the infection, whereas none of the normal kittens survived.

3. The survival times of young rabbits in both the normal and passively immunized groups was influenced by their age, very young animals dying several days earlier than rabbits 4 and 6 weeks old.

4. The possible epidemiological consequences of passive immunity in the behaviour of myxomatosis in populations of wild rabbits are discussed.

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