THE EPIZOOTIC BEHAVIOUR OF MOUSEPOX (INFECTIOUS ECTROMELIA OF MICE)

II. THE COURSE OF EVENTS IN LONG-CONTINUED EPIDEMICS

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(With 3 Figures in the Text)

The pioneer studies on the experimental epidemiology of virus diseases were those reported by Greenwood, Hill, Topley & Wilson (1936). They studied two long-continued epidemics of infectious ectromelia of mice lasting for $1\frac{3}{4}$ and $3\frac{1}{4}$ years respectively in herds maintained by the regular addition of normal mice. The disease continued to occur throughout the experiments with no apparent change in the virulence of the parasite, although there were considerable fluctuations of mortality associated with a heat-wave. The heightened resistance of mice of higher cage-ages to the effects of the virus were ascribed to immunization from non-lethal infections, and the results of closed epidemics set up with mice immunized with killed and then a small dose of living ectromelia virus supported this conclusion.

Following the demonstration by Burnet & Boake (1946) of the systematic status of ectromelia virus, and their development of a simple *in vitro* method of antibody titration, investigations on the experimental epidemiology of infectious ectromelia were commenced in these laboratories. Studies were directed first to the portal of entry of the virus, which was shown to be through small abrasions of the skin (Fenner, 1947*a*), and then to the methods of elimination of the virus and the duration of infectivity of infected animals (Fenner, 1947*b*). While small amounts of virus were sometimes excreted in the faeces, urine and saliva, the important mode of elimination of the virus and contamination of the environment was through lesions of the skin, both the primary lesion and, more important, the lesions of the secondary rash. The duration of infectivity was found to correspond with the period of active skin lesions, extending from about the 7th to the 21st day in natural infections. Infectivity was greatest during the first 7 days after the appearance of the primary lesion.

The disease has been called mousepox because of the relationship of ectromelia virus to the animal pox viruses and the resemblance between its clinical features (incubation period and rash) and those of smallpox (Fenner, 1948*b*). Having established the portal of entry and the methods of elimination of the virus, the epizootic behaviour of mousepox was investigated by means of closed epidemics (Fenner, 1948*a*). A much more accurate picture of the progress of infection through the herd was obtained by means of regular examination of the living mice for primary and secondary lesions than by consideration of fatal cases only. Morbidity curves were constructed showing the number of new cases found at each examination. The 'Moscow' strain of ectromelia virus proved to be much more virulent and much more infectious than was the egg-passaged 'Hampstead' strain.

In order to obtain results of the same type as those reported by Greenwood *et al.* (1936), two long-continued epidemics were set up by adding five inoculated mice to ten normal mice and then adding three normal mice every 4th day. One experiment (CVII), in which the egg-passaged 'Hampstead' strain of virus was used, was terminated on the 190th day and the other (Exp. CVI), in which the 'Moscow' strain of virus was used, was continued until the 290th day. This paper consists of a description of the results of these two experiments.

METHODS AND MATERIALS

Virus

The 'Moscow' strain of ectromelia virus was used as first egg-passage material and the 'Hampstead' strain had been passed chorioallantoically fifty to sixty times.

Mice

Male 8-week-old mice from the Hall Institute mouse-room were used throughout. They were multicoloured and have been continuously outbred for the last 10 years. The only enzootic disease of any consequence was a chronic pneumonia associated with a Gram-negative bacillus. This never caused symptoms in young mice, but sometimes a chronic pneumonia which was occasionally fatal in mice more than 6 months old.

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Antibody titrations

Ectromelia antihaemagglutinin (E-AHA) was titrated by determining the dilution of serum in 1% susceptible fowl cells which would inhibit their agglutination by four haemagglutinating doses of ectromelia virus.

Management of epidemics

The two epidemics were set up simultaneously by the inoculation of groups of five mice in the left hind pad with a 1:100 dilution of stock egg membrane suspension of each strain of virus. These five infected mice were added to two normal mice in a 'Topley' tin (Topley, 1923), and three normal suitably marked 8-week-old mice were added to the herd every 4th day. The mice were examined for primary and secondary lesions every 2nd day and at the same time were changed into clean sterile tins. The state of crowding of the mice was maintained approximately constant by adding one tin to the battery for every increase of twenty in the population. Cage litter consisted of sawdust and the mice were fed on an ample diet of 'Barastoc' dog cubes and tap water from overhead bottles. Dead mice were examined post-mortem as soon as possible after death. In the first experiment (Hampstead strain) one mouse was eaten to such an extent that examination was impossible and in the second experiment three mice were eaten. All of these animals had been seen to have primary lesions within the previous 3 days and the cause of death has been assumed to be mousepox. With the aid of the clinical examination of the mice it was found possible to assess in almost every case the specific or other nature of the cause of death. Only two conditions other than mousepox were of any importance, namely pneumonia and overheating.

When the experiments were ended all surviving mice were killed and post-mortem examinations were carried out and serum was collected for the determination of the E-AHA titres. In Exp. CVI (Moscow strain) suspensions of the lungs and spleen of the majority of mice killed at the conclusion of the experiment were inoculated into normal mice.

RESULTS

Determination of the specific nature of death

Greenwood *et al.* (1936) divided deaths occurring in epidemics of mouse typhoid and mouse pasteurellosis into specific and non-specific on the basis of autopsy findings and the results of bacteriological tests, but they were unable to decide upon any criteria concerning the specific nature of deaths in ectromelia. Correlation of the clinical history and the post-mortem findings has enabled such a distinction to be made here. Two types of specific deaths occurred. In the first, which accounted for 95% of all specific deaths in Exp. CVI and 91% in Exp. CVII, the mouse died within 0–4 days of the detection of the primary lesion, and the liver and spleen showed very extensive necrosis. In the second type the animal lived for several days after the appearance of the primary lesion, and after the development of a severe rash became sick and thin and finally died. In these animals, extensive necrosis of the liver and spleen was not found. The liver was usually small and in sections showed numerous focal collections of round cells, and the spleen showed patchy necrosis tending to become fibrotic. There was usually great wasting and a severe unhealed rash.

Other fatal cases fell into one of two groups. First, between days 230 and 266 in Exp. CVI there were twelve deaths in mice varying in cage-age from 104 to 203 days. All these mice had suffered from typical attacks of mousepox within the 3 weeks of entry to the herd. In eight of them the mice had become thin and shown rapid respiration for about a fortnight before their death. Autopsy showed no lesions of the liver or spleen other than the occasional occurrence of fibrotic patches on the spleen (evidence of past mousepox infection), and extensive consolidation of the lungs, sometimes chronic with abscess formation and sometimes acute. Culture of such lungs was sometimes sterile, but more usually there was a prolific growth of a Gram-negative bacillus.

In six of these twelve fatal cases suspensions of the liver, spleen and lung were subinoculated intraperitoneally and intranasally in mice, undiluted 20% suspensions and 1:100 dilutions being used, but ectromelia virus could not be isolated.

The other fatal cases consisted of seven in Exp. CVII and two in Exp. CVI which were found dead on the 175th and 176th days of the two experiments. The 175th day, 12 February 1948, was the hottest for the summer, the maximum shade temperature being 104·4° F. The temperature on the previous 3 days was 90·4, 95·1, and 102.1° F.; and on the succeeding day 68·2° F. Post-mortem changes had occurred before the autopsies were performed in these cases, but the cage-ages of the mice varied from 3 to 176 days and the deaths occurred on the days of the heat-wave (that is on the days and nights of the 174th and 175th days) and in mice which had either probably not yet contracted the disease (one case—3 days after entry) or had recovered from a typical attack many weeks earlier. No characteristic changes were seen at autopsy, except for the fibrotic patches on the spleen indicative of past mousepox infection which occurred in four cases.

In the graphs showing the mortality rates (Figs. 1 and 2) specific deaths due to mousepox have been separated from non-specific deaths, and in the life tables a similar separation has been made.

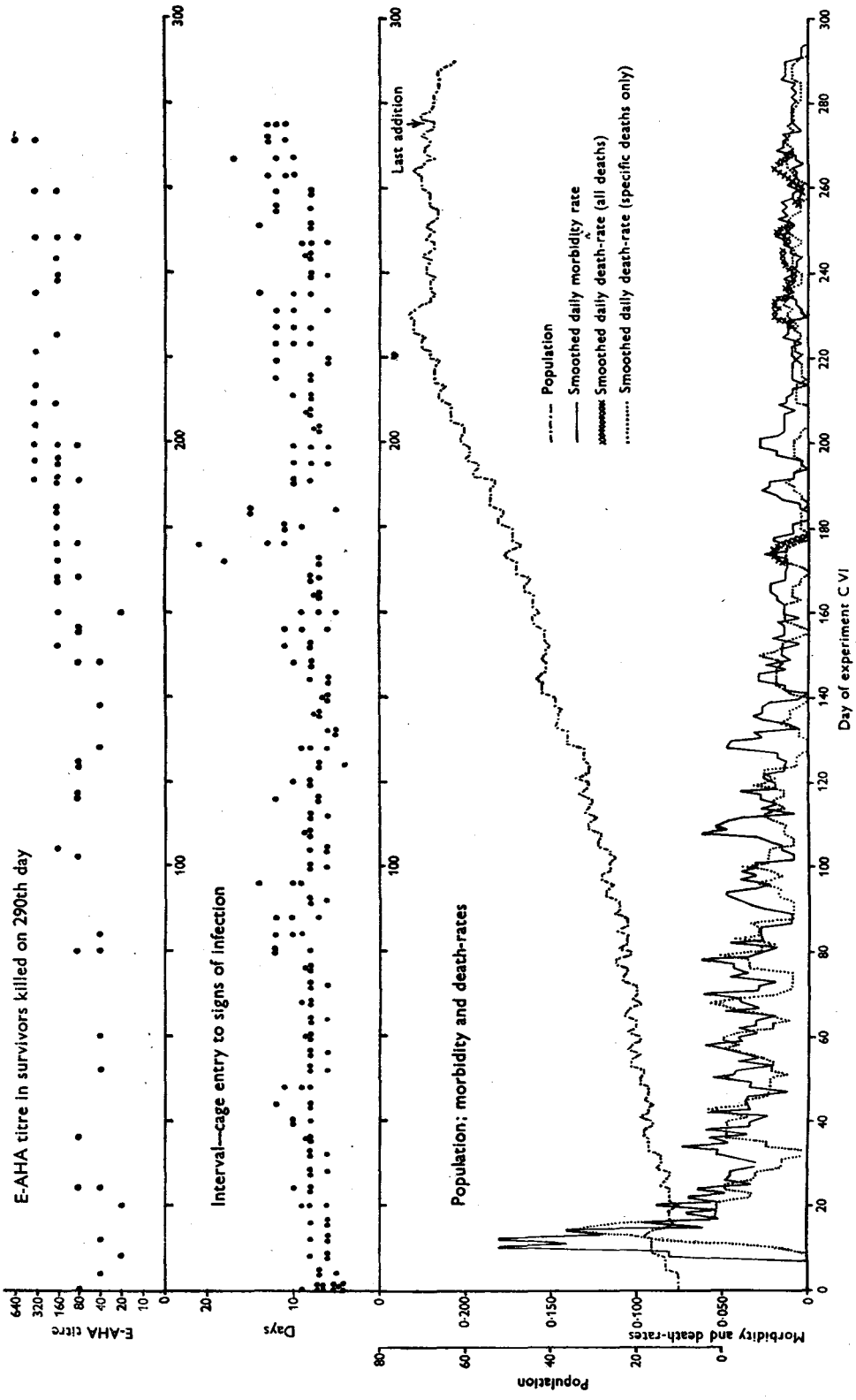

Fig. 1. The secular trend in population, morbidity rates, and total and specific death-rates in an epidemic of Moscow mousepox maintained by adding three normal mice to the herd every fourth day. In the upper part of the figure are shown the interval between entry to the herd and the occurrence of signs of infection, and the E-AHA titre of survivors, for each group of three mice added on the indicated day of the experiment.

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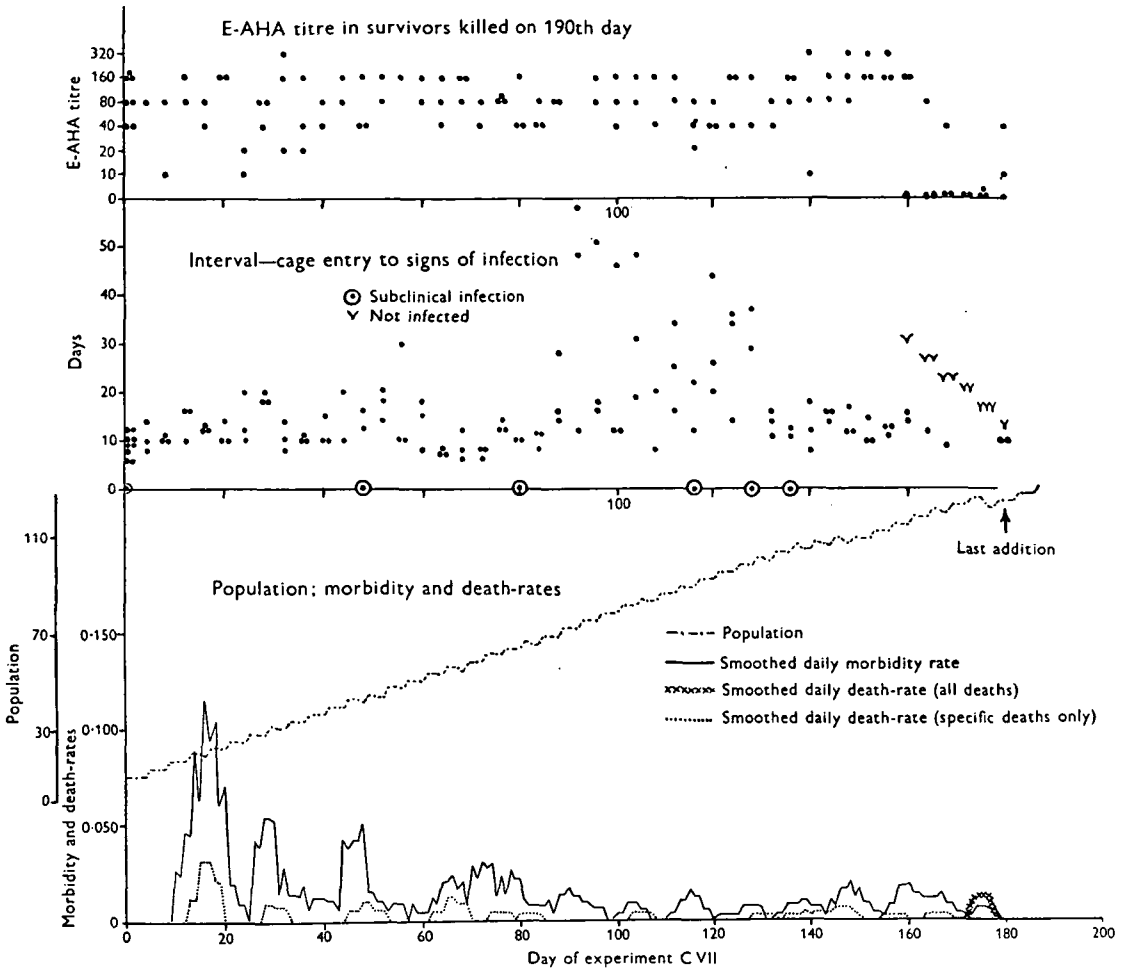

Fig. 2. The secular trend in population, morbidity rates, and total and specific death-rates in an epidemic of Hampstead mousepox maintained by adding three normal mice to the herd every fourth day. In the upper part of the figure are shown the interval between entry to the herd and the occurrence of signs of infection, and the E-AHA titre of survivors, for each group of three mice added on the indicated day of the experiment. Note that the scales used for population and interval between cage entry and infection are different from those used in Fig. 1.

Secular trend of morbidity and mortality

Figs. 1 and 2 show the secular trend in morbidity and specific and total mortality in the two experiments. I have followed the practice of Greenwood and used smoothed mortality and morbidity rates. The mortality (specific and total) and the morbidity recorded on any day is the ratio formed by dividing the sum of the deaths (or new cases) for the 5 days of which that day is central by the sum of the populations at the beginning of each of those 5 days. The morbidity rate was determined by the appearance of a primary lesion, or in the absence of this by the occurrence of death from acute mousepox or by the appearance of the secondary rash. In the last case the day of onset was taken as 2 days before the rash was detected. There were six subclinical cases in Exp. C VII in which the positive serological response and sometimes the appearance of the spleen at the end of the experiment were the only evidence of infection. No subclinical cases occurred in Exp. C VI.

There was a considerable difference in the mortality rates in the two epidemics, a fact which has been noted in previous experiments (Fenner, 1948*a*, *c*).

In contrast to this difference in mortality curves is the resemblance between the curves of morbidity rates. While there were only twenty-two specific

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deaths in Exp. CVII compared with ninety-six in the same period of Exp. CVI, the difference between the number of new cases recorded was only seventeen, consisting of six subclinical cases in Exp. CVII and eleven of the last eighteen mice added which had failed to acquire infection, or at least show any evidence of it, when the experiment was terminated. The consistently lower rates recorded in Exp. CVII are due to the larger population which occurred at all cage-ages. However, the intervals between entry to herd and development of infection varied greatly in the two experiments, as may be seen from the scatter diagrams showing the interval between entry to the herd and the development of signs of infection in each group of mice. Whereas, with the Moscow strain, the mean cage-age at the time of appearance of the primary lesion was $8{\cdot}4 \pm 2{\cdot}5$ days, with a range of 4–21 days, with the Hampstead strain the corresponding figures were $17{\cdot}3 \pm 10{\cdot}2$ days and 5–58 days. In both experiments there is a trend towards larger intervals between entry and infection as the population becomes larger. This is easily understood, for only mice with primary or secondary lesions were infectious and these became more and more diluted as the population increased, so that the chance of infection amongst the newer cage entrants became smaller. It was shown previously that mice which survived the acute disease and development of a rash were more important in the transmission of infection than were the acute fatal cases. Study of the secular variation in the duration and severity of the rashes and the secular variation between the interval from cage entry to primary lesion suggested that there was a relationship between the number of mice with severe rashes and the interval between entry and infection, but the position was complicated and not susceptible to satisfactory analysis.

Three factors were concerned in the differences in period between entry to the cage and infection in the two experiments. The rarity of severe skin lesions in Exp. CVII and their frequent occurrence in Exp. CVI was associated with much more massive contamination of the environment in the latter experiment. The higher mortality occurring with the Moscow strain kept the population smaller, and thus diminished the dilution of susceptible and infectious mice by healthy immune mice. The most important factor, however, was the considerable difference in the infectivity of the two strains even when the degree of contamination of the environment and the state of aggregation of the mice was the same (Fenner, 1948*a*).

In neither epidemic was any permanent change in the mortality rate seen, and both strains of virus maintained their original characters of virulence and infectivity after about twenty-four and thirteen serial passages by natural means. There were two periods in Exp. CVI, between the 122nd and 142nd and the 180th and 230th days, when the death-rate was lower than at other times, but many of the mice which survived had very severe rashes and the mortality rate after the 230th day returned to its original level. Two factors may have been concerned, chance variation in the natural resistance of the added mice or a decreased dosage of virus. There does not appear to have been any alteration in virulence of the type found in certain experiments with mouse pasteurellosis (Greenwood *et al.* 1936).

The life tables

The life tables (Tables 1–4) were kindly constructed by Mr J. B. Fletcher, M.A., F.F.A., of the Colonial Mutual Life Assurance Company, Melbourne, from the data of Exps. CVI (Moscow) and CVII (Hampstead). For the latter separate tables are presented for specific deaths and for all deaths. In Exp. CVI no specific deaths occurred after the 19th day and no non-specific deaths before the 48th day, so that both

Table 1. *Basic data for construction of life tables (Tables 2–4)*

	Moscow strain (Exp. CVI) 22. viii. 47– 6. vi. 48	Hampstead strain (Exp. CVII) 22. viii. 47– 27. ii. 48
Number of mice entering during experiment	214	145
Specific deaths from acute mousepox	129	20
Specific deaths from subacute mousepox	6	2
Non-specific deaths from bacterial pneumonia	12	2
Non-specific deaths during heat-wave	2	7
Probable reinfection	3	0
Surviving at end of experiment	65	114
Not infected* at end of experiment	0	11
Number of mouse days exposed to risk	11,933	12,817
Expectation of life at entry in days (all deaths):		
(*a*) Limited to 140 days	57·3	118·1
(*b*) Limited to 60 days	29·1	53·3
(*c*) Limited to 20 days	14·4	19·2

* Not infected = no clinical, autopsy, or serological evidence of infection

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Table 2. *Exp. C VI. Moscow strain*

Specific deaths only (to cage-age 20), all deaths (to cage-age 290)

Cage-age (days)	qx	lx	dx	$270Ex$	$140Ex$	$60Ex$	$20Ex$	$5qx$	Cage-age (days)
0	—	10000	—	90·1	57·3	29·1	14·4	—	0
1	—	10000	—	89·3	56·7	28·5	13·8	—	1
2	—	10000	—	88·5	56·0	27·9	13·2	—	2
3	—	10000	—	87·7	55·3	27·2	12·6	0·0140	3
4	—	10000	—	86·9	54·6	26·6	11·9	0·1215	4
5	—	10000	—	86·1	53·9	26·0	11·3	0·2710	5
6	—	10000	—	85·3	53·2	25·3	10·7	0·3925	6
7	0·01402	10000	140	84·5	52·6	24·7	10·0	0·4439	7
8	0·10900	9860	1075	84·9	52·6	24·4	9·5	0·4739	8
9	0·17021	8785	1495	94·4	58·3	24·7	10·1	0·4575	9
10	0·16667	7290	1215	113·0	69·6	31·6	11·5	0·3975	10
11	0·08462	6075	514	134·8	83·0	37·4	13·3	0·3156	11
12	0·06723	5561	374	146·5	90·2	40·5	14·2	0·2859	12
13	0·08108	5187	421	156·5	96·2	43·1	14·9	0·2614	13
14	0·07843	4766	374	169·7	104·3	46·6	15·9	0·2157	14
15	0·05319	4392	234	183·5	112·9	50·4	17·1	0·1598	15
16	0·04494	4158	187	193·3	119·0	53·1	17·9	0·1126	16
17	0·03529	3971	140	201·9	124·3	55·5	18·7	0·0708	17
18	0·02439	3831	93	208·8	128·7	57·4	19·3	0·0368	18
19	0·01282	3738	48	213·5	131·7	58·8	19·7	0·0128	19
20	—	3690	—	215·8	133·3	59·6	20·0	—	20
21	—	3690	—	—	133·1	59·5	20·0	—	21
22	—	3690	—	—	132·9	59·5	20·0	—	22
23	—	3690	—	—	132·8	59·5	20·0	—	23
24	—	3690	—	—	132·6	59·5	20·0	—	24
25	—	3690	—	—	132·4	59·5	20·0	—	25
26	—	3690	—	—	132·2	59·5	20·0	—	26
27	—	3690	—	—	132·0	59·5	20·0	—	27
28	—	3690	—	—	131·8	59·4	20·0	—	28
29	—	3690	—	—	131·5	59·4	20·0	—	29
30	—	3690	—	—	131·3	59·4	20·0	—	30
35	—	3690	—	—	130·2	59·3	19·9	—	35
40	—	3690	—	—	129·0	59·3	19·8	—	40
45	—	3690	—	—	128·0	59·2	19·8	0·0139	45
50	—	3638	—	—	128·4	59·9	20·0	—	50
55	—	3638	—	—	126·8	59·7	20·0	—	55
60	—	3638	—	—	125·1	59·5	20·0	—	60
65	—	3638	—	—	123·4	59·2	20·0	—	65
70	—	3638	—	—	121·5	58·8	20·0	—	70
75	—	3638	—	—	119·6	58·2	20·0	—	75
80	—	3638	—	—	117·7	57·6	20·0	—	80
85	—	3638	—	—	115·8	56·9	20·0	—	85
90	—	3638	—	—	113·9	56·2	19·9	—	90
95	—	3638	—	—	112·0	55·5	19·7	—	95
100	—	3638	—	—	110·1	54·7	19·5	0·0200	100
110	—	3565	—	—	108·6	54·1	19·3	0·0217	110
120	—	3404	—	—	109·4	54·9	19·3	—	120
130	—	3224	—	—	111·3	55·9	19·6	—	130
140	—	3120	—	—	111·2	55·4	20·0	—	140
150	—	3120	—	—	107·6	52·9	19·5	—	150
160	—	3120	—	—	—	50·1	18·8	0·0741	160
170	—	2880	—	—	—	52·0	19·5	—	170
180	—	2880	—	—	—	49·6	18·0	0·0500	180
190	—	2584	—	—	—	53·5	18·5	0·0625	190
200	—	2423	—	—	—	55·2	18·9	0·0667	200
210	—	2261	—	—	—	57·8	20·0	—	210
220	—	2261	—	—	—	56·7	20·0	—	220
230	—	2261	—	—	—	55·6	20·0	—	230
240	—	2261	—	—	—	—	18·9	—	240
250	0·11111	2261	151	—	—	—	17·8	0·1111	250
260	—	2010	—	—	—	—	20·0	—	260
270	—	2010	—	—	—	—	20·0	—	270
280	—	2010	—	—	—	—	—	—	280
290	—	2010	—	—	—	—	—	—	290

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Table 3. *Exp. C VII. Hampstead strain*

All deaths

Cage-age (days)	qx	lx	dx	$170Ex$	$140Ex$	$60Ex$	$20Ex$	$5qx$	Cage-age (days)
0	—	10000	—	141·3	118·1	53·3	19·2	0·0075	0
1	—	10000	—	141·0	117·9	53·1	19·1	0·0075	1
2	—	10000	—	140·7	117·7	52·9	19·0	0·0075	2
3	0·00752	10000	75	140·4	117·4	52·7	18·8	0·0075	3
4	—	9925	—	141·2	118·1	52·9	18·9	—	4
5	—	9925	—	140·9	117·9	52·8	18·8	—	5
6	—	9925	—	140·6	117·7	52·6	18·6	0·0231	6
7	—	9925	—	140·3	117·5	52·4	18·5	0·0616	7
8	—	9925	—	139·9	117·3	52·2	18·4	0·0616	8
9	—	9925	—	139·5	117·1	52·1	18·3	0·0770	9
10	0·02308	9925	229	139·1	116·9	51·9	18·2	0·0846	10
11	0·03937	9696	382	142·0	119·4	52·9	18·5	0·0630	11
12	—	9314	—	147·5	124·2	55·0	19·1	0·0327	12
13	0·01639	9314	153	147·1	124·0	54·9	19·1	0·0327	13
14	0·00833	9161	76	149·3	125·9	55·7	19·3	0·0166	14
15	—	9085	—	150·2	126·8	56·0	19·4	0·0084	15
16	0·00840	9085	76	149·9	126·7	55·9	19·4	0·0167	16
17	—	9009	—	150·8	127·7	56·3	19·5	0·0084	17
18	—	9009	—	150·5	127·5	56·2	19·4	0·0084	18
19	—	9009	—	150·2	127·4	56·1	19·4	0·0255	19
20	0·00847	9009	76	149·8	127·3	56·0	19·3	0·0255	20
21	—	8933	—	—	128·2	56·4	19·4	0·0172	21
22	—	8933	—	—	128·0	56·3	19·4	0·0172	22
23	0·01724	8933	154	—	127·9	56·2	19·3	0·0260	23
24	—	8779	—	—	130·0	57·2	19·6	0·0087	24
25	—	8779	—	—	129·8	57·1	19·6	0·0178	25
26	—	8779	—	—	129·7	57·0	19·6	0·0178	26
27	0·00885	8779	78	—	129·5	57·0	19·5	0·0178	27
28	—	8701	—	—	130·6	57·4	19·7	0·0090	28
29	0·00893	8701	78	—	130·4	57·3	19·6	0·0090	29
30	—	8623	—	—	131·4	57·8	19·8	—	30
35	—	8623	—	—	130·5	57·6	19·6	0·0094	35
40	—	8542	—	—	130·5	57·9	19·5	0·0100	40
45	—	8457	—	—	130·4	58·3	19·5	0·0104	45
50	—	8369	—	—	130·4	58·7	19·6	0·0217	50
55	—	8187	—	—	—	60·0	20·0	—	55
60	—	8187	—	—	—	59·9	20·0	—	60
65	—	8187	—	—	—	59·8	20·0	—	65
70	—	8187	—	—	—	59·6	20·0	—	70
75	—	8187	—	—	—	59·4	20·0	—	75
80	—	8187	—	—	—	59·2	20·0	—	80
85	—	8187	—	—	—	59·0	20·0	—	85
90	—	8187	—	—	—	58·8	20·0	—	90
95	—	8187	—	—	—	58·6	20·0	—	95
100	—	8187	—	—	—	58·4	19·9	—	100
110	—	8187	—	—	—	57·5	19·6	—	110
120	0·02000	8033	161	—	—	56·9	19·6	0·0200	120
130	—	7872	—	—	—	55·8	20·0	—	130
140	—	7872	—	—	—	—	20·0	—	140
150	—	7872	—	—	—	—	19·5	—	150
160	—	7557	—	—	—	—	18·8	—	160
170	—	7159	—	—	—	—	18·0	—	170
180	—	6137	—	—	—	—	—	—	180
190	—	6137	—	—	—	—	—	—	190

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Table 4. *Exp. CVII. Hampstead strain*

Specific deaths only

Cage-age (days)	qx	lx	dx	$60Ex$	$20Ex$	$5qx$	Cage-age (days)
0	—	10000	—	54·0	19·3	—	0
1	—	10000	—	53·8	19·2	—	1
2	—	10000	—	53·6	19·1	—	2
3	—	10000	—	53·5	19·0	—	3
4	—	10000	—	53·3	18·9	—	4
5	—	10000	—	53·2	18·8	—	5
6	—	10000	—	53·0	18·6	0·0231	6
7	—	10000	—	52·8	18·5	0·0616	7
8	—	10000	—	52·7	18·4	0·0616	8
9	—	10000	—	52·5	18·3	0·0770	9
10	0·02308	10000	231	52·4	18·2	0·0847	10
11	0·03937	9769	385	53·5	18·5	0·0631	11
12	—	9384	—	55·5	19·2	0·0328	12
13	0·01639	9384	154	55·4	19·1	0·0328	13
14	0·00833	9230	77	56·3	19·4	0·0167	14
15	—	9153	—	56·6	19·5	0·0084	15
16	0·00840	9153	77	56·6	19·4	0·0168	16
17	—	9076	—	57·0	19·6	0·0085	17
18	—	9076	—	56·9	19·5	0·0085	18
19	—	9076	—	56·8	19·5	0·0256	19
20	0·00847	9076	77	56·7	19·4	0·0256	20
21	—	8999	—	57·2	19·6	0·0172	21
22	—	8999	—	57·1	19·5	0·0172	22
23	0·01724	8999	155	57·0	19·5	0·0172	23
24	—	8844	—	58·0	19·8	—	24
25	—	8844	—	57·9	19·8	0·0089	25
26	—	8844	—	57·9	19·7	0·0089	26
27	—	8844	—	57·8	19·7	0·0089	27
28	—	8844	—	57·8	19·7	0·0089	28
29	0·00893	8844	79	57·7	19·6	0·0089	29
30	—	8765	—	58·2	19·8	—	30
35	—	8765	—	58·0	19·6	0·0095	35
40	—	8682	—	58·4	19·6	0·0100	40
45	—	8595	—	58·8	19·7	0·0105	45
50	—	8505	—	59·4	19·8	0·0108	50
55	—	8413	—	60·0	20·0	—	55
60	—	8413	—	60·0	20·0	—	60

specific and total deaths have been included in one table. The experiments were concluded too soon to allow the estimation of the unlimited expectation of life, but expectations limited to 20, 60, 140 and 270 days were determined for the data from Exp. CVI, and 20, 60, 140 and 170 days from the data from Exp. CVII.

The ways in which our experience differed from that of Greenwood *et al.* (1936) can be clearly seen from Fig. 3, which shows the probability of dying in the next 5 days ($5qx$) for the first 60 days of Exps. CVI and CVII, and Exps. Ectromelia 1 and Ectromelia 2 of Greenwood.

With the Moscow strain $5qx$ rose sharply to the high figure of 0·47 and returned to zero by the 20th day. Apart from a small rise due to a non-specific death on the 48th day it remained at zero. Mortality in the Hampstead epidemic (Exp. CVII) was very much lower and less regular; the curve remained very near the base line before the 5th and after the 25th days and rose to a maximum of 0·085 on the 10th day. The curves for the two epidemics described by Greenwood rise to a broad peak at about 0·2 and remain at that level between the 10th and 20th days and then decline slowly but in neither experiment reach the base line. The difference in the heights of the curves and the period for which they remain above the base line probably depends upon a number of factors, of which three can be defined. The low curve of the Hampstead (Exp. CVII) epidemic is due to its low virulence and its prolongation above zero until the 55th day to its low infectivity. The virus used in Greenwood's experiments was more virulent, but it also was of a relatively low order of infectivity (see also Fenner, 1948*c*), and the dilution of susceptible and infectious

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mice by healthy immune animals lowered its apparent infectivity still further, for these experiments included many more animals than did either Exp. CVI or Exp. CVII.

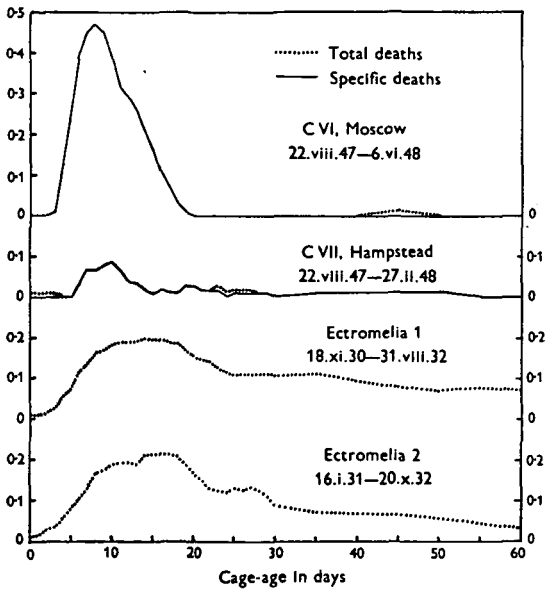

Fig. 3. The probability of dying in the next 5 days ($5qx$) for the first 60 days of cage-life for Exps. C VI (Moscow) and C VII (Hampstead) and for epidemics Ectromelia 1 and Ectromelia 2 of Greenwood *et al.* (1936).

Immunity in mousepox

Both experiments show that immunity to reinfection after recovery from an attack of mousepox is of a high order. No deaths from mousepox were recorded in mice which had suffered an earlier attack, and in only five mice (all in Exp. CVI) were signs which might be interpreted as evidence of reinfection seen. All these animals developed swollen feet about 100 days after admission to the herd, that is about 80 days after recovery from their original attack of mousepox. In two cases the swelling affected the periarticular tissues around the ankle joint, but in the other three it resembled the swelling associated with primary lesions of mousepox. One of these animals had entered the herd on day 0 and another on day 36, and these two mice had E-AHA titres of 80 when killed on day 290. This titre was higher than that found in five of their six surviving contemporaries, providing some support for the view that virus multiplication had occurred. Within the time limits of these experiments, therefore, reinfection or clinical recurrence was very rare.

Further support for this view is provided by the low antibody titres found in most mice which had been in the herd (Exp. CVI) for more than 190 days (see Fig. 1). Examination of 25 mice which had not been exposed to reinfection for 12 months after recovery from mousepox showed that the mean E-AHA titre was 40, with a range of 10–80. When such mice were reinfected by the inoculation of large doses of virus the titre always rose, usually to 160 and sometimes to 640 (Fenner, 1948*d*). The absence of such rises in the old survivors of Exp. C VI (except perhaps for the two mice mentioned earlier in which the E-AHA titre was 80), and the regular and gradual fall in E-AHA titres as the cage-age of the survivors increased, suggests that reinfection rarely occurred amongst these mice. The trend in E-AHA titres in Exp. C VII is similar (Fig. 2), but owing to its shorter duration the fall is not as great as in Exp. C VI.

Persistence of the virus in mousepox

Most of the investigators who have described the interruption of experiments with mouse-lung passage of influenza virus by contamination and eventual replacement of the influenza virus by ectromelia have ascribed this to latent ectromelia infection of the mouse stock associated with persistence of virus in the lungs (Fairbrother & Hoyle, 1937; Kikuth & Gönnert, 1940; Andrewes, 1939). Hornus & Thibault (1939) described the occurrence of fatal cases of ectromelia amongst mice inoculated with a variety of agents, and considered that this was due to activation of a latent infection.

This view seems to be incompatible with the conception that mousepox is like smallpox, a disease with a defined incubation period and regular course which may vary greatly in severity, but which is followed by disappearance of the virus and very nearly complete immunity to reinfection. In an earlier paper (Fenner, 1948*a*) some experiments were described which were designed to determine whether ectromelia could be produced by 'provocation' in mice which had recovered from mousepox, as claimed by Kikuth & Gönnert (1940). The results were completely negative. In the present experiment the spleens of all survivors of Exp. CVI, and the lungs of all survivors except those which had lived in the herd for more than 140 days, were ground and suspensions inoculated undiluted and at a 1:100 dilution in mice, the spleen suspensions intraperitoneally and the lung suspensions intranasally. Material from three mice contained virus, and the clinical histories of these mice are shown briefly in Table 5.

The positive result obtained with mouse 882 was not unexpected, for this animal was still suffering from active mousepox. The other two mice had completely recovered from their original infections and showed no sign of disease when they were killed. The discovery of small amounts of virus in the spleen of one mouse and the lungs of both suggests that occasionally a true latent carrier state may occur.

Table 5. *Clinical histories of mice from the lungs or spleen of which virus was isolated (Exp. C VI).*

Mouse no.	Subinoculations of: Lung	Subinoculations of: Spleen	No. of days since: Appearance of primary lesion	No. of days since: Healing of secondary rash	Autopsy appearance of: Spleen	Autopsy appearance of: Lungs	E-AHA titre of serum
58831	1/2*	2/2	87	75	—	—	160
58865	1/2	0/2	39	28	—	—	320
58882	2/2	0/2	6	Unhealed	+	—	640

* Numerator = mice infected; denominator = mice inoculated with undiluted organ suspensions.

It appears to be rare, since virus was found in the spleen of only one out of 65 mice tested and in the lungs of two out of 43 mice. In other experiments the livers and spleens of mice which had recovered from mousepox were tested, and no positive results were obtained with 20 animals in which 3 weeks or more had elapsed since their infection. The recurrence of the foot swelling, and the isolation of virus from the swollen foot, of mice which had recovered from infection 2 and 7 months earlier by inoculation of that foot, and had not subsequently been exposed to infection, was recorded earlier (Fenner, 1948*a*). No further instances of this occurred in another 25 mice which were regularly examined for 10 months.

There is some support, therefore, for the view that a latent carrier state may occur in ectromelia, but it appears to be rare. As a rule recovery from infection is accompanied by the elimination of virus from the body, and a high degree of immunity to reinfection. The occurrence of mousepox during serial passage of mouse lungs would not, then, be the result of provocation of latent virus but of inclusion in the passage material of the lung of one of the rare latent cases or from a mild unrecognized case of the disease. Serial passage of such material would increase its titre and therefore apparently its virulence until inoculated mice died from mousepox. Past infection of the inoculated mice would not prevent death when large doses of virus were inoculated intranasally (Fenner, 1948*d*).

The results of Hornus & Thibault (1939) are perhaps best explained by assuming that in mice which were incubating or suffering from mild mousepox a non-specific irritation, such as an intraperitoneal inoculation, so depressed the animal's defences that it succumbed to mousepox. Gönnert's (1942) results show the ill-effects of attempted mechanical or chemical stimulation during the incubation period.

DISCUSSION

The picture of mousepox emerging from these and other experiments is that of a disease which presents many analogies to smallpox, that is a disease in which there is a definite incubation period, followed by a period of patent or latent infection, followed in turn by elimination of the virus and almost complete immunity to reinfection. This corresponds fairly accurately with the conclusion reached by Kermack & McKendrick (1937) for their analysis of Greenwood's data. Transmission is due to the entry of virus through minute skin abrasions, and virus is excreted principally through the lesions of the secondary rash. Chronic carriers of the virus are very rare but they do occur. There is no evidence that they are able to transmit the disease.

The length of the incubation period in the early stages of these experiments was approximately the same as in the formal experiments described earlier (Fenner, 1948*a*), that is 7 days for the Moscow strain and 8 days for the Hampstead strain. The slow increase in the interval between entry to the cage and infection in the Moscow epidemic and the rapid increase in the Hampstead epidemic was due to the decreasing chance of infection which occurred when infective and susceptible mice were increasingly diluted by healthy immune animals. The same factor acted on a larger scale in Greenwood's experiments, and probably accounts for the occasional occurrence of deaths from mousepox several weeks after entry to the herd.

SUMMARY

1. Two long-continued epidemics of mousepox were set up with different strains of the virus and maintained for 190 and 290 days respectively. Considerable differences were observed between the behaviour of the two strains of virus, the Moscow strain being much more virulent and more highly infective than the Hampstead strain.

2. The two strains of virus maintained their original characters through the experiments.

3. Life tables were constructed for both epidemics, specific and non-specific deaths being dealt with separately. They show that high and durable immunity follows recovery from infection.

4. Two mice which had recovered from infection were found to harbour small quantities of the virus in the lungs, and in one case in the spleen also, suggesting that chronic latent carriers of the virus may occur.

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