CONTRIBUTIONS TO THE EXPERIMENTAL STUDY OF EPIDEMIOLOGY. FURTHER OBSERVATIONS ON THE EFFECT OF VACCINATION ON HERD MORTALITY.

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(*From the London School of Hygiene and Tropical Medicine.*)

(With two Graphs.)

In the last memoir of this series (Greenwood, Topley and Wilson, 1931) we discussed at some length the modifications of the course of herd sickness effected by immunisation of entrants to the herds. Briefly, these were our conclusions: efficient artificial immunisation (the criteria of efficiency being the usual standardising methods of a laboratory) confers a considerable temporary advantage upon mice entering an infected herd. The risk of death during the period of herd life when the rate of mortality is especially high is greatly reduced, so that when one has the case of a herd or group temporarily exposed to special risks, the value of the expedient is very great. But no method of artificial immunisation we have tried will render the immunised animals impervious to the risks which their membership of the herd entails.

The experiments now to be described were designed to test two points. First of all we wished to study the course of herd mortality when recruitment was confined to immunised animals. Secondly we wished to compare the effects of immunisation by different routes.

Exp. 1. Taking the former problem first, if the reasoning of our last paper were just, we should expect that the rate of mortality in a herd recruited from immunised animals, or any function of the rate of mortality, would over a fair range of herd age be much more favourable than that of a herd recruited in the usual way from unsalted stock, but that mortality from the specific cause would not be extinguished. The experiment was inaugurated on 14. iii. 29 by bringing together 25 mice, each inoculated with 1000 *Bact. aertrycke*, and 100 normal mice. Three normal mice were added daily for 59 days. On 13. v. 29 the normal immigrants were replaced by 3 mice daily which had been vaccinated.

As in our previous experiments the mice were vaccinated by intraperitoneal injection of a killed formolised suspension of *Bact. aertrycke*, which contained both the "H" and the "O" antigens. Two doses of 500×10^6 bacilli were given, with an interval of 1 week between the two doses. The interval between the administration of the second dose of vaccine and addition to the infected herd varied from 7 to 13 days. A certain number of mice were vaccinated each week, over and above those required for the daily additions; these were anaesthetised, bled to death from the jugular vein, and their sera tested for the presence of agglutinins. As it was known from previous experience (Topley 1929) that "H" agglutinins would almost invariably be

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present, 45 out of 67 specimens of serum were put up against the "O" antigen of *Bact. aertrycke* only, so that it was possible to start at a low dilution of serum (1 in 5). The remaining 22 specimens were tested against both forms of antigen, starting at a dilution of 1 in 20. The results are given below.

Number of sera tested against "O" only = 45
" agglutinating "O" antigen = 9 (20 per cent.)
" tested against "O" and "H" = 22
" agglutinating "H" alone = 20 (90·9 per cent.)
" " "O" " = 0
" " both "O" and "H" = 2 (9·1 per cent.)

Three mice continued to be added until 21. x. 29 from which date 1 vaccinated mouse was added daily until 29. vi. 30 when immigration ceased. On 29. viii. 30 the survivors were killed. Table I records the general experience of the normal mice which entered from 15. iii. 29 to 12. v. 29; Table II that of the vaccinated mice.

Table I. *Cage V. Normal mice, entrants* 15. *iii.* 29–12. *v.* 29.

No. of mice	177
No. who die	177
No. who die of specific (*Bact. aertrycke* + N.E.) deaths	169
No. of mouse days exposed to risk	5870
Expectation of life at entry in days:	
(*a*) From specific deaths only	34·82
(*b*) From all deaths	32·66
Life table death-rate daily:	
(*a*) From specific deaths	0·0287
(*b*) From all deaths	0·0306
Expectation of life, limited to 60 days, at entry:	
(*a*) Specific deaths	27·00 ± 1·019
(*b*) All deaths	26·35 ± 1·003

Table II. *Cage V. Data used in the life tables.*

	Three mouse period	One mouse period
Period during which mice were entered	13. v. 29 to 20. x. 29	21. x. 29 to 29. vi. 30
No. of calendar days	161	252
Period covered by life table	13. v. 29 to 29. viii. 30	21. x. 29 to 29. viii. 30
No. of mice concerned	483	252
No. who die	482	242
No. who die of specific (*Bact. aertrycke* + N.E.) deaths	396	220
No. surviving at the end of the period	1	10
No. of mouse days exposed to risk	19,207	10,461
Expectation of life at entry in days:		
(*a*) From specific deaths (*Bact. aertrycke* + N.E.) only	47·04	48·16
(*b*) From all deaths	39·34	43·25
Life table death-rate daily:		
(*a*) From specific (*Bact. aertrycke* + N.E.) only	0·0213	0·0208
(*b*) From all deaths	0·0254	0·0231
Expectation of life at entry limited to 60 days:		
(*a*) From specific (*Bact. aertrycke* + N.E.) deaths	34·92 ± 0·684	36·38 ± 0·938
(*b*) From all deaths	32·11 ± 0·661	35·35 ± 0·932

For reasons discussed in earlier papers we think that the most useful standard of comparison is the expectation of life limited to 60 days and, although the estimated standard errors of this measure are only approxi-

mations, and we must remember that all survivors were killed, we think that a comparison is fair. In Table III and the graphs we show the probability of dying within the next 5 days for the various categories over the first 60 days of cage life.

From the nature of the experiment a strict control in time against unimmunised mice is not possible, the comparison with the normal additions *may* be unfair to the latter since their experience was during the epoch when the ratio of deliberately infected animals to all exposed to risk was large. But the general run of events is unmistakable. Putting the advantage of immunisation at its highest, *i.e.* by admitting the comparison of immunised and normals to be valid, we see that the immunised enjoyed in respect of limited expectation of life an advantage of the order of 50 per cent., but that during the time when all immigrants had been previously immunised the specific rate of mortality was still high and the vital statistics of the community very different from those of an uninfected herd. Restriction of immigrants to immunised animals did not bring the epidemic sickness to an end. This special experiment fully confirms the conclusions drawn from our earlier work. It may also be noticed that although reduction of the daily number of immigrants was associated with a decrease of the rate of mortality, the decrease was not large.

Exp. 2. We now pass to the experiment designed to test the relative advantages of intra-peritoneal and oral administration of vaccine.

Here again the vaccine used was a killed formolised suspension of *Bact. aertrycke* containing both the "O" and the "H" antigens. The P mice (see below) received two doses of 500×10^6 bacilli; the O mice were given two doses of 5000×10^6 bacilli introduced directly into the stomach by means of a fine ureteric catheter attached to a syringe. As before, a small number of extra mice were vaccinated each week, and the results of testing their sera for agglutinins are shown below.

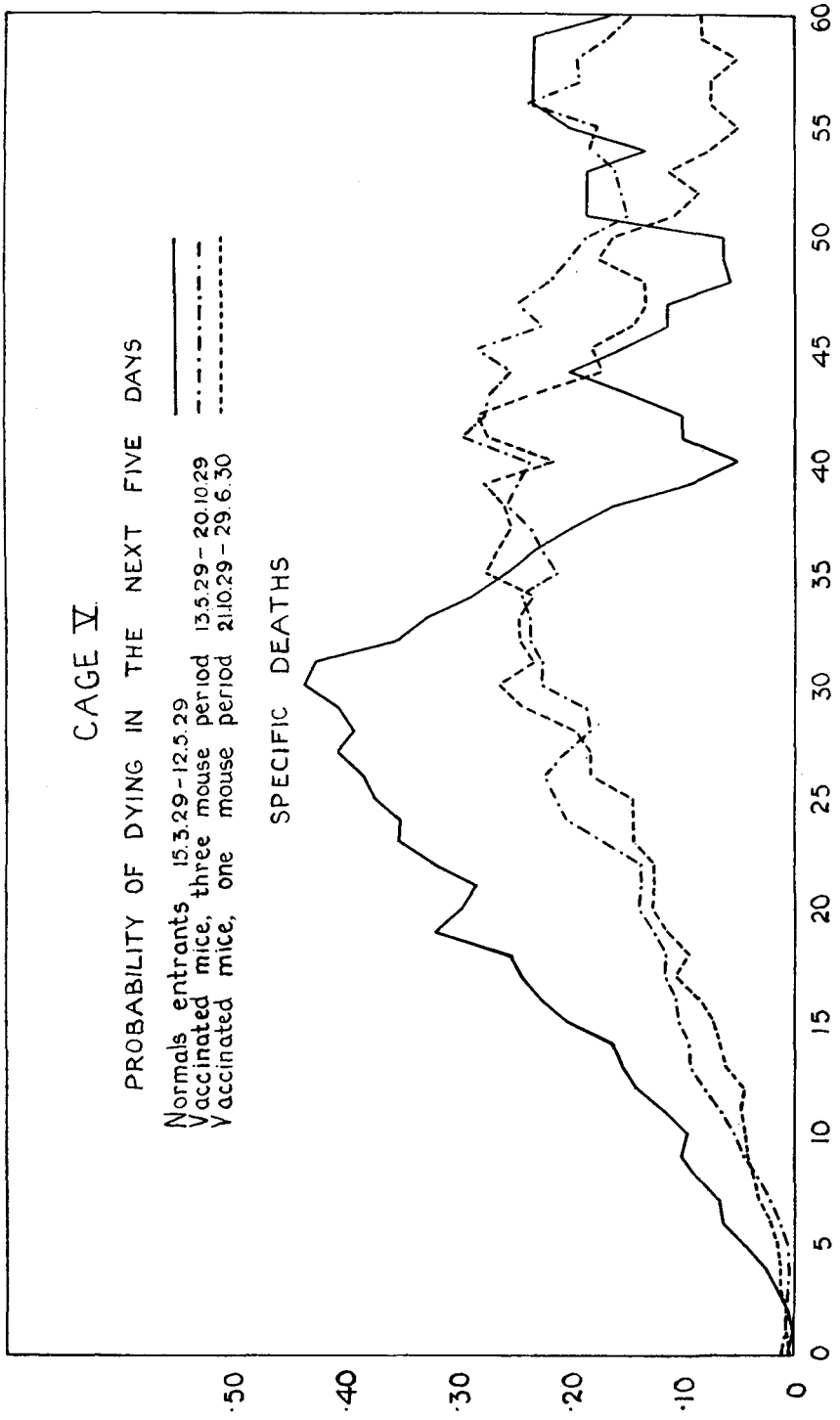
Route	No. tested against "O" only (lowest dilution 1/5)	No. positive		
I.P.	14	4 (28·6 %)		
Per os	20	6 (30·0 %)		
	No. tested against "O" and "H" (lowest dilution 1/20)	"H" + only	"O" + only	"O" and "H"
I.P.	14	12	0	2
Per os	23	8	0	0

It appears therefore that intraperitoneal vaccination produced "H" agglutinins in every mouse tested, whereas the "O" form was only found in about one-third of the sera. *Per os* inoculation, on the other hand, did not produce "H" agglutinins nearly so frequently—only 8 of 23 mice gave positive reactions, and those mostly to low titre. As regards "O" agglutinins there was no significant difference between the effects of oral and intraperitoneal administration.

These observations are in general accord with those of Pijper and Dau (1930), who noted the development of "O," but not of "H" agglutinins in human subjects who had received typhoid vaccine by the mouth. Since, as

Table III. *Cage V. Probability of dying in the next 5 days.*

Cage age in days	Total deaths			Specific deaths		
		Vaccinated mice			Vaccinated mice	
	Normals entrants 15. iii. 29– 12. v. 29	Three mouse period 13. v. 29– 20. x. 29	One mouse period 21. x. 29– 29. vi. 30	Normals entrants 15. iii. 29– 12. v. 29	Three mouse period 13. v. 29– 20. x. 29	One mouse period 21. x. 29– 29. vi. 30
0	0·0057	0·0104	0·0079	0·0057	0·0104	0·0040
1	0	0·0104	0·0119	0	0·0083	0·0080
2	0·0057	0·0104	0·0119	0·0057	0·0063	0·0080
3	0·0170	0·0104	0·0159	0·0170	0·0063	0·0159
4	0·0341	0·0084	0·0120	0·0284	0·0042	0·0120
5	0·0511	0·0105	0·0160	0·0456	0·0063	0·0160
6	0·0739	0·0147	0·0201	0·0627	0·0126	0·0201
7	0·0800	0·0274	0·0321	0·0689	0·0232	0·0321
8	0·0983	0·0358	0·0364	0·0874	0·0316	0·0364
9	0·1118	0·0526	0·0445	0·1004	0·0464	0·0405
10	0·1138	0·0613	0·0488	0·0964	0·0530	0·0447
11	0·1288	0·0768	0·0533	0·1171	0·0665	0·0493
12	0·1615	0·0887	0·0498	0·1439	0·0804	0·0458
13	0·1731	0·1135	0·0672	0·1558	0·0966	0·0633
14	0·1722	0·1133	0·0678	0·1604	0·0961	0·0678
15	0·2095	0·1239	0·0769	0·2036	0·1043	0·0726
16	0·2324	0·1386	0·0909	0·2267	0·1072	0·0867
17	0·2444	0·1591	0·1092	0·2444	0·1183	0·1050
18	0·2558	0·1601	0·0991	0·2558	0·1177	0·0949
19	0·3200	0·1754	0·1227	0·3200	0·1254	0·1141
20	0·2991	0·2031	0·1343	0·2991	0·1401	0·1298
21	0·2844	0·1957	0·1381	0·2844	0·1373	0·1288
22	0·3235	0·1977	0·1422	0·3235	0·1396	0·1280
23	0·3542	0·2287	0·1650	0·3542	0·1731	0·1461
24	0·3529	0·2523	0·1606	0·3529	0·2047	0·1461
25	0·3780	0·2548	0·1604	0·3780	0·2149	0·1459
26	0·3974	0·2600	0·1934	0·3846	0·2211	0·1839
27	0·4203	0·2359	0·1886	0·4080	0·2069	0·1837
28	0·4032	0·2015	0·1976	0·3905	0·1806	0·1976
29	0·4182	0·2154	0·2469	0·4058	0·1898	0·2407
30	0·4706	0·2467	0·2675	0·4393	0·2277	0·2615
31	0·4468	0·2613	0·2534	0·4263	0·2264	0·2335
32	0·3750	0·2811	0·2676	0·3519	0·2376	0·2481
33	0·3514	0·2905	0·2687	0·3273	0·2374	0·2491
34	0·3125	0·3005	0·2541	0·2870	0·2449	0·2321
35	0·2593	0·2701	0·3130	0·2593	0·2104	0·2748
36	0·2308	0·2622	0·2936	0·2308	0·2254	0·2677
37	0·2000	0·2628	0·2788	0·2000	0·2359	0·2525
38	0·1667	0·2886	0·2857	0·1667	0·2586	0·2596
39	0·0909	0·2815	0·2967	0·0909	0·2469	0·2789
40	0·0500	0·2756	0·2278	0·0500	0·2385	0·2152
41	0·1000	0·3306	0·2857	0·1000	0·2963	0·2740
42	0·1000	0·3217	0·2933	0·1000	0·2779	0·2817
43	0·1500	0·3113	0·2429	0·1500	0·2705	0·2304
44	0·2000	0·2784	0·1875	0·2000	0·2510	0·1742
45	0·1579	0·3043	0·1967	0·1579	0·2858	0·1803
46	0·1111	0·2469	0·1636	0·1111	0·2268	0·1466
47	0·1111	0·2564	0·1509	0·1111	0·2462	0·1336
48	0·0588	0·2329	0·1509	0·0588	0·2192	0·1336
49	0·0625	0·2143	0·2115	0·0625	0·2003	0·1758
50	0·0625	0·2031	0·1837	0·0625	0·1889	0·1638
51	0·1875	0·1639	0·1304	0·1875	0·1490	0·1092
52	0·1875	0·1724	0·1111	0·1875	0·1576	0·0894
53	0·1875	0·1786	0·1333	0·1875	0·1607	0·1122
54	0·1333	0·2000	0·0732	0·1333	0·1826	0·0732
55	0·2000	0·1961	0·0500	0·2000	0·1786	0·0500
56	0·2308	0·2549	0·0750	0·2308	0·2387	0·0750
57	0·2308	0·2083	0·0750	0·2308	0·1911	0·0750
58	0·2308	0·2174	0·0513	0·2308	0·1957	0·0513
59	0·2308	0·1818	0·0804	0·2308	0·1591	0·0804
60	0·1667	0·1707	0·0804	0·1667	0·1477	0·0804



Graph I.

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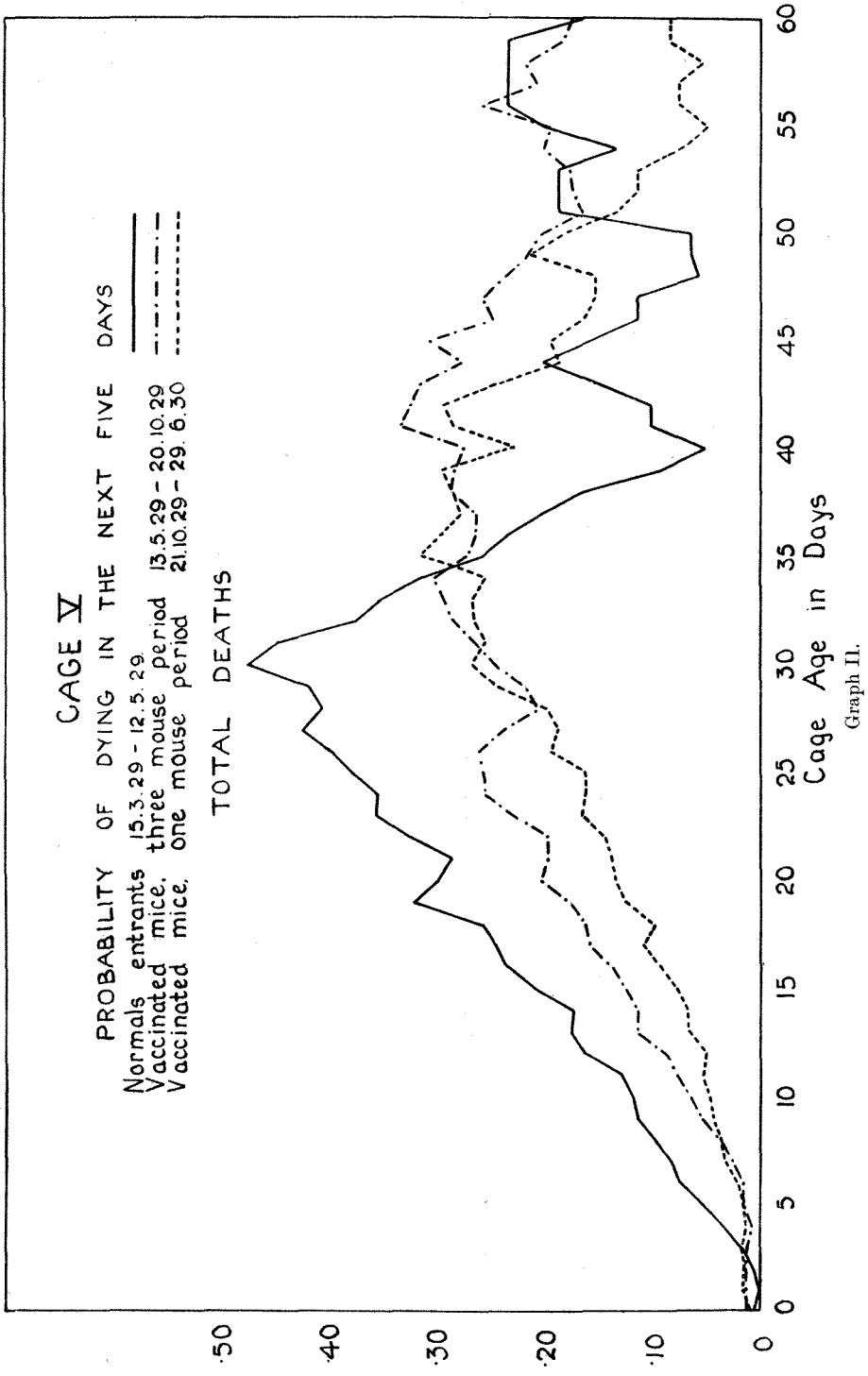

Graph II.

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will be seen, the advantage of the mice vaccinated *per os* was but little inferior to that of those vaccinated by the intraperitoneal route, the results of this experiment afford additional evidence of the preponderating rôle of the "O" antibodies in immunity.

The experiment was inaugurated on 3. x. 29 by bringing together 25 mice each inoculated with 1000 *Bact. aertrycke* and 100 normals. Three normals were added daily down to 12. xii. 29. Thenceforward 2 normals were added daily and at weekly intervals three batches of 10 each were added, viz.:

O—mice vaccinated orally;

P—mice vaccinated intraperitoneally;

N—normal mice.

On 24. iv. 30 the last of the weekly batches was added.

On 22. vi. 30 the last daily addition was made.

On 23. vi. 30 all survivors were killed.

Table IV contains the summarised particulars. Table V contains a detailed comparison of the limited expectations of the several batches. Table VI compares, account being taken of estimated error of sampling, the limited expectations of life at various herd ages from day 0 to day 40.

Table IV.

	N	O	P	Added daily
Period covered	12. xii. 29–23. vi. 30	12. xii. 29–23. vi. 30	12. xii. 29–23. vi. 30	9. xii. 29–23. vi. 30
No. of calendar days	193	193	193	196
No. of mice concerned	200	200	200	283
No. who die of *Bact. aertrycke*	135	129	117	190
No. who die of N.E.	44	34	37	59
No. who die of nil found	11	9	9	7
No. surviving at end of period	10	28	37	27
No. of mouse days exposed to risk ...	7397	9137	10,064	10,804
Expectation of life at entry in days:				
(*a*) Unlimited from specific deaths only	40·41	55·24	65·02	44·65
(*b*) From all deaths	38·38	52·61	61·46	43·44
Limited to 60 days:				
(*a*) From specific deaths only	31·18	35·30	38·19	30·75
(*b*) From all deaths	30·16	34·45	37·32	30·23
Life table death-rates:				
(*a*) From specific deaths only	0·0247	0·0181	0·0154	0·0224
(*b*) From all deaths	0·0261	0·0190	0·0163	0·0230

It will be seen that both P and O mice have more favourable experiences than either N or Added Daily mice, over a considerable range of herd life. Take for instance the limited expectation at entrance (specific deaths), the difference between the P mice and the normals is 7·01 days or more than 22 per cent. of the expectation of the normals. The standard error of this difference is $\pm$ 1·57, so that it is unlikely to be a mere random fluctuation. The advantage is consistently maintained to the 40th day of cage life, although we have then reached a stage where the ratio of the difference to its standard error is much smaller than before. The difference between the P mice and the best of the normals is 6·21 days with a standard error of 3·18. At later

ages the advantage of the vaccinated mice is negative or imperceptible. Throughout, the mice vaccinated *per os* have a smaller advantage than the mice vaccinated intraperitoneally.

Table V. *Expectation of life of each batch limited to* 60 *days. Specific deaths only.*

Date of entry	Added daily (against midday of the 7 days)	N	O	P
12. xii. 29	33·89	32·05	25·70	36·90
19. xii. 29	25·22	22·00	43·50	37·80
26. xii. 29	30·07	37·50	38·25	28·80
2. i. 30	27·82	26·55	37·98	43·76
9. i. 30	40·35	32·60	50·20	41·17
16. i. 30	26·96	30·10	25·65	38·70
23. i. 30	39·29	38·11	31·50	38·75
30. i. 30	33·43	29·62	28·15	37·70
6. ii. 30	37·72	42·00	45·10	47·90
13. ii. 30	35·69	31·50	43·85	41·69
20. ii. 30	26·96	42·24	38·25	42·80
27. ii. 30	28·08	34·61	35·15	31·45
6. iii. 30	34·96	31·05	36·12	40·40
13. iii. 30	27·11	29·30	38·45	43·50
20. iii. 30	31·00	27·20	28·70	38·78
27. iii. 30	31·43	22·50	25·40	38·53
3. iv. 30	27·29	34·60	40·75	34·17
10. iv. 30	29·90	33·00	28·46	42·10
17. iv. 30	23·11	20·00	36·15	28·35
24. iv. 30	20·07	25·60	26·00	37·00

Table VI. *Expectation of life limited to* 60 *days.*

Cage age in days	N	O	P	Added daily
		Specific deaths.		
0	31·18 ±1·04	35·30 ±1·22	38·19 ±1·17	30·75 ±0·93
10	22·94 ±1·06	28·07 ±1·23	31·22 ±1·18	23·08 ±0·95
20	18·34 ±1·19	25·09 ±1·36	26·67 ±1·25	19·36 ±1·07
30	23·56 ±1·69	29·81 ±1·71	30·27 ±1·51	25·67 ±1·49
40	39·17 ±2·49	44·04 ±2·23	45·38 ±1·97	38·92 ±2·03
50	39·79 ±2·73	45·06 ±2·37	47·97 ±2·15	45·07 ±2·32
60	40·19 ±2·94	49·21 ±2·63	49·69 ±2·38	48·36 ±2·49
70	42·40 ±3·21	44·87 ±2·73	49·52 ±2·49	50·91 ±2·63
80	42·12 ±3·57	44·47 ±2·92	48·90 ±2·68	51·19 ±2·89
90	46·81 ±4·08	45·78 ±3·26	47·38 ±2·83	50·59 ±3·11
		Total deaths.		
0	30·16 ±1·02	34·45 ±1·20	37·32 ±1·15	30·23 ±0·92
10	22·03 ±1·04	27·28 ±1·21	30·66 ±1·17	22·85 ±0·94
20	17·70 ±1·17	24·70 ±1·33	26·21 ±1·23	19·00 ±1·06
30	22·35 ±1·66	29·58 ±1·68	29·40 ±1·49	25·09 ±1·48
40	38·02 ±2·44	43·64 ±2·19	43·58 ±1·95	38·26 ±2·01
50	39·79 ±2·68	44·23 ±2·33	47·16 ±2·12	45·07 ±2·30
60	40·19 ±2·89	47·83 ±2·58	49·69 ±2·37	48·36 ±2·47
70	42·40 ±3·15	43·13 ±2·68	49·52 ±2·46	50·91 ±2·60
80	42·12 ±3·50	43·52 ±2·86	48·72 ±2·64	51·19 ±2·86
90	46·81 ±4·00	44·47 ±3·20	46·62 ±2·79	50·59 ±3·08

It will be seen, therefore, that these two experiments are fully accordant with those described in our last memoir and the conclusions therein drawn need no modification.

With regard to the other point at issue—the relative effectiveness of vaccination by the alimentary tract as compared with direct inoculation into

the tissues—the results are quite clear cut, and are in accord with those of many other observers. It is obviously possible to produce a significant degree of active immunisation by administering large doses of *Bact. aertrycke* vaccine by the mouth, although this route would appear to be slightly less effective than the intraperitoneal. There is no evidence that the immunity induced by oral administration differs in kind from that induced by inoculation into the tissues; had the former method of administration been followed by any local immunisation of the intestinal mucosa it would have been reasonable to expect the immunity produced to be appreciably greater than that following intraperitoneal administration.

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