APPENDIX

TO THE PAPER BY M. GREENWOOD, E. M. NEWBOLD, W. W C. TOPLEY AND J. WILSON "ON THE MECHANISMS BY WHICH PROTECTION AGAINST INFECTIOUS DISEASE IS ACQUIRED IN 'NATURAL' EPIDEMICS." (*Journ. of Hygiene*, XXV. no. 3, Aug. 1926, pp. 336–353.)

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(With Graphs I–IV.)

The joint paper to which this note is an appendix dealt with the question of acquired immunity in the light of experimental results. In particular an experiment was described in which mice were exposed for varying lengths of time in a cage (called there cage *A*) in which a pasteurellosis epidemic was running, and then transferred to a second cage (called cage *B*) in which a similar epidemic was kept up. The after-history of these mice was compared with that of equal numbers of normal mice who were put into cage *B* at the same time with no previous exposure.

The analysis of the results of cages *A* and *B* by partial correlation showed that length of previous exposure to infection in cage *A* was on the whole positively associated with length of after-life in cage *B*, and that this association remained significant when the "specific" death-rate during the exposure both before and after transfer was made constant. Severity of the death-rate during previous exposure in *A* showed a smaller gross association with length of after-life, and this became negligible when allowance was made for length of previous exposure and for varying death-rate during after-life in *B*.

A check on these results may be made by a re-analysis of the data given by one of a series of earlier experiments, in which a similar epidemic was kept up by the regular introduction of normal mice to an infected cage. The longest of these experiments (described as Experiment 2 *a*) was chosen for this purpose. This experiment began on 6. iii. 21 and from that date till 30. iv. 23, three mice were added each day, the rate of addition was then decreased to one a day. For the present purpose only those mice were used who entered the cage during the period when three mice were added each day.

The life-table analysis of this experiment already published (Greenwood and Topley, 1925) shows that, broadly speaking, length of previous exposure was here also associated with length of after-life—since (up to a certain point) the expectation of life increased with cage-age. This method, however, makes no allowance for varying death-rate, and gives no information about the relative value of length and severity of previous exposure, and is therefore less able to throw any light on the difficult question of relative effect of selection and acquired immunity. The method of partial correlation—in spite

of its obvious limitations—does go a little further in this direction. The data of Experiment 2 *a* have therefore now been analysed in exactly the same way as those of cages *A* and *B*, but with the necessary difference explained below.

In the *A* and *B* experiment previous exposure to infection and the after-life studied took place in different cages in each of which an infection was running. Mice previously exposed in cage *A* for differing periods were then transferred to cage *B* and their after-life in that cage noted. In Experiment 2 *a* the whole experience took place in a single cage. Although there was thus no real break in a mouse's life, artificial breaks were made at arbitrary periods to correspond with the transfer from *A* to *B*, and the conditions before and after this point of imaginary division studied separately—the time before corresponding to life in *A* and the time after to life in *B*. As the breaks were only imaginary, they could be repeated during the mouse's life, so that any one mouse furnished several observations. The breaks were made at intervals of ten days' exposure; thus a mouse who lived 45 days in cage 2 *a* give five observations, for which the lengths of previous exposure were 0, 10, 20, 30 and 40 days respectively, and the corresponding lengths of after-life were 45, 35, 25, 15 and 5 days. The previous exposure was always in the period when three mice were added each day, and in the majority of cases so also was the after-life. In the case of some forty odd survivors from the first period, the after-life extended partly into the period of only one daily addition.

The average cage death-rates during previous exposure and after-life had of course to be calculated separately for each of these five observations. The zero exposures corresponded to the *C* mice in the *A* and *B* experiment, *i.e.* the normal mice put into *B* with no previous exposure in *A*. For these mice, the death-rate during previous exposure was taken as zero. As in the *A* and *B* experiment "specific deaths"[1] only were used and the after-life limited to 60 days, this procedure being, perhaps, open to less objection than the alternatives. Possible fallacies arising from these limitations have been considered, but do not appear to be of any practical importance. In cage 2 *a* only those

Table I.

Length of previous exposure	No. of mice surviving this length of exposure	No. of mice surviving this but not the next length of exposure	No. of observations contributed by each mouse	Total no. of observations contributed
0	1867	768	1	768
10	1099	545	2	1090
20	554	211	3	633
30	343	76	4	304
40	267	53	5	265
50	214	49	6	294
60	165	43	7	301
70	122	122	8	976
Totals	4631	1867		4631

[1] "Specific" deaths means deaths caused either by a *Pasteurella* infection, or by a mixed infection of which *Pasteurella* was part (in both cases verified by a post-morten examination), or deaths not verified owing to destruction of the corpse by cannibals.

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Table II. *Correlation Coefficients in Cages A and B and Cage 2 a.*

Total correlations. (Specific deaths only.)

	Cages *A* and *B*			Cage 2 *a*		
	r	η	$\eta^2 - r^2$	r	η	$\eta^2 - r^2$
Length of after-life and length of previous exposure	·214±·016	·244±·016	·014±·004	*·015* ·227±·009	*·015* ·252±·009	*·003* ·012±·002
Length of after-life and average death-rate during previous exposure	·119±·017	·254±·016	·052±·008	*·016* −·054±·010	*·014* ·328±·009	*·010* ·105±·006
Length of after-life and average death-rate during after-life ...	−·152±·017	·237±·016	·033±·006	*·012* −·469±·008	*·012* ·508±·007	*·006* ·039±·004
Length of after-life and "total" previous exposure	·237±·016	·241±·016	·002±·001	*·016* ·083±·010	*·015* ·259±·009	*·008* ·060±·005
Length of after-life and average death-rate during previous exposure for the last 10 days before "transfer"	·110±·017	·186±·016	·022±·005	*·016* −·078±·010	*·013* ·383±·009	*·012* ·141±·007
Length of previous exposure and average death-rate during previous exposure	·410±·014	·802±·006	·475±·024	*·014* ·326±·009	*·007* ·721±·005	*·020* ·414±·013
Length of previous exposure and average death rate during after-life	·047±·017	·158±·017	·023±·005	*·015* −·198±·010	*·015* ·256±·009	*·005* ·026±·003
Length of previous exposure and average death-rate during previous exposure for the last 10 days before "transfer"	·453±·014	·758±·007	·370±·021	*·014* ·343±·009	*·008* ·701±·005	*·019* ·373±·012
Average death-rate during after-life and average death-rate during previous exposure for the period of exposure	·026±·017	·422±·014	·178±·014	*·015* ·193±·010	*·012* ·468±·008	*·013* ·182±·009
Average death-rate during after-life and "total" previous exposure	−·083±·017	·285±·016	·075±·009	*·016* ·075±·010	*·014* ·336±·009	*·010* ·107±·007
Average death-rate during after-life and average death-rate during previous exposure for the last 10 days before "transfer" ...	−·051±·017	·339±·015	·113±·015	*·015* ·193±·010	*·012* ·483±·008	*·014* ·196±·009

The variable that was found in terms of the other is given in italics.

η's were corrected by the formula $\eta^2 = \dfrac{(\text{crude } \eta^2) - \frac{K-3}{N}}{1 - \frac{K-3}{N}}$, where K = no. of groups.

mice were used whose previous exposure took place in the three-mouse period; this gave 1867 individual mice, who contributed altogether 4631 observations, as shown in Table I.

The correlation results are shown in Tables II and III side by side with those of cages *A* and *B*[1].

Table III. *Partial Correlations for Cages A and B and Cage 2 a.*

	Cages *A* and *B* *r*	Cage 2 *a* *r*
1st order.		
Length of after-life and length of previous exposure (keeping constant death-rate during previous exposure)	·185±·016	·259±·009 *·015*
Length of after-life and death-rate during previous exposure (keeping constant length of previous exposure)	·027±·017	−·139±·010 *·015*
Length of after-life and length of previous exposure (keeping constant death-rate during after-life)	·224±·016	·155±·010 *·015*
Length of after-life and death-rate during previous exposure (keeping constant death-rate during after-life)	·117±·017	·042±·010 *·016*
Length of after-life and "total" previous exposure (keeping constant death-rate during after-life)	·228±·016	·134±·010 *·015*
Length of after-life and death-rate during previous exposure for 10 days before "transfer" (keeping constant death-rate during after-life)	·119±·017	·014±·010 *·016*
Length of after-life and death-rate during previous exposure for 10 days before "transfer" (keeping constant length of previous exposure)	·015±·017	−·171±·010 *·015*
Length of previous exposure and death-rate during previous exposure for 10 days before "transfer" (keeping constant death-rate during after-life)	·452±·014	·396±·008 *·013*
Length of previous exposure and average death-rate for previous exposure (keeping constant the death-rate during after-life) ...	·410±·014	·379±·009 ·013
2nd order.		
Length of after-life and length of previous exposure (keeping constant death-rate during previous exposure and death-rate during after-life)	·194±·016	·150±·010 *·015*
Length of after-life and death-rate during previous exposure for the last 10 days before "transfer" (keeping constant length of previous exposure and death-rate during after-life)	·021±·017	−·052±·010 *·016*
Length of after-life and death-rate during previous exposure (keeping constant length of previous exposure and death-rate during after-life)	·029±·017	−·018±·010 *·016*
Multiple correlations.		
Length of after-life with length of previous exposure and average death-rate during previous exposure	·215*	·228†
Multiple partial correlation‡.		
Length of after-life with length of previous exposure and average death-rate during previous exposure (keeping constant average death-rate during after-life)	·225	·396

* Root mean square value ρ_0 for independence by Yule's formula $\sqrt{\frac{n-1}{N}}$, where n = no. of variables and N = no. of observations is ·036.

† Root mean square value ρ_0 for independence by Yule's formula $\sqrt{\frac{n-1}{N}}$, where n = no. of variables and N = no. of observations is ·021.

‡ Found from the formula $r_{2(13).4} = \frac{r_{2(13)} - r_{42} r_{4(13)}}{\sqrt{(1-r^2_{24})(1-r^2_{4(13)})}}$.

[1] In the tables two probable errors are given for each coefficient for cage 2 *a* as upper and lower limits between which the true probable error probably lies. The usual formula for the P.E. of r assumes that all the observations are independent. This is clearly not the case here, hence

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Taken on the whole the results for cage 2 *a* agree with those found for cages *A* and *B* as regards the correlations that are of any practical interest.

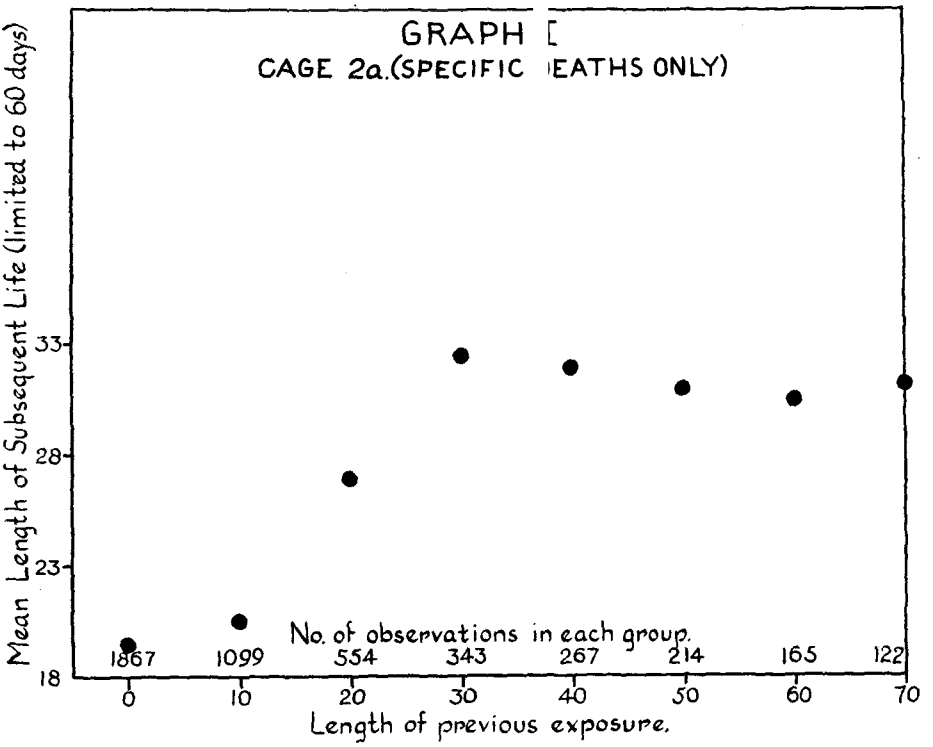
Two minor points relating to the subsidiary correlations may be noted first. The large positive correlation appearing in both cases between length of previous exposure and the average cage death-rate during this exposure is at first sight surprising but it has no real significance as it is chiefly due to the normal *C* mice, or the corresponding 0, 0 mice, *i.e.* those who had no previous exposure and hence were subject to no previous death-rate. The same remark of course applies when the death-rate is only taken over the last ten days before "transfer."

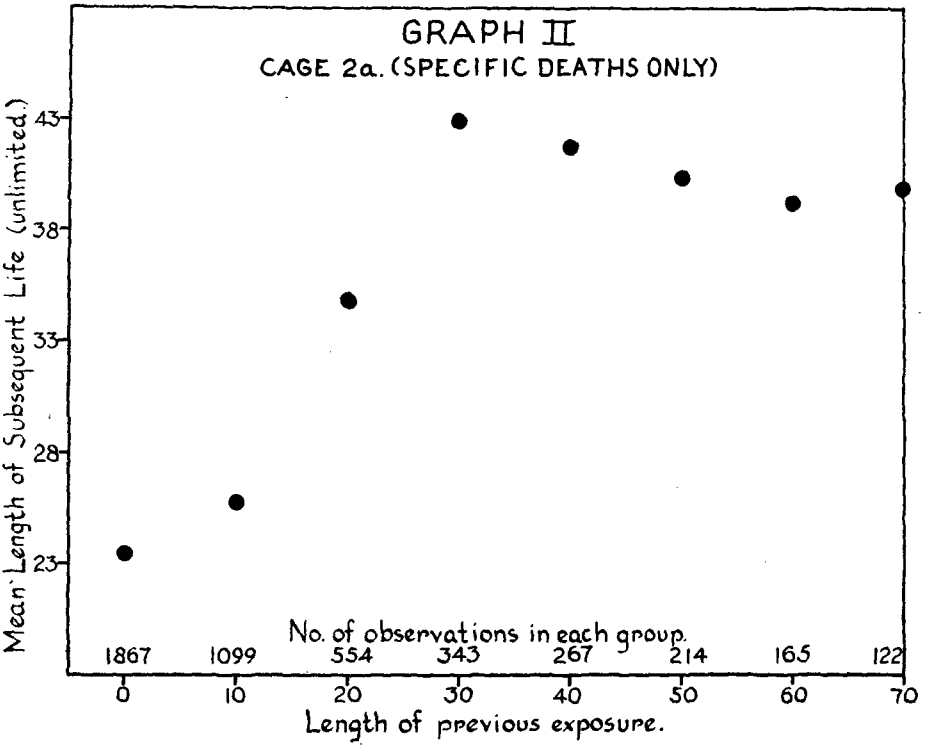
The corresponding correlations between length of after-life and average death-rate during the after-life are—as would be expected—negative. The fact that the correlation between the average cage death-rates before and after "transfer" is significantly positive in Experiment 2 *a*, but negligible in the *A* and *B* experiment, is probably due to the two exposures being in the same cage in one case and in different cages in the other—the death rate in any one cage being roughly continuous. This again applies when the previous death-rate is only taken over the last ten days.

The main result of any interest is that the subsequent length of life has in Experiment 2 *a* just as in *A* and *B*, a closer connection with the *length* of the previous exposure than with severity of the specific death-rate during this exposure. The correlation with the latter variate is indeed negative in Experiment 2 *a* both in gross value and when length of exposure is kept constant, if we keep constant the death-rate during after-life it becomes positive, but is very small, ·042; if we keep both these constant, it is again negative but remains small. The multiple correlation coefficient is slightly higher in cage 2 *a* than in cages *A* and *B*, whether the after-life death-rate is kept constant or not. It measures the correlation between the length of after-life and the "best" linear combination of the two variables—length and severity of previous exposure; the word "best" denotes that these two variables are given the relative weights which make this correlation a maximum. Owing to the negative relation just mentioned between length of after-life and the average death-rate during previous exposure in cage 2 *a*, the latter variable has a negative weight in the "best" linear combination, while in the *A* and *B* experiment it had a positive weight. In each case its weight is relatively small.

Hence still more than for cages *A* and *B* the most probable interpretation seems to be that exposure to a severe death-rate tends to neutralise whatever immunising effect it may have by causing mice to enter the subsequent

the lower limit given—the usual value taking $N = 4631$ (the total number of observations)—is probably an under-estimate of the error, but on the other hand to put $N = 1867$ (the actual number of different mice) which was done to get the higher limit, is probably to over-estimate the error. The two limits are near enough for their range of uncertainty to be immaterial for the present purposes.





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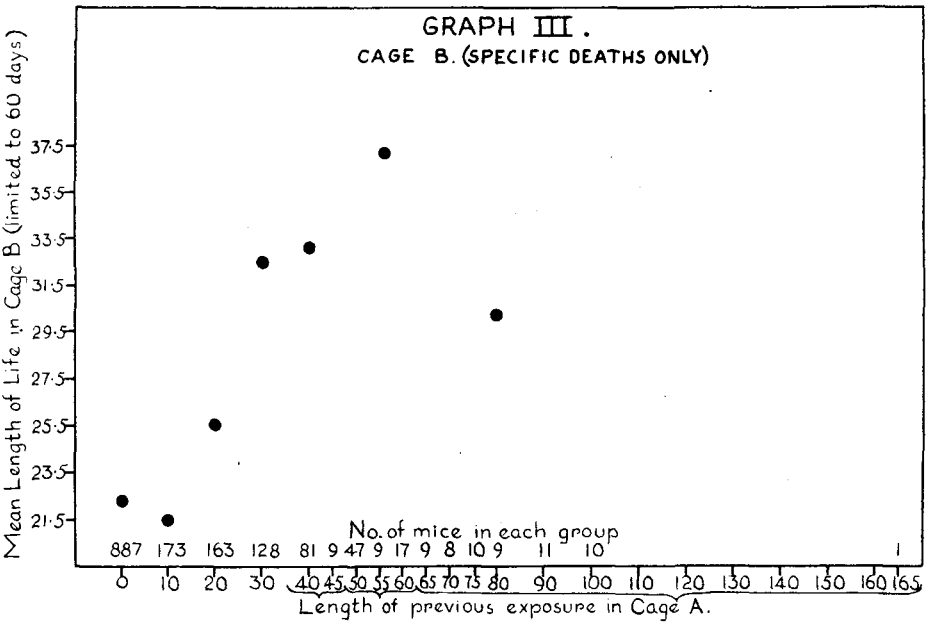

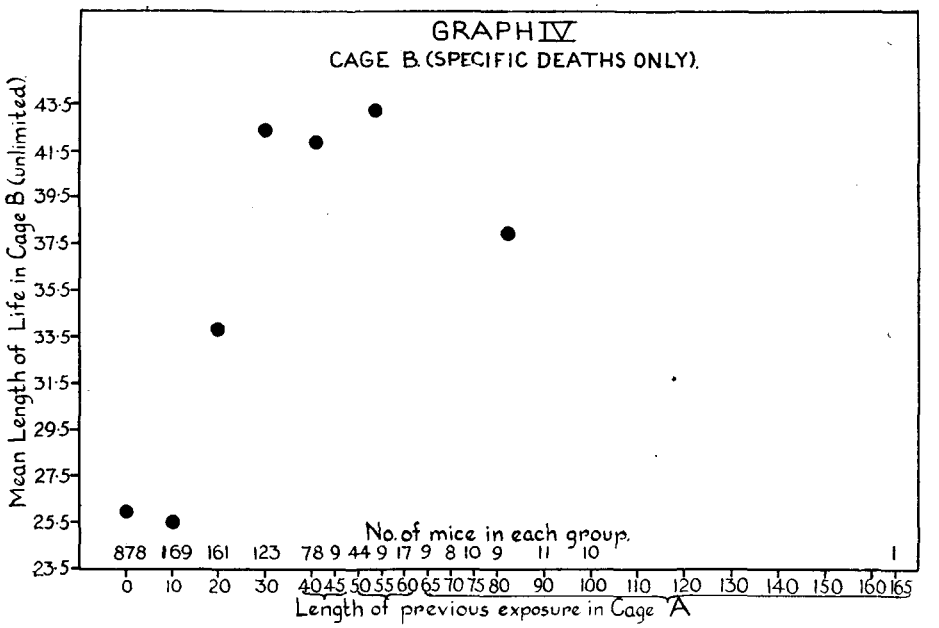

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period of exposure already mortally infected, while the long exposure to a less severe infection is connected with a favourable expectation of life.

It has been noted in previous work that it is only up to a point that increased length of exposure is associated with increased length of after-life. This point seems to come earlier in Experiment 2 *a* than in the *A* and *B* experiment (see Graphs I and III); in 2 *a* 30 days' exposure gives the maximum advantage, and in cages *A* and *B* the advantage increases till about 60 days' exposure and then seems to decline. Graphs II and IV repeat the results shown in Graphs I and III for unlimited after-life instead of for the after-life limited to 60 days. No essential difference appears. Apart from the fact of the previous and subsequent exposure being in different cages in the *A* and *B* experiment, and in the same cage in Experiment 2 *a*, the chief measurable difference in the conditions of the two experiments is that in cage 2 *a* the average death-rate (whether taken as a whole, or previous and subsequent exposure compared separately) was rather higher than in cages *A* and *B* (see Table IV).

Table IV. *Average Specific Death-rate per Mouse per Day.*

	Whole period	Average of all periods of previous exposure	Average of all periods of subsequent exposure
Cage 2 *a*	·0366	·019*	·042
Cage *A*	·0212	·009*	—
Cage *B*	·0297	—	·031

* The smallness of these values is due to the large groups with no previous exposure and therefore zero death-rate. These do not affect the rates in the first and third columns.

Incidentally it is of interest to note that this lower specific death-rate in cage *A* agrees with the tendency previously noted in other experiments of this series, viz. that a higher death-rate seems to be associated with a constant high circulation rate of non-immunes rather than with discontinuous introduction of fresh immigrants at longer intervals even though in large batches. In cage *A* the entries were at the rate of 50 mice per 10 days for the first 2 months, then for a little over 2 months at 100 per 10 days, and after that 4 batches of 200 and 2 of 100 were put in at irregular intervals of 1 to 2 months. Besides these batches a regular three a day entry was made for 2 months only in the earlier part of the experiment—as opposed to a continuous entry of three each day throughout the experiment 2 *a*.

Thanks are again due to Mr J. W. Martin and Miss C. Thomas for the greater part of the statistical computation involved in this analysis.

Summary.

An experiment recently described in this *Journal* (Greenwood, Newbold, Topley and Wilson, 1926) dealt with the after-life of mice that had been previously exposed to epidemic pasteurellosis, and led to the following deductions:

"We think we have proved—

(1) That the survivors of herd-exposure to epidemic pasteurellosis are more resistant to subsequent exposure than healthy animals not previously exposed to risk.

(2) That this superiority is significantly correlated both with length of previous exposure independently of its severity, and with severity of previous exposure apart from its duration.

We think it is probable that—

(3) The severity of the prior exposure as measured by the average death-rate during the period of exposure, is less important than the length of exposure.

(4) The advantage of exposure at first increases with its duration and then decreases, so that mice who have been exposed for a moderate time are more resistant to subsequent exposure than mice who have been exposed for a very short or a very long time."

The results of an independent experiment, similar in kind, with the difference that the after-life and previous exposure took place in a single cage, and covering 1867 mice, support (1), (3) and (4) of the above deductions, also (2) so far as it relates to length of previous exposure, but the severity of previous exposure apart from its duration shows a negative but insignificant association with improved expectation of after-life. These results are in agreement with the suggestion inspired by the experiment previously described. "That these facts are difficult to interpret in terms of pure selection and more easily reconciled with a process of active immunisation during the primary exposure, but that the nature of this process will remain obscure until we have more experimental data at our disposal."

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(*MS. received for publication* 5. x. 1926.—Ed.)