ON THE MECHANISMS BY WHICH PROTECTION AGAINST INFECTIOUS DISEASE IS ACQUIRED IN "NATURAL" EPIDEMICS.

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(With 3 Graphs.)

Introduction.

The problem which we shall discuss in this communication is of great importance, is perhaps the most important of all epidemiological problems, and we are far indeed from supposing that we have solved it. We think, however, that the work we have done is certainly useful in enabling us, and others, to state the problem correctly, and possibly useful in suggesting a first approximation to its solution.

With very rare exceptions, naturally occurring epidemics among men or other animals pass leaving a sensible proportion of seemingly unaffected survivors. Even in non-fatal, highly contagious diseases, such as measles, which only an inconsiderable proportion of survivors to adult age finally escape, the attack rate upon exposed susceptibles in particular epidemics is usually well below 100 per cent. Thus Butler in his paper on Measles reports on 3059 house contacts (the contacts being persons stated not to have been previously attacked) of measles. The total attack rate was 66·1 per cent., the maximum value was 90·1 per cent. for the age group of 3–4 years (323 observations) and the rate for the whole age group 0–4 (1792 observations) was 77·6 per cent. Butler cited the experience of Marlborough College in an epidemic occurring in 1912. Of the total number of 632 boarders, 190, or 30·0 per cent., were attacked. Of 217 said not to have been attacked previously 83·4 per cent. were attacked and of 415 previously attacked 2·16 per cent. The problem proposed is, to what special qualities or particular circumstances do those who pass successfully through an epidemic owe their success? The simplest answer is, of course, that they escape by "chance." This is, *verbally*, the simplest answer, but actually it assumes a quite complex hypothesis. It assumes that a herd subjected to an infectious disease may be likened to a host within which a number of cannon are discharging "shots" at random, those who are hit by the shots enter the class of infected and may themselves be transmuted into biological cannon discharging "shots." An individual escapes infection because a shot has not happened to come his way. As an allegorical description of the machinery, this simile might well be criticised, but we need do no more than point out one absolutely fatal objection to it, *so far as our own experimental work is concerned.* We italicise the proviso

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because we are not acquainted with any sufficient evidence that the attack rate upon, say, survivors of one epidemic of measles again exposed to infection is different from that upon persons of the same age not previously exposed; but our experience—as will shortly appear—is that those who survive one epidemic are on the average and for a certain time, definitely more resistant than those not before at risk. In other words the survivors differ, on the average, from the rest of the herd by something more than the fact that they *have* survived; so we dismiss the explanation of "luck."

We now have to consider whether the peculiarity of the average survivor is antecedent or consequent to his exposure. For the moment, but only for the moment, we shall regard these as true alternatives because to do so simplifies exposition. The former alternative is based upon the extension to this branch of biology of the principle of innate variability. We may surmise that, just as in any herd of animals we shall find differences of measurable external characters, weight, length, etc., we shall have differences of physiological make-up, so that when submitted to a common peril by infection some will be destroyed, others gravely injured, others slightly injured, others not scathed. In the process, of course, there will not be a one-to-one correspondence between natural resistance and the event—the "luck" of our previous case will not be excluded—some very resistant animals will be overwhelmed by heavy fire, some feeble animals will survive—but, on the average, the exposure will destroy a larger proportion of the naturally weak and spare a larger proportion of the naturally strong; the mortality will, in the familiar phrase, be largely *selective*. This is, of course, the application to epidemiology of the classical formula of Darwinism, but whether the variations of physiological make-up are continuous or discontinuous, *i.e.* whether or not we are Mendelians, is impertinent to our enquiry at this stage. We merely note that notorious variations of susceptibility to particular infections, separating different nearly allied races or even varieties of animals, can most easily be explained on the hypothesis of innate variability; that in fact there is abundant reason for entertaining this hypothesis seriously. The reason that it has, on the whole, tended to lose credit in practical medicine is really psychological, due partly, perhaps, to the exaggerations of its more extreme advocates but, still more, to the difficulty of making a crucial test and the apparent hopelessness of devising any practicable measures of epidemiological prophylaxis based upon it. The latter objection is probably, consciously or unconsciously, the strongest argument against "Eugenics" as a practical gospel. It is not that rational persons disbelieve the doctrine that like begets like, but that they do not see how in such a world as ours the principle can be applied to the eradication of disease.

The other alternative is that animals exposed to an infection, even if their innate powers are all precisely equal, will, by "luck," receive unequal doses, doses unequal in amount, in quality and in frequency of repetition. A large dose may kill at once, or a large dose followed by a small dose may be destruc-

tive. But a small dose received first may protect against a subsequent large dose; two small doses within a certain interval may be mortal, spaced out they may be protective; and so on, through all the permutations and combinations afforded by variation of dosage, quality, quantity and frequency. At the end we shall reach a surviving population, on the average, more resistant than an unexposed population, just as a selected population would be more resistant than an unexposed population, not by virtue of the preservation of favourable variations but by virtue of successful education. To point the antithesis, we might take this exaggeration. If the permanent civil service of a state were recruited in three different ways, (*a*) by appointing the first 100 men aged 25 years who sent in applications, (*b*) by appointing the first 100 men who produced proof of having attended an honours university course (without requiring proof that they had passed any examination), (*c*) by appointing the first 100 in order of marks in a common examination, *on the average,* we should expect to find the intellectual level of the (*b*'s) and (*c*'s) above that of the (*a*'s), but there might be some doubt as to whether the (*c*'s) would be better than the (*b*'s) and still greater doubt if the (*c*) method were, not to take the *best* 100 but to take a *random* 100 out of those who had obtained at least 60 per cent. of the marks in the examination. Under method (*b*) we ensure a certain educational exposure, under (*c*) apply a certain educational selection. What we wish to know is whether the principle of (*b*) or of (*c*) is the more important in the problem of survivorship.

We have just used the words "more important" and they bring us back to the preliminary caution, viz. that, in reality, there cannot be a dichotomy of selection and education—or, in our particular case, acquired immunity. It is arbitrary, and indeed contradicted by experience, to assume that the success of an immunising process is wholly a function of the dosage and quality of the inoculum; the amenability of the tissues of the receiving animal to the process of education is a factor too and perhaps an innately variable factor. Selection may be working here, we might almost say, *must* be working.

All we can expect a perfectly clean experiment to tell us is whether the observed facts are completely explained by the selection hypothesis and if not so, whether the part played by the educational process is an important one. If we can go so far as this, however, we shall have advanced a step and it is to a study of the possibilities of such an advance that we have directed our attention. As we proceed the difficulties encountered will be explained.

Of recent work bearing upon the mechanism of natural immunity that of Hirszfeld is noteworthy.

It has long been known that the property of isoagglutination or isohaemolysis is not uniformly distributed. Thus, to take a usual schema, in a sample of men we shall find (1) a subgroup whose corpuscles are not affected by immune sera *a* and *b*, we may call these the *o* class; (2) a subgroup responding to *a*, the *A* class; (3) a subgroup responding to *b*, the *B* class; (4) a subgroup responding to both *a* and *b*, the *AB* class.

Fairly extensive sampling has shown that the repartition of these classes in different nations is characteristically different. There is evidence (v. Dungern and Hirszfeld, Landsteiner and van Scheer, Bernstein and others) that these characters are heritable and transmitted in a manner suggesting that the Mendelian principles are directly applicable. Hirszfeld and his collaborators have made the interesting observation that if both Schick reactions and blood tests are employed, the children of a positive reactor whose blood grouping is the same as that of the positively reacting parent are positive reactors too, but that the same correlation is not found when the parent is a negative reactor, *i.e.* that although a majority of the children of the same haemological type react negatively they do not all do so. Hirszfeld has accordingly made the suggestion that the potentiality of becoming effectively immunised under suitable environmental conditions is a character linked in inheritance with that upon which the blood grouping depends.

At present the evidence is not sufficient to allow one to discuss the subject in detail, but it is important as affording a clue to the mechanism of natural immunity.

Direct evidence that the survivors of an infection are relatively immune to subsequent doses of infective material has often been furnished, *e.g.* by Webster for mouse-typhoid.

Webster has also brought forward evidence that the property is to some extent non-specific, *e.g.* he found that the survivors of mice exposed to enteric infection were relatively immune to toxic doses of mercuric chloride. The data upon which this conclusion was based were somewhat scanty and Topley, Wilson and Lewis found that survivors of exposure to *Aertrycke* (mutton) infection were not more resistant than controls to *Pasteurella*. We should not, however, attach any great significance to the latter observation, as indicating the specificity of the resistance exhibited by the surviving mice, since the results obtained in many other experiments have convinced us of the unwisdom of accepting the response to artificial infection as a criterion of the presence or absence of resistance, of a type and degree which may be highly effective under natural conditions. Certain observations which have been recorded in a previous report (Greenwood and Topley, 1925) do however suggest, though they by no means prove, that the increased resistance of mice to pasteurellosis, on the one hand, and to *B. aertrycke* infection, on the other, which results from exposure to the risk of infection, may develop independently one of another.

In our opinion, having regard to the variability of rates of mortality in control series, the experimental evidence of non-specific immunity in mice is not wholly convincing. We also doubt whether, in any of the experimental series with which we are acquainted, the possibility that the success of survivors re-exposed to infection was a consequence not of selection by death but of active immunisation was or could be excluded.

Nature of the experiment.

For the purpose of this experiment, we have had under observation two herds which we will call *A* and *B*. The nucleus of each was a group of infected animals and the populations were maintained by the introduction of immigrants, the difference between the two being that while *A* was only recruited from healthy immigrants, not previously exposed to infection, *B* received healthy unexposed immigrants (whom we term *C*'s) and also mice which had survived a certain time in colony *A*; these immigrants to *B* we term *A*'s. The details of the method of immigration (partly continuous, partly the introduction of large batches) and the movements of the death-rates associated with immigrations, although of importance to our general programme of work, will not be discussed in this paper, which will deal exclusively with the problem above proposed. It is, however, material to remark that in this experiment no difficulty has arisen through the introduction of *enteric* infection, so that from the epidemiological point of view the case is of a pure *Pasteurella* epidemic. Graphs I and II, recording the smoothed specific and total rates of mortality, indicate that in both colony *A* and colony *B* epidemic conditions of the type common to all our experiments prevailed. Tables I A and I B record the general facts and show the scale of the observations.

Table I A. (*Cage A.*)

Period 19. xi. 24 to 30. xii. 25

	No. of mouse-days exposed to risk	Deaths		Death-rates (per mouse per day)	
		Specific*	Total	Specific	Total
All mice in cage *A*	62,731	1333	1484	·0212	·0237

* Specific deaths include deaths of mice which could not be examined *post mortem* as well as those in which pasteurellosis was definitely found.

Table I B. (*Cage B.*)

Period 19. xi. 24 to 27. xii. 25

	No. of mouse-days exposed to risk	Deaths		Death-rates (per mouse per day)	
		Specific	Total	Specific	Total
A mice	26,909	676	728	·0251	·0271
C mice	28,804	1096	1152	·0381	·0400
Total (including other entries)	86,281	2566	2734	·0297	·0317

The comparison of *A*'s and *C*'s.

We must first show that, whatever the criterion, *A*'s exposed in *B* had a superiority over *C*'s exposed in *B*.

This is established by the following comparisons:—

(*a*) The general and specific death-rates of *A*'s compared with *C*'s (Table I B).

(*b*) The proportion of animals which survived at least 28 days in *B* (Table II).

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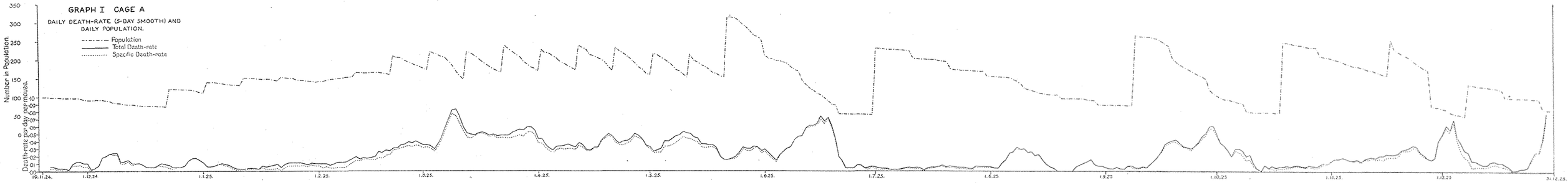

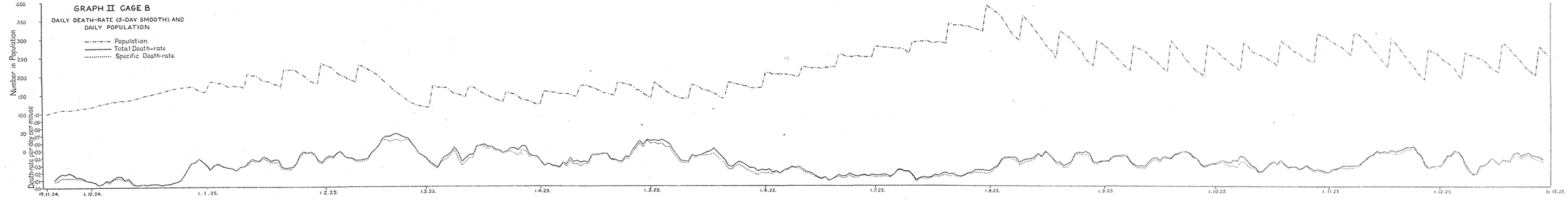

Graphs I and II.

The smoothed daily death-rate for day x is taken as

$$\frac{\text{sum of the deaths in the 5 days of which day } x \text{ is the middle one}}{\text{sum of the populations in the same 5 days}}.$$

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(*c*) A comparison of the average lengths of life limited to 60 days (*i.e.* the partial expectation of life for the first 60 days in *B*, which amounts to ignoring the variations of length of life beyond 60 days). (Table III.)

Table II.

	Length of exposure in *A* (days)	Number in group	Percentage surviving at least 28 days in *B*
C mice	0	887	23·3 ± ·96
A mice	10	173	26·7 ± 2·27
	20	163	36·0 ± 2·54
	30	128	46·8 ± 2·98
	40–45	90	49·7 ± 3·55
	45–	131	55·6 ± 2·93

Table III.

Cage B. Specific deaths only (and survivors at 60 days up to end of Dec. 1925).

Standard deviation of length of life in cage *B* limited to 60 days

(i)	All *C* mice	15·99 days
(ii)	All *A* mice	21·44 „
(iii)	*A* mice who have had a time of low exposure in *A*	18·22 „

All A mice and all C mice. (Specific deaths only.)

	Length of exposure in cage *A* (days)	Number in group	Length of life in cage *B* limited to 60 days	
C mice	0	887	22·37 ± ·36	
A mice	10	173	21·34 ± 1·10	All *A*'s 28·43 ± ·55
	20	163	25·50 ± 1·13	
	30	128	32·55 ± 1·28	
	40 & 45	90	33·08 ± 1·52	
	50, 55 & 60	73	37·39 ± 1·69	
	65, 70, 75, 80, 90 & 165	58	30·29 ± 1·90	

∴ Difference between *A*'s and *C*'s = 6·07 ± ·66.

The general effect of these comparisons is, we submit, quite decisive, viz. that on the average the *A* mice enjoyed a considerable advantage.

The above tables provided mass comparisons of all *A* mice with all *C* mice. But, as will be seen in Graph II, the conditions in *B* were varying and it might be that the advantage of the *A*'s was due to a greater proportion of them having had the luck to pass their lives in *B* under more favourable conditions than the average of the *C*'s. This is not, *a priori*, likely, but we can test the point by comparing the simultaneous death-rates and by a confrontation of the mice of different categories entering *B* on the same day. Tables IV and V provide the necessary information. It was to be expected, and has happened, that when only small absolute numbers are available there should be irregularities, but it will be seen that the instances when the after-history of an individual batch of *C*'s was more favourable than that of any batch of associated *A*'s were few. Of the 19 sets in Table V carrying us down to the period when mice still survived at the time of writing up the record, *i.e.* to a period when the statement ceases to be complete, there are only two cases where the average length of life of the *C*'s was greater than that of any associated batch of *A*'s. We note too—its importance will be pointed out

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Table IV. (*Cage B.*)

Period of exposure in cage *B*	Group	Mouse-days exposed to risk	Deaths		Average daily death-rates per mouse	
			Specific*	Total	Specific	Total
19. xi. 24 to 31. xii. 24	*A*	—	—	—	—	—
	C	—	—	—	—	—
	Total	5867	65	71	·0111	·0121
1. i. 25 to 31. i. 25	*A*	1192	35	37	·0294	·0310
	C	366	20	20	·0546	·0546
	Total	5839	188	197	·0322	·0337
1. ii. 25 to 28. ii. 25	*A*	2201	103	106	·0468	·0482
	C	358	24	24	·0670	·0670
	Total	5160	242	253	·0469	·0490
1. iii. 25 to 31. iii. 25	*A*	1943	80	83	·0412	·0427
	C	393	21	24	·0534	·0611
	Total	4559	191	205	·0419	·0450
1. iv. 25 to 29. iv. 25	*A*	1711	70	73	·0409	·0427
	C	626	27	27	·0431	·0431
	Total	4589	165	174	·0360	·0379
30. iv. 25 to 30. v. 25	*A*	2260	69	75	·0305	·0332
	C	521	24	29	·0461	·0557
	Total	4905	183	202	·0373	·0412
31. v. 25 to 29. vi. 25	*A*	2987	36	41	·0121	·0137
	C	727	18	20	·0248	·0275
	Total	6610	100	111	·0151	·0168
30. vi. 25 to 30. vii. 25	*A*	3867	38	46	·0098	·0119
	C	1328	28	28	·0211	·0211
	Total	9153	130	147	·0142	·0161
31. vii. 25 to 30. viii. 25	*A*	3083	68	79	·0221	·0256
	C	3968	165	173	·0416	·0436
	Total	9552	319	341	·0334	·0357
31. viii. 25 to 29. ix. 25	*A*	2152	52	57	·0242	·0265
	C	4310	187	191	·0434	·0443
	Total	7439	263	272	·0354	·0366
30. ix. 25 to 28. x. 25	*A*	1838	45	46	·0245	·0250
	C	5096	151	166	·0296	·0326
	Total	7360	205	221	·0279	·0300
29. x. 25 to 27. xi. 25	*A*	1872	44	47	·0235	·0251
	C	5885	227	238	·0386	·0404
	Total	8034	274	288	·0341	·0358
28. xi. 25 to 27. xii. 25	*A*	1803	36	38	·0200	·0211
	C	5226	204	212	·0390	·0406
	Total	7214	241	252	·0334	·0349

* Specific = *Pasteurella* and not examined.

later—that in 10 of the 19 instances the best of the *A*'s were not those who had had the longest exposure in *A* and that in only two instances were the *A*'s with least exposure in *A* the longest lived.

The detailed comparison, therefore, confirms the general comparison; we hold it to be proven that exposure in *A* led to the survivors passing into *B* having a definite superiority over the previously unexposed *C*'s.

These, then, are the facts, the explanation of which we must seek. Can we explain them wholly on the principle of selection? We attempted to settle

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Table V. *Cage A mice transferred to cage B and normals (C) put into cage B.*

Batch	No. of days exposed in *A* before transfer to *B*	Date* of entry in *A*	Date of entry in *B*	No. in batch at date of transfer to *B*	Average age at death. All deaths unlimited	Average specific death-rate during exposure in *A* before transfer to *B*
C 1	0	—	45	10	*19·9*	—
A 2	10	35	45	10	23·8	·0103
A 1	45	0	45	10	27·5	·0090
C 2	0	—	55	10	*13·0*	—
A 3	10	45	55	10	19·5	·0052
A 2/A	20	35	55	10	20·1	·0076
A 1 A	55	0	55	10	24·9	·0081
C 3	0	—	65	10	*15·1*	—
A 4	10	55	65	10	22·5	·0027
A 3/A	20	45	65	10	19·1	·0039
A 2/B	30	35	65	10	35·6	·0058
A 1 B	65	0	65	10	22·0	·0069
C 4	0	—	75	10	*17·8*	—
A 5	10	65	75	10	18·8	·0062
A 4/A	20	55	75	10	26·4	·0044
A 3/B	30	45	75	10	36·8	·0047
A 2/C	40	35	75	10	45·1	·0059
A 1/C	75	0	75	10	17·2	·0068
C 5	0	—	85	10	*14·5*	—
A 6	10	75	85	10	8·1	·0054
A 5 A	20	65	85	10	19·0	·0058
A 4 B	30	55	85	10	23·2	·0048
A 3 C	40	45	85	10	16·7	·0049
C 6	0	—	105	10	*13·2*	—
A 8	10	95	105	10	34·3	·0323
A 7	20	85	105	10	27·1	·0250
A 6/A	30	75	105	10	26·5	·0193
A 5/B	40	65	105	10	34·6	·0163
A 4/C	50	55	105	10	41·8	·0138
C 7	0	—	115	10	*8·4*	—
A 9	10	105	115	10	9·5	·0510
A 8/A	20	95	115	9	27·22	·0417
C 8	0	—	125	10	*38·4*	—
A 10	10	115	125	10	26·6	·0473
A 9/A	20	105	125	10	13·5	·0492
C 9	0	—	135	10	*20·0*	—
A 11	10	125	135	10	33·7	·0483
A 10/A	20	115	135	10	22·1	·0478
A 9/B	30	105	135	6	31·0	·0489
C 10	0	—	145	10	*37·4*	—
A 12	10	135	145	10	14·4	·0285
A 11/A	20	125	145	10	35·2	·0385
A 10/B	30	115	145	4	21·75	·0413
C 11	0	—	155	10	*13·5*	—
A 13	10	145	155	10	10·1	·0351
A 12/A	20	135	155	10	35·2	·0319
A 11/B	30	125	155	10	16·4	·0373
C 12	0	—	165	10	*22·4*	—
A 14	10	155	165	10	44·0	·0380
A 13/A	20	145	165	10	46·8	·0365
A 12/B	30	135	165	10	47·1	·0339
A 11/C	40	125	165	4	9·25	·0374
A 6/D	90	75	165	1	39·0	·0347
A 5/C	100	65	165	1	96·0	·0325
A 1/D	165	0	165	1	169	·0255

* The first day of the experiment is counted as day 0, and so on.

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Table V (*contd.*).

Cage A mice transferred to cage B and normals (C) put into cage B.

Batch	No. of days exposed in *A* before transfer to *B*	Date of entry in *A*	Date of entry in *B*	No. in batch at date of transfer to *B*	Average age at death. All deaths unlimited	Average specific death-rate during exposure in *A* before transfer to *B*
C 13	0	—	175	10	*38·5*	—
A 15	10	165	175	10	17·2	·0327
A 14/A	20	155	175	10	43·6	·0354
A 13/B	30	145	175	10	69·5	·0353
C 14	0	—	185	10	*34·1*	—
A 16	10	175	185	10	41·3	·0338
A 15/A	20	165	185	10	53·6	·0332
A 14/B	30	155	185	10	49·6	·0349
A 13/C	40	145	185	3	16·67	·0350
C 15	0	—	195	10	*40·8*	—
A 17	10	185	195	10	32·0	·0232
A 16/A	20	175	195	10	51·9	·0273
A 15/B	30	165	195	4	52·25	·0288
A 14/C	40	155	195	8	44·5	·0309
C 16	0	—	205	10	*44·4*	—
A 17/A	20	185	205	10	52·7	·0236
A 16/B	30	175	205	10	63·7	·0263
C 17	0	—	215	10	*12·1*	—
A 17/B	30	185	215	10	62·0	·0309
A 16/C	40	175	215	10	70·2	·0315
C 18	0	—	225	10	—	—
A 17/C	40	185	225	10	—	·0286
A 16/D	50	175	225	10	73·8	·0298
C 19	0	—	235	10	*22·0*	—
A 18	10	225	235	10	—	·0018
A 17/D	50	185	235	10	—	·0216
C 20	0	—	245	40	*26·725*	—
A 18/A	20	225	245	10	36·2	·0030
A 17/E	60	185	245	9	25·22	·0184
C 21	0	—	255	70	—	—
A 18/B	30	225	255	10	29·6	·0033
C 22	0	—	265	70	*18·97*	—
A 18/C	40	225	265	10	33·4	·0056
C 23	0	—	275	70	—	—
A 18/D	50	225	275	10	34·3	·0070
C 24	0	—	285	70	—	—
A 18/E	60	225	285	10	—	·0069
C 25	0	—	295	70	—	—
A 18/F	70	225	295	10	27·6	·0067
C 26	0	—	305	70	—	—
A 19	10	295	305	10	—	·0152
A 18/G	80	225	305	10	—	·0084
C 27	0	—	315	70	—	—
A 19/A	20	295	315	10	—	·0237
A 18/H	90	225	315	10	42·3	·0117

the matter by a resort to the *ad absurdum* method. A batch of immigrants to colony *A* must, from the nature of the case, be exposed to risk of death and some will perish in *A*. Suppose that of *n* mice *entering A*, 1/*m*th die in *A*, and so the survivors leaving *A* for *B*, *i.e.* the mice we call *A*'s, form only

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$(m-1)/m$th of the company of immigrants to A of which they formed part. Then we might compare their after-fate *in* B not with the average of the C's accompanying them but with the average of the best $(m-1)/m$th part of those C's; *e.g.* if the A's comprised 90 per cent. of their initial group, we might compare them with the average of the 90 per cent. of the associated C's who lived longest in B. This comparison obviously gives the principle of selection its best chance. In fact it assumes that the whole of the mortality is selective and that survivorship is due to mortuary selection and nothing else. It might, indeed, be reasonably argued that, in comparing the fate of a given batch of entrants from A with that of the longest lived $(m-1)/m$th of the C's, we should expect, on the average, to obtain essentially similar results, even on the hypothesis of immunisation without selection; for the best $(m-1)/m$th of the C's will have passed through exactly the same experience in B as the A's have in A, so far as the value of this experience can be assessed on the degree of the preceding mortality among the samples under comparison, disregarding the length of exposure, and the death-rates prevailing during that period. If tried by this method the A's still show an advantage in comparison with the C's, a statistically significant advantage, we have proved that selective mortality does not completely explain the facts, for the compared C's are equally select. If, however, the comparison is indecisive, the opposite conclusion does not follow. Table VI shows the results; they are indecisive.

Table VI. *Cage B. Comparison of A mice with the corresponding "best" selection of C mice for the larger C groups.*

Length of life in cage B limited to 60 days

	A mice		C mice			
			Whole group.	"Best" selection		Proportion of C group in the selection
Group of A mice	Specific deaths	Total deaths	Specific deaths	Specific deaths	Total deaths	
A 18/B	23·5	23·5	19·2	21·5	21·5	·878
A 18/C	31·1	31·1	19·2	22·9	25·0	·741
A 18/D	34·6	35·0	21·9	29·4	30·0	·613
A 18/E	33·7	33·7	18·1	28·0	28·8	·547
A 18/F	31·5	27·3	25·2	39·9	39·9	·493
A 18/G	34·8	33·3	22·3	38·9	38·9	·354
A 19	19·5	19·5	22·3	24·4	24·9	·865
A 18/H	41·4	41·4	18·5	35·8	37·1	·192
A 19/A	31·9	31·9	18·5	24·1	24·9	·579
A 18/J	30·2	30·2	32·7	60·0	60·0	·082
A 19/B	43·6	43·6	32·7	48·2	49·2	·367
A 19/C	45·1	45·1	27·1	46·5	46·5	·306
A 20	25·9	25·9	21·9	22·5	22·8	·935
A 19/D	38·9	38·9	21·9	41·1	41·1	·244
Total	33·3	32·9	—	29·5	30·1	—

The general average of the A's is indeed still slightly better than that of the best C's, but in the individual batches the advantage is as often one way as the other. The attempt to prove by a short cut that selection is not a sufficient explanation has failed, although the result certainly does not strengthen the case in favour of selection.

THE DIFFERENT EFFECTS OF LENGTH OF EXPOSURE AND SEVERITY OF EXPOSURE.

In considering the way in which improvement by selection and improvement by immunisation would be likely to act, it seems reasonable to suppose that, the greater the importance of the selective factor the greater would be the importance attaching to the stringency of the selective process carried out in *A*, and the less the importance of a sojourn in *A* when the mortality in *A* was trifling. As always happens in the real problems of biology, as distinct from mere paper speculations, there is not a perfect antithesis. When mortality is very high, even if we assume that it is wholly or mainly selective mortality, it is probable that a certain proportion of the exposed to risk will acquire the infection and will die in *B* not in consequence of the new exposure but of what they have carried across in their bodies. That is, the great selective value of a high mortality rate might be masked by the existence among the transfers of a high proportion of mortally infected animals. Passing to the opposite extreme, if animals exposed to a not fatal infection (*i.e.* if during their sojourn in *A* no deaths at all occurred) showed an advantage, it would not absolutely exclude the cherishing by the conditions in *A* of favourable innate variations. Assuming that these innate variations consisted, at least in part, of differences in immunisability, so that the result of non-lethal infection would be greatly to increase the resistance of some mice, while leaving that of others unaffected or very slightly increased, the preliminary exposure might tune up the different natural types to respond to a second exposure more exactly so that among them the mortality due to the second exposure would be less marred by mere chance variations—non-selective mortality—than in an untuned sample of *C*'s. But it does seem broadly reasonable to suppose, if selection is not only involved but is of chief importance, that superiority in *B* should be more closely correlated with *severity* of exposure in *A* than with duration of exposure. This is a question we have examined in detail by the method of multiple and partial correlation, but before dealing with the somewhat intricate details of this we may note a trial which does not involve the use of this calculus. We have taken out separately (Table VII) the experiences in *B* of mice who while in *A* were never exposed to rates of mortality in excess of 7·5 per mille per diem, less than one-third of the average rate of mortality in *A*. Confronted with the associated *C*'s it will be seen that these *A* mice possessed an advantage of the same order of magnitude as all *A* mice and that, related to duration of exposure in *A*, the result exhibited the feature which we have noted as being not invariable but frequent in the general *A* experience, viz. a more or less regular improvement with increasing duration of exposure in *A* up to a certain point and thereafter an irregular decrease, the mice exposed at more than 70 days' duration proving inferior to the *C*'s themselves. This result seems to us more easy to interpret upon the hypothesis of an active immunisation, an educational process the effect of

Table VII. *A mice whose time of exposure in cage A was at an average death-rate of* $< \cdot 00755$ *and C mice.*

	Length of exposure in cage A (days)	Number in group	Length of life in cage B limited to 60 days	
All C mice	0	887	$22 \cdot 4 \pm \cdot 36$	
C mice corresponding to these A's	0	493	$20 \cdot 9 \pm \cdot 49$	
A mice	10	59	$23 \cdot 3 \pm 1 \cdot 60$	All A mice $25 \cdot 08 \pm \cdot 84$
	20	38	$23 \cdot 8 \pm 1 \cdot 99$	
	30	39	$25 \cdot 4 \pm 1 \cdot 97$	
	40	30	$26 \cdot 6 \pm 2 \cdot 24$	
	50	9	$34 \cdot 6 \pm 4 \cdot 10$	
	60	10	$33 \cdot 7 \pm 3 \cdot 89$	
	65	9	$22 \cdot 4 \pm 4 \cdot 10$	
	70	8	$31 \cdot 5 \pm 4 \cdot 35$	
	75	10	$14 \cdot 9 \pm 3 \cdot 89$	

$\therefore$ Difference between these A's and all C's $= 2 \cdot 71 \pm \cdot 92$ days.
$\therefore$ Difference between these A's and contemporary C's $= 4 \cdot 2 \pm \cdot 97$.

which wears off, than upon the hypothesis of selection. Mortality was occurring indeed but it never reached a high intensity (and so was little subject to the fallacy of a carry over), and did not show any sign of cumulative favourable effect, by the greater and greater elimination of the "unfit."

We pass now to the more elaborate analysis.

Great as is the value of the calculus of correlations, the difficulty of its employment and, still more, of its interpretation, when we have to deal with biological data, is not small. One is seldom, in these classes of investigation, in the biometrician's paradise where the characters are not very variable—have coefficients of variation not more than 15 or 20 per cent.—regressions are linear and the dependent variable is highly correlated with a number of independent variables which among themselves are not highly correlated. It is much more usual (as in this case) to have coefficients of variation ranging up to or beyond 100 per cent., regressions of more than dubious linearity and "independent" variables displaying obstinately high correlations one with another.

In the system of variables employed by us, two must be defined. By "Total Exposure" we mean the product of the number of days' exposure in A with the average specific death-rate during that exposure.

By "Selection Rate" we mean the ratio of the deaths in A of a given batch divided by the difference between the total number of the batch entering A and the number of that batch already transferred to B before the transfer of the individual in question. Thus if an individual mouse formed one of a batch of 50 entering A, if 10 of these died while he was in A and if 15 were transferred to B before his turn came, the "Selection Rate" applicable to him would be 10/(50–15).

We had no very high hopes of the value of this ratio as an independent measure of selection, but we need scarcely discuss the theoretical pros and cons because this ratio was found to be highly correlated ($r = 0 \cdot 935$) with "Total Exposure" so that its independent value is negligible.

In Table VIII the various total and partial correlations are set out. If we pass straight to the highest partials, it would seem clearly established that length of exposure in A freed from variations of severity of disease in either A or B is distinctly more closely related to length of life in B than is the final death-rate in A. Looking at the gross correlations it will be seen that length of life in B is more highly correlated with length of previous exposure in A than with average death-rate in A; but it will also be seen that the regression in the latter case departs much more widely from linearity and that the two correlation ratios are sensibly equal. It follows that a comparison of the partials is dangerous, in that the conditions of strict comparability are not fulfilled. We cannot in fact prove, by this method, that length of exposure divested of selective mortality is more important as a factor of survival in B than selective mortality when other variables are held constant. We can, however, prove that when the death-rates in A and B are held constant, there is a statistically significant positive relation between variations of length of exposure in A and variations of length of life in B. Using as a measure of events in B not length of life but proportion of entrants surviving 28 or more

Table VIII.

Total correlations.

		r	η	$\eta^2 - r^2$
(21)*	*Length of life in* B *and length of previous exposure in* A	*·214±·016*	*·244±·016*	*·014±·004*
(23)	Length of life in B and average death-rate in A	·119±·017	·254±·016	·052±·008
(24)	Length of life in B and average death-rate in B	−·152±·017	·237±·016	·033±·006
(25)	*Length of life in* B *and "total exposure" in* A ...	*·237±·016*	*·241±·016*	*·002±·001*
(26)	*Length of life in* B *and "selection rate" in* A ...	*·219±·016*	*·228±·016*	*·004±·002*
(27)	Length of life in B and average death-rate in A for the last 10 days before transfer	·110±·017	·186±·016	·022±·005
(13)	Length of exposure in A and average death-rate in A...	·410±·014	·802±·006	·475±·024
(14)	Length of exposure in A and average death-rate in B...	·047±·017	·158±·017	·023±·005
(17)	Length of exposure in A and average death-rate in A for the last 10 days before transfer ...	·453±·014	·758±·007	·370±·021
(16)	Length of exposure in A and "selection rate" in A...	·784±·007	·827±·005	·068±·009
(43)	Average death-rate in B and average death-rate in A for period of exposure	·026±·017	·422±·014	·178±·014
(45)	Average death-rate in B and "total exposure" in A...	−·083±·017	·285±·016	·075±·009
(47)	Average death-rate in B and average death-rate in A for the last 10 days before transfer ...	−·051±·017	·339±·015	·113±·011
(56)	"Total exposure" in A and "selection rate" in A	·935±·002	·941±·002	·012±·004

* The variable that was found in terms of the other is given first (*e.g.* for (21) η is the η of 2 on 1). η's were corrected by the formula

$$\eta^2 = \frac{\text{crude } \eta^2 - \frac{\kappa - 3}{n}}{1 - \frac{\kappa - 3}{n}}, \text{ where } \kappa = \text{no. of groups.}$$

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Table VIII (*contd.*).

Partial correlations.

		r
1st order		
(21·3)	*Length of life in* B *and length of exposure in* A (*keeping death-rate in* A *constant*)	*·185±·016*
(23·1)	Length of life in *B* and death-rate in *A* (keeping length of life in *B* constant)	·027±·017
(21·4)	*Length of life in* B *and length of exposure in* A (*keeping death-rate in* B *constant*)	*·224±·016*
(23·4)	Length of life in *B* and death-rate in *A* (keeping death-rate in *B* constant)	·117±·017
(25·4)	**Length of life in B and "total exposure" in A (keeping death rate in B constant)**	**·228±·016**
(27·4)	Length of life in *B* and death-rate in *A* for 10 days before transfer (keeping death-rate in *B* constant)	·119±·017
(27·1)	Length of life in *B* and death-rate in *A* for 10 days before transfer (keeping length of life in *A* constant)	·015±·017
(21·6)	Length of life in *B* and length of exposure in *A* (keeping "selection rate" constant)	·070±·017
(25·6)	Length of life in *B* and "total exposure" in *A* (keeping "selection rate" constant)	·094±·017
(17·4)	Length of exposure in *A* and death-rate in *A* for 10 days before transfer (keeping death-rate in *B* constant)	·452±·014
(13·4)	Length of exposure in *A* and average death-rate in *A* (keeping constant the death-rate in *B*)	·410±·014
2nd order		
(21·34)	*Length of life in* B *and length of exposure in* A (*keeping constant death-rate in* A *and death-rate in* B)	*·194±·016*
(27·41)	Length of life in *B* and death-rate in *A* for the 10 days previous to transfer (keeping constant length of exposure in *A* and death-rate in *B*) ...	·021±·017

Multiple correlation.

(2·(13))	*Length of life in* B *with length of exposure in* A *and average death-rate in* A	*·215**

Multiple partial correlation.

(2(13)·4)†	**Length of life in B with length of exposure in A and average death rate in A (keeping average death rate in B constant)**	**·225**

* Value for independence by Yule's formula $\sqrt{\frac{n-1}{N}}$, where n = no. of dependent variables and N = no. of observations, is ·025.

† Found from the formula $r_{2(13)\cdot 4} = \frac{r_{2(13)} - r_{42}\, r_{4(13)}}{\sqrt{(1-r_{2}^{2}{}_{4})\,(1-r_{4}^{2}{}_{(13)})}}$.

days (see Table IX) the same result emerges. This result does not enable us to differentiate selection from education. The method, assuming all the conditions of complete comparability are fulfilled, merely expresses the fact that when the variable, rate of mortality, is held constant, length of survival in *B* increases significantly with length of exposure in *A*; it is *not* equivalent to saying that, were the rate of mortality given the *particular* constant value zero, the relation between survival in *B* and exposure in *A* would be that prescribed by the equation. We can, of course, substitute zero in the regression equation and we shall reach a result which will be *formally* equivalent to the statement just made, but we have no observations of the partial array in

Table IX.

Total correlations.

	r	η	$\eta^2 - r^2$
Proportion surviving 28 days in B and length of exposure in A	·475±·049	·518±·047	·043±·027
Proportion surviving 28 days in B and average specific death-rate in A	·222±·061	·530±·046	·232±·061
Proportion surviving 28 days in B and average specific death-rate in B	−·581±·042	·563±·043	Corrected $\eta^2 > r^2$
Proportion surviving 28 days in B and total exposure in A	·481±·049	·493±·048	·012±·014
Average specific death-rate in B and length of exposure in A	−·041±·064	Not significant	—
Average specific death-rate in B and total exposure in A	−·236±·060	,,	—
Average specific death-rate in B and average specific death-rate in A	−·110±·063	·462±·050	·202±·057
Average specific death-rate in A and length of exposure in A	·231±·060	·674±·035	·401±·081

Partial correlations.

	r
Proportion surviving 28 days in B and length of exposure in A (keeping constant average specific death-rate in B)	·554±·044
Proportion surviving 28 days in B and average specific death-rate in A (keeping constant average specific death-rate in B)	·195±·061
Proportion surviving 28 days in B and total exposure in A (keeping constant average specific death-rate in B)	·435±·052

Multiple r's.

Proportion surviving 28 days in B, with length of exposure in A and average specific death-rate in A	$r_{2(13)}$	·488
Average specific death-rate in B with ditto	$r_{4(13)}$	·111

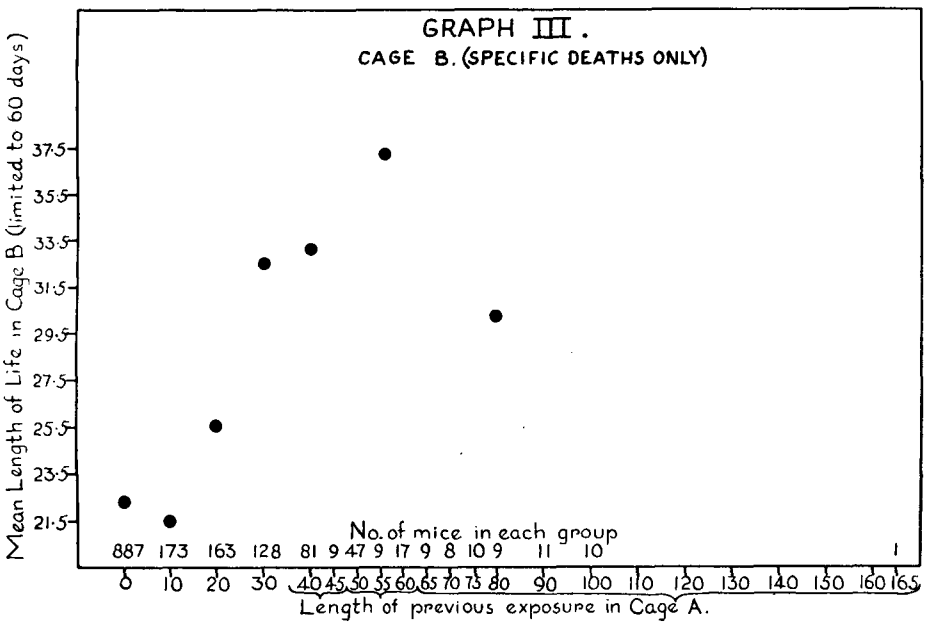
Multiple partial r.

Proportion surviving 28 days in B, with average specific death-rate in A and length of exposure in A, keeping constant the average specific death-rate in B ...	$r_{2(13)\cdot 4}$	·684

question so that we are in fact extrapolating, always a dangerous undertaking. But if our results only apply to partial arrays for constant rate of mortality different from zero, then even on a strict selectionist hypothesis there should be a positive association between exposure in A and survival in B, because the positive death-rate in A will have had a longer time to operate, *i.e.* to weed out the unfit.

The two considerations which, not decisively but appreciably, tell in favour of education rather than selection are first that already noted, viz. the enjoyment of virtually the same superiority of A's over C's when consideration is confined to A's never exposed to a high death-rate in A, and second, the point also briefly indicated, that the advantage of exposure in A tends to fall off as the exposure is prolonged. We have already pointed out in earlier communications that survivors of epidemic conditions always tend eventually to succumb to the particular infection. A similar phenomenon is present here and strikingly so. The regression chart of survival on exposure (Graph III) brings this out clearly enough. The rule is subject to particular exceptions,

but it *is* the rule that exposure beyond a certain point is not an advantage. This is not easy to understand on a pure principle of selection. We might indeed suppose that the selective mortality produces all its effects within a short time and so account, on a pure selection doctrine, for a regression curve with an asymptote parallel to the axis of exposure in *A*, but we could hardly account on any selection hypothesis for a regression curve which returns towards the axis at a finite angle. It is true that the data for longer durations are scanty and the form of the distribution (partly for this reason) irregular, so that the proof is incomplete. But it is easier to explain the facts we have by the hypothesis that mice who survive in *A* undergo an immunisation process



the effectiveness of which increases with duration and is then gradually lost. There is, however, an objection to this argument. Let us suppose that what happens is this. On entering *A* a mouse is exposed to a desultory bombardment of doses of various size. The mice who receive large doses are killed, those who receive small doses are in the way of developing an active immunity. It is well known that the immunity conferred by most artificial processes wears off and there is no reason why the method of conveying the dose should alter this. We might suppose that a mouse immunised in the first 10 days of stay, say, will, if left alone (say taken out of the cage) gradually lose his immunity. But he is *not* taken out of the cage, he is continuously exposed and yet, on the average, his powers wane. The explanation of the facts by wearing off of acquired immunity is insufficient. Various explanations are possible. It might be that—to take a hint from Dudley's work—*pari passu* with increasing tissue resistance, whether a cellular or a humoral immunity,

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there is an accumulation within the body of a *materies morbi*, that over against increasing tissue resistance there is an accumulation of infective material and that ultimately the latter overpowers the former. The point that a temporary immunity may be associated with an ultimate weakening has, indeed, impressed itself upon us in the course of our previous work. The fact, which we think we have established, that at least one form of epidemic disease can be maintained indefinitely in a herd at the price of merely admitting to it healthy susceptibles and that the vast majority of the survivors of prolonged exposure themselves die of the disease, is really a cogent argument against the all sufficiency of selection. Continuous immigration of susceptibles does not, as we have shown, lead to overwhelming outbursts of epidemic disease, to the establishment of death-rates so high that few could survive, but to moderate waves of disease. Yet ultimately nearly all succumb. We say again that this does *not* prove that selection is impotent, it only proves that if there be mortuary selection, the select are subject to a casual specific mortality which is important. Also, it is perfectly consistent with the temporary advantage secured not being a result of mortuary selection at all but of active immunisation the effects of which are counter-balanced by another process. It is, perhaps, to a variation of our experimental method, rather than to a more elaborate statistical analysis of such data as might be presented by a continuation of the present experiment, that we must look for an answer to this problem. It is in the possibility of defining the problems at issue, and of obtaining adequate data along the lines indicated, that the particular advantages of the combined statistical and experimental method appear to us to lie. The differentiation of natural resistance into those forms which are specifically acquired, and those forms which are due to some other factor, presents great technical difficulties. So far as those factors which are not specifically acquired are heritable, we may in time solve our problem by adequate experiments in selective breeding; but we see no reason for believing that all forms of natural resistance, other than those due to previous non-lethal infection with the specific parasite, are the result of ancestral endowment. It appears to us very possible that the past history of any particular individual, quite apart from his genetic make-up, may place him in a more, or less, resistant class, in respect of attack by a micro-organism which he has not previously encountered. Such an individual would be selected, under the conditions obtaining in the experiments we have just described, no less certainly than another individual who possessed the same degree of resistance as the result of innate characters. If acquired immunity be an important factor in survival through epidemic periods, it should not be impossible to produce, by proceedings more quantitatively controlled than exposure to risk of infection, an increase in resistance which is of the same order as that we have observed in our surviving mice, and which could be shown to be immediately due to immunisation without selection. Whether we can take the further step of assessing the part played by innate variations in immunisability only the

future can decide. The present experiment has brought us to the following position:—

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.

We suggest:—

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.

We have again the pleasant duty of acknowledging how much we owe to the collaborators who render these researches practicable.

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