LATENT INFECTION WITH *C. DIPHTHERIAE* IN ASSOCIATION WITH BACTERIAL EXPERIENCE AND SCHICK IMMUNITY.

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(With 2 Figures in the Text.)

CONTENTS.

I. Introduction.

The observations described in this paper were made at Greenwich Hospital School (G.H.S.), the population of which averages 1000 boys aged $11\frac{1}{2}$ to $15\frac{1}{2}$ years. The school year consists of three terms, the first or winter term (January to April), the second or summer term (May to July), and the third or autumn term (September to December).

In place of K.L.B., the common abbreviation for the diphtheria bacillus, four other sets of initials will be employed:

(*a*) C.D., for *Corynebacterium diphtheriae*, refers to specific organisms which have been isolated in pure culture, but have not necessarily been tested for virulence. C.D. therefore includes

(*b*) T.C.D., toxigenic C.D., and

(*c*) A.C.D., avirulent C.D.

A fourth abbreviation, M.D., is used for organisms which have not necessarily been isolated, but which resemble C.D. in their morphology and staining characters. M.D. may also be used to include all the above classes of bacterial cultures.

Schick positive, or susceptible, means that a subject has less than about 0·03 unit of diphtheria antitoxin in his blood. It does not necessarily mean he is susceptible to diphtheria, since many Schick susceptibles must have a high resistance to clinical diphtheria. On the other hand, Schick immunes are, for practical purposes, immune to typical diphtheria (see Glenny, 1925, and Dudley, 1929).

II. Incidence of cases and carriers of diphtheria.

Fig. 1 gives graphs of the carrier rates for M.D., and of the incidence of the cases which were notified as diphtheria, each term during the same 9 years' period. These graphs show that:

(*a*) From 1923 to 1927 the carrier rates tended to rise after the yearly case incidence had become steady.

(*b*) The seasonal peaks of symptomatic and symptomless infection with C.D. generally coincided in the autumn terms.

(*c*) The incidence of diphtheria was most often lowest in the summer terms, while the carrier rate tended to a minimum in the winter terms.

(*d*) Active immunisation of the school in 1928 was followed by the disappearance of symptomatic diphtheria and a marked drop in the carrier rate.

Table I. *Seasonal variation, in residential, and day, schools, of cases, carriers, and carrier-case ratio in diphtheria.*

	G.H.S.			L.C.C.S.		
Season of year or school term	A Cases per 1000 per term	B Carrier rate per 1000	Ratio B : A	A Cases per 1000 per term	B Carrier rate per 1000	Ratio B : A
Winter	14·2	33	2·3	2·9	58	20·0
Summer	7·7	62	8·0	2·2	53	24·1
Autumn	23·5	107	4·5	3·4	69	20·3
Mean rates	15·1	67	4·9	2·8	60	21·5
Coefficient of variation	43	48	49	17	11	19

Table I is made from the data in Fig. 1 and L.C.C. reports, and compares the average seasonal incidence per term, of carriers and cases in Greenwich Hospital School (G.H.S.) and the London County Council Schools (L.C.C.S.). Although there was little difference in the mean yearly carrier rate of the two groups, yet the incidence of notified diphtheria was five times as great in G.H.S. as in the L.C.C.S., in spite of the latter's population having a lower average age. As a result, the preponderance of carriers over cases in the L.C.C.S. must have been over fourfold that found in G.H.S. The marked difference in diphtheria morbidity is attributed to the special nature of the institutional environment (see Dudley, 1926). The seasonal fluctuations in both symptomatic and symptomless infection was much greater in the residential school. In both groups the ratio of the carrier rate to the incidence of

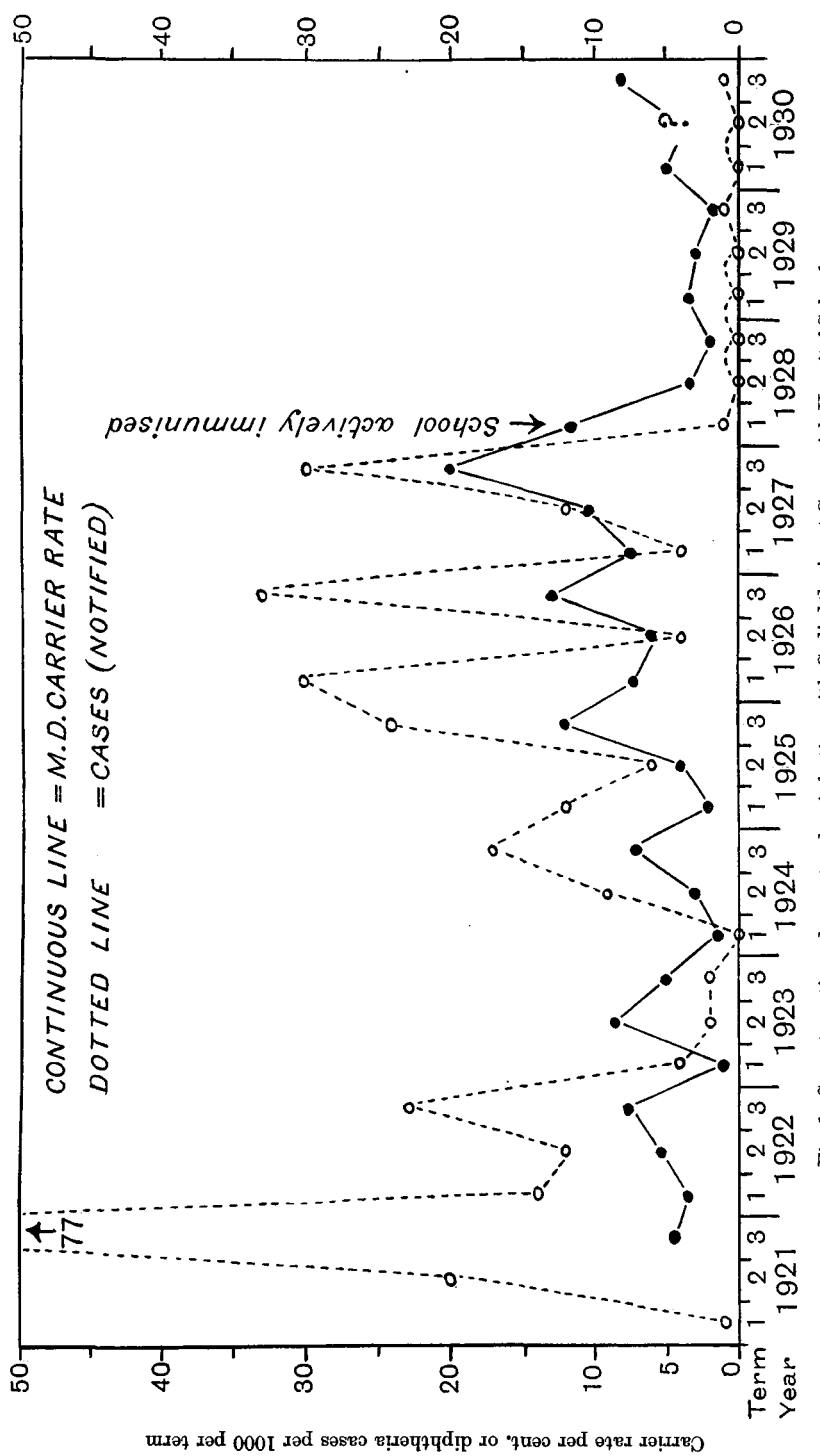

Fig. 1. Symptomatic and symptomless infection with *C. diphtheriae* at Greenwich Hospital School.

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cases was highest in the summer, and in G.H.S. the absolute number of carriers present in the hottest term of the year must have been about double the number present in the coldest term—the reverse of what was observed in the number of cases. This increase of carrier, and decline of case, infection in hot weather is of interest in view of Doull's (1928) observations that the incidence of diphtheria in America declines with latitude, and with the fact that the carrier rates and Schick immunity in some large towns in the tropics may be very high, although clinical diphtheria is not in evidence.

III. Virulent and avirulent carrier rates.

Table II, which summarises the cultures containing M.D. which were obtained from random samples of apparently healthy boys between 1922 and 1930, shows that:

(*a*) Of 328 C.D. cultures isolated 42 per cent. were T.C.D.

(*b*) The mean carrier rate for T.C.D. was 2·9.

(*c*) The total M.D. rate, and the ratio of toxigenic to avirulent cultures, was extraordinarily variable at different times in the same environment.

(*d*) The T.C.D. rate was more consistent than the A.C.D. rate.

(*e*) The active immunisation of the school in January and February, 1928, was followed by the decline and ultimate extinction of A.C.D. infection, while the amount of symptomless infection with T.C.D. was not significantly altered.

When taken in conjunction with Fig. 1, Table II demonstrates that there was no constant association of any carrier rate, or of the percentage of C.D.

Table II. *Virulent and avirulent carrier rates. Greenwich Hospital School*, 1922–30.

Period	A No. C.D. cultures	B T.C.D.	C T.C.D. or C.D. %	A M.D. carrier rate	B T.C.D. %	C A.C.D. %
Jan.–July 1922	35	17	49	4·5	2·2	2·3
Sept.–Dec. 1922	22	13	59	7·7	4·5	3·2
Jan.–March 1923	—	—	—	0·0	—	—
April–Dec. 1923	30	20	67	6·3	4·2	2·1
Jan.–July 1924	13	10	76	2·0	1·5	0·5
*Sept. 1924–Sept. 1927	—	—	—	7·5	—	—
Oct.–Dec. 1927	42	2	5	20·0	1·0	19·0
†Jan. 1928	89	18	21	15·8	3·2	12·6
Feb. 1928	19	8	42	12·8	3·3	9·5
March 1928	12	5	42	11·5	5·4	6·1
2nd Term, 1928	8	2	25	3·5	0·9	2·6
3rd Term, 1928	9	6	67	3·3	2·1	1·2
1st Term, 1929	20	13	65	4·7	3·1	1·6
2nd Term, 1929	14	10	72	4·0	2·9	1·1
3rd Term, 1929	6	6	100	1·5	1·5	0·0
1st Term, 1930	9	9	100	4·8	4·8	0·0
Totals...	328	139	42	(109·9)	(40·6)	(66·6)
Mean rates				6·9	2·9	4·8
Standard deviation ...				±3·0	±1·3	±5·4
Coefficient of variation...				44	45	112

* No virulence tests recorded for 3 years.

† School immunised in January and February, 1928: note subsequent decline and disappearance of A.C.D. and persistence of T.C.D.

cultures which were toxigenic, with the number of cases notified as diphtheria from G.H.S. For example, in July, 1923, 2 cases of notified diphtheria were accompanied by a carrier rate for M.D. of 12 per cent., and over 80 per cent. of the C.D. cultures isolated at the time were toxigenic. During October and November, 1927, 30 cases of diphtheria were notified at G.H.S., and the carrier rate for M.D. (20 per cent.) was the highest ever recorded in this school, but only 2 (5 per cent.) out of 42 carrier strains of C.D. proved toxigenic. These two combinations of events represent highest and lowest frequencies of T.C.D. among total M.D. carrier infections found in G.H.S. It is noteworthy that the low case rate in conjunction with the high virulent carrier rate occurred in the summer, while the combination was reversed in the winter.

IV. Absolute number of carriers.

The absolute number of the population which get an M.D. infection without symptoms during any period must depend on the *real* carrier rate and duration of the infection. It should be realised that the number of carriers which were discovered, whenever a sample of G.H.S. boys was swabbed, must always have been less than the real number of boys infected at the time with M.D. The *real* rate exceeded the *recorded* rate because:

(*a*) No nasal swabs were taken.

(*b*) All "doubtful" cultures were recorded "negative M.D."

(*c*) Plain coagulated ox serum was always used for the primary throat cultures, and Alison and Ayling (1929), among others, have shown that the substitution of special selective culture media for the above medium increases the number of carrier infections found in a group.

The average of the recorded carrier rates for M.D. in both G.H.S. and the L.C.C.S. for the last 10 years has been, in both groups, roughly 6 per cent., and about half of the isolated cultures of C.D. were found virulent in both environments. If this rate remained constant, and the duration of carrying was always one month, then the absolute number of "discoverable" carriers of M.D. would be equivalent to 72 per cent. of the population per annum, and there would be enough "discoverable" symptomless diphtheria infection to enable each child at the age of 15 to have been infected five times with virulent, and five times with avirulent, diphtheria bacilli. Unfortunately such a simple deduction does not allow for chronic carriers, who may remain infected for many months or even years, nor for an excessive number of reinfections of the same subject. In this respect one may note that most of the literature on carriers refers to the "chronic" and "convalescent" carrier. A recent paper by McCartney and Harvey (1928) shows that "chronic" carriers of M.D. nearly always have abnormal throats or noses, and are most resistant to treatment. For this reason there is an impression, which I hope to prove false, but which seems to be gaining rather than losing ground, that symptomless diphtheria infections are limited to a small section of the community with abnormal throats or noses; and that therefore carrier rates as high as 5 or 6 per cent. do not neces-

sarily imply that a large proportion of the healthy child population must have been infected frequently with C.D. At G.H.S. chronic carriers were rare, most of the discovered carriers had healthy throats, and all septic noses and tonsils were attended to by a surgical specialist. At least four-fifths of sub-clinical infections with C.D. at G.H.S. were of the type generally called "contact carriers," among whom the duration of "discoverable" infection rarely lasted over two weekly swabs.

The investigation which is summarised in Table III was deliberately made to show how many different individuals in a school may become infected with C.D. in the course of a year. The inhabitants of two school dormitories were swabbed eight times, at roughly monthly intervals (excluding holidays). Each dormitory contained over 100 beds. Altogether 284 individuals were examined, but only 139 of these were present at seven or eight of the sessions. The remaining 145 boys joined or left school during the year, or for some other reason missed more than one session.

Table III. *Results of repeated swabbings of same groups.*

		"Individuals"				"Swabs"		
No. of swabbings per boy	Dormitory	No. in group	No. infected M.D.	% infected M.D.	% infected more than once	Total number	No. infected with M.D.	"Carrier rate" infected %
7 or 8	No. 5	75	30	40	8·0	567	39	6·9
Average 7·6	No. 7	64	25	39	9·4	489	33	6·7
Total		139	55	40	8·7	1056	72	6·8
Less than 7	No. 5	81	15	19	0·0	320	15	4·7
Average 4·1	No. 7	64	13	20	6·5	274	22	7·3
Total		145	28	19	2·8	594	37	6·2
Total boys Average 5·8	Nos. 5, 7	284	83	29	5·6	1650	109	6·6

Table III shows that:

(*a*) The average recorded "carrier rate," *i.e.* the percentage of total primary cultures in which M.D. were seen, was 6·6.

(*b*) Swabbing the throats of a random sample of the G.H.S. boys seven or eight times proved that 40 per cent. of the boys were carriers of M.D. within a year.

(*c*) Only 9 per cent. of the above sample were found infected on more than one occasion.

(*d*) By comparing the lower sub-group of boys, who were swabbed on the average four times in the year, with the upper sub-group, it is seen that, when the number of examinations was roughly doubled, the number of different individuals discovered to be infected with M.D. was also doubled, though the average "carrier rate" in both groups was approximately equal.

(*e*) Both dormitories returned similar figures, which lessens the risk that the total group formed an exceptional sample of the G.H.S. boys.

Two further points, not shown in Table III, are also relevant. No case of

diphtheria was notified from these 284 boys during the year of the investigation. About half the cultures of C.D. isolated during the period were virulent to guinea-pigs. This mass experiment more than justifies the belief, that between 1919 and 1928, when the average diphtheria morbidity was 4·5 per cent. per annum, the *real* number of latent infections in different boys must have been considerably more than tenfold the number of case infections. And, if the same excess of "contact" over "chronic" carriers should exist in the L.C.C.S., where the morbidity of notified diphtheria was about 0·8 per cent., the number of "discoverable" latent C.D. infections (and unnotified cases) per year must have been at least thirty times as great as the number of notified cases.

V. CHANGES IN VIRULENCE OF "CARRIED" *C. DIPHTHERIAE*.

Between 1922 and 1924, of twenty-one cultures of A.C.D. which were isolated from Schick tested subjects, thirteen came from random samples of the population. Five of the latter cultures were isolated from immune, and eight from susceptible, Schick reactors. At this time only about 20 per cent. of the whole community were Schick susceptible. Therefore, if these thirteen A.C.D. cultures had been distributed evenly throughout the community, one would have expected to find only two or three, instead of eight, among the Schick susceptible members of a random sample. The eight other carriers of A.C.D. were Schick immune, and found in a selected sample of 100 boys who had been infected recently with T.C.D.—as cases or as carriers of diphtheria. This sample therefore had an avirulent carrier rate of 8 per cent. against an expected rate of 3 per cent. as found in random samples examined at the same time, and because of their history of previous virulent infection, practically all the members of the selected sample must have been Schick immune. Therefore, during this period although the probability of finding carriers of avirulent bacilli in random samples of the population was greater in the Schick-susceptible than Schick-immune fraction of the community, yet the probability of finding A.C.D. was greatest in that selected sub-group of immunes who had recently been infected with T.C.D.

These earlier investigations were repeated more carefully in 1928 when A.C.D. infection had become much more common. Twenty-three carriers of T.C.D. who were found in January and February were reswabbed in March and April. These re-examinations produced eight cultures of A.C.D., and seven of T.C.D. (These figures include two cases of mixed infection with both A.C.D. and T.C.D.) The expected numbers as estimated from the random samples which were examined at the same time would have been 2·6 T.C.D. and 2·3 A.C.D. cultures. The excess of the found over the expected number of T.C.D. cultures is easily explained as due to the persistence of the original T.C.D. infection, but the fact that the number of A.C.D. cultures was three times that expected needs some other explanation. The reverse sequence of events, namely, T.C.D. infection followed by A.C.D. infection, was not so common. In early 1928 the carrier rate for avirulent diphtheria bacilli was very high (see

Table II) and fifty-three boys, who had been carriers of A.C.D. in January and February, when reswabbed about 6 weeks later produced eight C.D. cultures of which only one was virulent. After a lapse of another 3 months forty-eight of this sample were reswabbed a second time—four A.C.D., and one T.C.D., cultures were isolated. The total 111 re-examinations produced two T.C.D., eleven A.C.D., and six "non-isolated" M.D. The estimated carrier rates were therefore 14 per cent. for A.C.D. and 2·7 per cent. for T.C.D. The rates obtained from the random samples, examined at the same time, were 4·7 per cent. for A.C.D. and 3·0 for T.C.D. The higher carrier rate of A.C.D. can be explained by the persistence of the original infection, whereas the T.C.D. rate was practically the same as in the unselected samples of the population examined at the same time. To summarise, in G.H.S., if a carrier of C.D. was re-examined within 3 months, he was more likely to be found still infected with C.D. than a boy selected at random. If the original infection was A.C.D. such a carrier was no more likely to become a T.C.D. carrier than the average boy. On the other hand, if the original infection was virulent, the probability of the carrier being found to harbour avirulent bacilli if reswabbed within 3 months was greater than in other members of the institution.

VI. School seniority and *C. diphtheriae* infection.

In a previous communication (Dudley, 1922) it was shown that the proportion of Schick immunes was greatest in those groups of boys who, irrespective of their age, had been longest at school. This increase of Schick herd immunity with school seniority occurred at a rate equivalent to about 50 per cent. of the Schick susceptibles present at the beginning of any year acquiring Schick immunity by the end of the year. (A yearly rate has been postulated as the rate of natural immunisation has a seasonal periodicity.) The incidence of diphtheria decreased more rapidly than the decline in Schick susceptibility, which suggests that a subject acquires some increase in his resistance to attack before his positive Schick reaction becomes negative. The incidence of M.D. carrier infection also declined with seniority, but, at first, less rapidly than Schick susceptibility. These facts are illustrated in Table IV which was made

Table IV. *Carrier rate, morbidity, and Schick immunity according to school seniority.*

No. years residence G.H.S.	−1	1–2	2–3	3+
Average case rate per 1000 per ann.	10	3·9	3·0	2·1
Average carrier rate per cent.	9·7	7·3	3·3	2·7
Average per cent. Schick positive	40 (10)	21 (5·2)	14 (3·8)	8·0 (2·0)

Figures in parenthesis = Per cent. Schick positive divided by 4, to compare the decline in susceptibility with that of the case and carrier rates.

from an analysis of 385 notified cases and 187 carriers which were discovered between 1919 and 1927, and the results of 2638 Schick tests made subsequent to 1921. These figures, demonstrating the increase of immunity with *seniority* at G.H.S., are consistent with the well-established fact that cases, carriers of

diphtheria, and Schick susceptibles, are all concentrated on the lower *age* groups of an urban population. Doull (1930) gives recent exact statistics confirming these facts as regards U.S.A.

VII. SCHICK REACTION OF THE CARRIER OF TOXIGENIC *C. DIPHTHERIAE*.

For practical purposes carriers of toxigenic diphtheria bacilli may be said always to have a negative Schick reaction, yet virulent carriers are most frequently found in the section of the population where the frequency of Schick immunes is least. Schick positive carriers of T.C.D. do exist, five such have been discovered in G.H.S. but in each case a subsequent Schick test, within a month of the positive test, was negative. The combination of a positive Schick reaction and of carrying T.C.D. is found so rarely, because, unless the Schick test synchronises with the onset of T.C.D. infection, the subject will generally have acquired sufficient antitoxin to neutralise a dose of Schick toxin before he is tested. Harries (1927) reports some cases which confirm this deduction.

Frost (1928), and Collins (1929), show that, in urban populations, only one case of clinical diphtheria is reported for every six to ten natural latent immunisations. In the diphtheria stricken G.H.S., where the intensity and risk of infection was so much greater, only three Schick susceptibles acquired Schick immunity naturally for every case of diphtheria which was notified. Observations at G.H.S. and elsewhere show that Schick immunity must often be acquired very shortly after a Schick susceptible becomes "discoverably" infected with T.C.D. Therefore, it is tolerably certain that latent infection with T.C.D. is the mechanism by which the high Schick herd immunity of adult town dwellers is acquired. From these premises it would seem logical to suppose that, in general, whenever a Schick susceptible is attacked by T.C.D. he shortly becomes a Schick immune, or a case of diphtheria. But, as I hope to show later, this hypothesis can only explain in part why carriers of T.C.D. are so rarely found to have positive Schick reactions.

VIII. SIMULTANEOUS DISTRIBUTION OF SCHICK IMMUNITY AND *C. DIPHTHERIAE* CARRIER INFECTION.

In order to try and elucidate some of the difficulties raised in the previous paragraphs, the whole of the residents in G.H.S. (except the new entrants) were swabbed and Schick tested synchronously. These examinations were completed within a month, and fortunately coincided with a time when the carrier rate for A.C.D. was high enough to obtain frequencies of some significance. Table V summarises the results of this experiment and the figures may be taken to represent the distribution of carrier infection and Schick immunity in relation to school seniority at the same instant of time. The table shows:

(*a*) The total M.D. rate declined with seniority.

(*b*) The T.C.D. rate declined more quickly than the A.C.D. rate.

(*c*) Schick-susceptible carriers of T.C.D. were confined to the most junior group.

(*d*) The A.C.D. infections in all the seniority groups were roughly double as frequent among the Schick-positive as among the Schick-negative fractions of the group.

(*e*) A.C.D. infections showed no association with school seniority when the susceptible and immune sub-groups were taken separately. Therefore, the decline of A.C.D. infection in the total seniority groups was wholly secondary to the increase in the herd immunity of the more senior groups.

Table V. *Seniority and Schick immunity, of carriers of C. diphtheriae from synchronous swabs and Schick tests.*

Length of residence in G.H.S.	Schick susceptibles			Schick immunes			% Schick positive	"Non-isolated" M.D. %
		Carrier rates %			Carrier rates %			
	No.	V	A	No.	V	A		
Under 1 year	115	1·7	21	187	7·8	12	38	5·6
1–2 years	52	0	23	228	3·1	· 8·3	19	5·7
Over 2 years	32	0	19	227	1·4	10	12	4·2
Totals...	199	1·0	22	642	3·8	10	24	5·2

V = Virulent, A = Avirulent, diphtheria bacilli.
The carrier rates are estimated from the M.D. rates, and frequency of T.C.D. among the cultures isolated from each group. C.D. was not isolated from 44 of the 133 M.D. cultures.

This distribution of A.C.D. showed that at the time of test all Schick-susceptible groups were twice as heavily infected as immune groups, irrespective of their experience of the diphtherial environment. Nevertheless, although the numbers in the total seniority groups and immune sub-groups are large enough for the frequencies to be of some statistical significance, yet the size of the two more senior Schick-susceptible sub-groups are too small for their frequencies of A.C.D. infection to carry much weight.

IX. Immunisability and avirulent *C. diphtheriae* infection.

If a number of Schick-positive reactors are all given the same course of a diphtheria prophylactic, and Schick tested subsequently at regular intervals, it will be found that some become Schick negative more quickly than others. Thus, there are different degrees of "immunisability." Just as it was found that the Schick herd immunity of a group increased with residence in G.H.S., so it was discovered that "herd immunisability" or the rapidity of response to a diphtheria antigen increased with school seniority (Dudley, 1928).

The same 199 Schick-positive reactors, to which the data in Table V refers, were all given two doses of a diphtheria prophylactic and Schick tested again 1 month, and 3 months, after the first prophylactic inoculation. By this means they were classed in three orders of immunisability—"rapid," "medium" or "slow"—according to their response to a constant antigenic stimulus. Table VI gives the frequencies of avirulent C.D. infections found in these three Schick-

susceptible groups. This table shows that avirulent carrying was twice as frequent in the "slow" as in the "rapid" group of Schick susceptibles. The "rapid" group, however, still had a higher carrier rate for A.C.D. infection than any of the Schick-immune groups. Thus, at G.H.S., the more "immunisable" a subject was, the less the probability of finding him an A.C.D. carrier. And, further, since T.C.D. carriers were only found in the "rapid" group, the more immunisable a Schick susceptible was, the more likely he was to be discovered latently infected with T.C.D.

Table VI. *Immunisability and avirulent carrying.*

Schick immunity acquired	No. of boys	A.C.D. carriers No.	A.C.D. carriers %
"Rapid" within 1 month	73	11	15 ±3·4
"Medium" between 2 and 3 months	89	21	24 ±3·4
"Slow" in over 3 months	37	11	30 ±4·9
Total...	199	43	22 ±2·0

NOTE. 2 T.C.D. carriers were also found in the most immunisable group.

X. HYPOTHESIS OF "MUTATING" TOXIGENIC *C. DIPHTHERIAE.*

Many observations make it probable that T.C.D. under natural conditions frequently "mutates" to A.C.D. There is, however, little reliable evidence that avirulent diphtheria bacilli ever acquire toxigenicity. ("Mutate" is here used in accordance with the common bacteriological practice and carries no Mendelian implications.) Some evidence in favour of the view that T.C.D. can acquire avirulence is:

(*a*) Some laboratory workers claim to have derived A.C.D. from well-established strains of T.C.D. Crowell (1926) for instance, working with a culture of T.C.D., which originated from a single cell, produced an avirulent strain of C.D.

(*b*) Besides the observations recorded in Section V, there are many others, such as those of McCartney and Harvey (1928), who noticed that subjects who have been recently infected with T.C.D. are prone to be found harbouring A.C.D. at subsequent examinations.

(*c*) T.C.D. and A.C.D. are sometimes both isolated from the same throat swab. Five such mixed infections are reported by Okell and Parish (1926). The discovery of such mixed cultures is more intelligible on the mutation than on the separate infection theory. Because A.C.D. and T.C.D. colonies are almost invariably indistinguishable, bacteriologists usually pick off only one colony, and rarely more than two, to test for virulence. Five mixed infections that were isolated from G.H.S., were only found because they were bacteriological curiosities, in which the virulent and avirulent colonies had a distinguishing character on special media, moreover, in each instance one variety of C.D. outnumbered the other by about fifty to one (see Parker, 1928). For these reasons it would be surprising that mixed infections have been reported so often, unless both the varieties of C.D. had originated as a single infection.

(*d*) Okell (1929) found that when A.C.D. replaced T.C.D. in the same subject, the pair of organisms generally fell into the same serological group. Since the number of serological families of C.D. seems to be almost unlimited, the probability that an infection with T.C.D. should be followed by one of A.C.D. having the same antigenic property is unbelievably small, unless the latter is frequently the direct descendant of the former.

By taking the three seniority groups of immunes given in Table V, and the three "immunisability" groups of Schick susceptibles shown in Table VI, the G.H.S. population can be divided into six orders of immunity. There is no simple test to distinguish different orders of "immuneness" among Schick-negative reactors, but the size of the Schick-immune groups is large enough to be tolerably certain that the more senior groups would have a greater antitoxin herd immunity than the junior groups, because although each immune group probably contained individuals of all orders of antitoxic immunity above the Schick test level, yet the proportion of subjects with the higher resistances will be greater in the senior groups owing to their longer residence in a diphtheria-stricken environment. In the susceptible groups, though the numbers are small, the rate of immunisation enabled each individual to be placed in a distinct immunity group. Therefore, all the members of one group were less or more resistant than any member of another group. The carrier rates for these six orders of herd-immunity are given in the form of a histogram in Fig. 2 which shows that:

(*a*) C.D. infections declined with increase of resistance.

(*b*) T.C.D. infection was concentrated on the Schick immunes, and declined with resistance.

(*c*) T.C.D. infection was only found in the most resistant Schick-susceptible group.

(*d*) A.C.D. infection declined regularly in the first four immunity groups.

(*e*) A.C.D. infection was practically equal in all three Schick-immune groups.

In the so-called "commensal" or "saprophytic" infections, and in some diseases such as influenza, which leave little or no permanent immunity, there is rarely any significant variation in the frequency distribution of infection by age and seniority, provided the whole community is living under identical environmental conditions. For example, an examination of 700 throat swabs from the boys living in G.H.S., showed the presence of Hofmann bacilli in 30, 29, and 31 per cent. of the groups who had been respectively, less than 1, 1 to 2, and over 2 years in residence. In contrast, such organisms as T.C.D., or diseases such as measles, capable of stimulating a durable immunity, tend to be concentrated on those seniority or age groups of least specific bacterial experience. According to Fig. 2 A.C.D. were distributed like a "commensal" infection among Schick immunes, and like an "antigenic" infection among Schick susceptibles. This, combined with the fact that the only T.C.D. infections discovered among the Schick susceptibles were confined to the most resistant of

the three sub-groups, and that T.C.D. infection declined with increase of resistance among the Schick immunes, is consistent with the following hypothesis: A.C.D. infections often arise as T.C.D. mutants. Once established, A.C.D. spread from host to host as "commensals," infecting all groups equally, whatever their degree of diphtheria antitoxic immunity. T.C.D., on the other

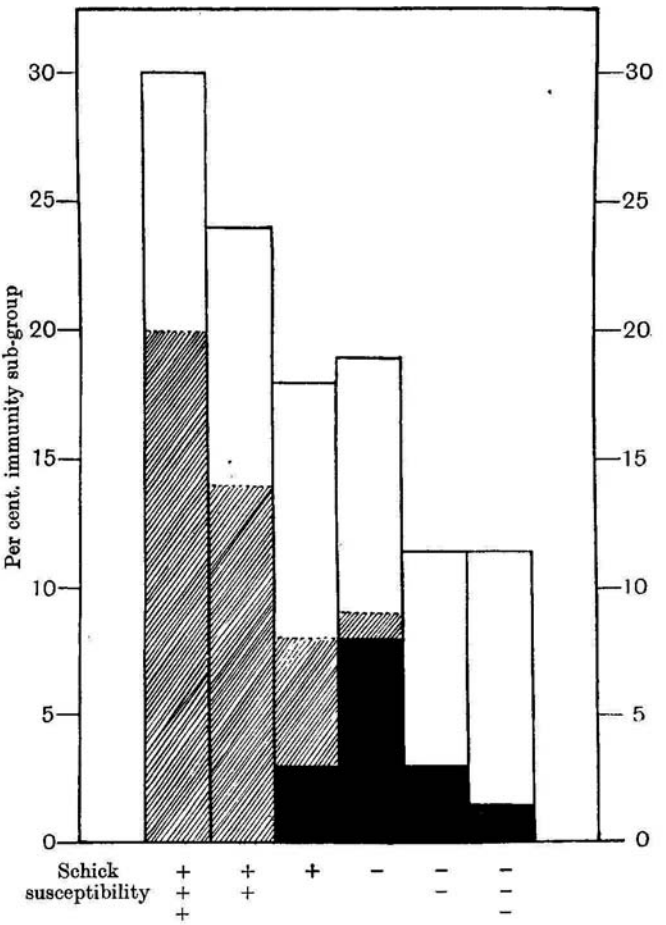

Fig. 2. Distribution of carriers among six orders of Schick immunity.
Black area = T.C.D. Remainder = A.C.D. as found.
White area = A.C.D. to A.C.D. infection.
Shaded area = "Mutated T.C.D." (by hypothesis).

hand, is distributed according to the degree of susceptibility of the group. If, however, the resistance of an attacked individual is below a certain degree, generally corresponding to the Schick test level, the infecting T.C.D. rapidly mutates to A.C.D.

Fig. 2 envisages a time and set of conditions, where 10 per cent. of all the immunity groups were infected "commensally" with A.C.D. This 10 per cent. is represented by the white parts of the columns. The shaded areas of the

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histogram then represent those A.C.D. carriers whose infection is supposed to have originated from T.C.D. These shaded areas representing "mutated T.C.D.," together with the black areas representing persistent T.C.D. infection, now form a characteristic "antigenic" distribution from the sub-group with least resistance to diphtheria to that with most, as judged by their Schick reactions, seniority, and immunisability.

If the mutation hypothesis, or some modification of it, is not accepted, one is driven to the absurd alternative that, in such environments as G.H.S. where the Schick susceptibles and immunes must be exposed to exactly the same risks of contact with T.C.D., Schick susceptibles are, in general, immune to T.C.D. *carrier infection*, and only become susceptible to such infection when they acquire diphtheria antitoxin in their blood. A working hypothesis is best judged by the difficulties it can explain. The mutating T.C.D. theory makes the following observations more intelligible:

(*a*) In the lower age-groups of certain environments, although Schick susceptibles can outnumber Schick immunes, yet during certain periods the real number of "initial" T.C.D. infections must, in turn, outnumber the sum of the immunes present at the beginning of the period, and the number of "latent immunisations" and cases of diphtheria which occur during the period. Hence many Schick susceptibles must be attacked by T.C.D. without getting diphtheria or acquiring Schick immunity. And the reason why such susceptible carriers are less frequently discovered than Schick-immune carriers, is that, in such subjects, the T.C.D. rapidly mutates to A.C.D.

(*b*) On the rare occasions when virulent carrying is discovered to be associated with a positive Schick reaction, the reaction almost invariably becomes rapidly negative. If such a rapid acquirement of antitoxic immunity is the only alternative to diphtheria when a Schick susceptible is attacked by T.C.D., it is hard to reconcile the rapidity of natural immunisation with the relative slowness with which Schick immunity is acquired by the majority of subjects who are artificially immunised. According to the mutation hypothesis the Schick susceptible, in an environment where T.C.D. infection is endemic, is from infancy onwards repeatedly infected with T.C.D. which, unless he gets diphtheria, each time changes to A.C.D. The repeated antigenic stimuli from these infections ultimately induces a degree of antitoxic immunity which permits the T.C.D. infection to persist without mutation. On this hypothesis, natural latent immunisation is really a longer process than artificial active immunisation. The peculiar fact that the majority of diphtheria patients remain Schick susceptible after recovery, unless they are subsequently re-exposed to risk of infection with T.C.D., when they gain Schick immunity rather than a second attack of diphtheria, is consistent with the above conclusion (Dudley, 1923).

(*c*) Under natural conditions, provided a significant number of M.D. cultures are examined, it is exceptional to find one variety of C.D. present to the complete exclusion of the other. On the other hand, T.C.D. and A.C.D. are

commonly absent together. For instance, during one year no M.D. were seen in the primary throat cultures of 316 consecutive new entrants to G.H.S.

After the school was actively immunised early in 1928, the carrier rate for A.C.D. fell gradually to zero, while the T.C.D. rate was not significantly affected. Between September 1929 and December 1930 no A.C.D. culture was discovered in G.H.S. during the time twenty-three T.C.D. cultures were isolated from apparently healthy boys. On the assumption that this fall in the C.D. carrier rate was because of, and not in spite of, the artificial immunisation of the school, it is difficult to see why only the non-toxic variety of C.D. should have been eradicated, unless the fact that there were practically no Schick susceptibles left in which T.C.D. strains could easily mutate, and among which A.C.D. are most frequently found, caused the supply of avirulent infections to fail.

(*d*) Doull, Stokes and McGinnes (1928) found that during the month following a carrier's discovery, family "contacts" with carriers of A.C.D. suffered a higher diphtheria incidence than a comparable sample of the rest of the community, but a lower rate than similar "contacts" with T.C.D. carriers. The authors suggest that this may mean that A.C.D. carriers may be the direct source of diphtheria cases. There is little evidence that A.C.D. can ever revert to T.C.D. and no urgent need for these observations to be explained in this way; because the examination of the protocols in the paper show that the time relations, between the symptomatic infection with T.C.D. and the discovery of the A.C.D. carrier, were such that both infections could generally have been contracted from an undiscovered source of T.C.D., and that sometimes even the discovered A.C.D. infection might itself have arisen as a recent mutation of a T.C.D. strain. Doull and his co-workers' observations suggest that the A.C.D. carrier is harmless when discovered, but may indicate the presence or recent existence of T.C.D. in the family.

(*e*) Finally, there is the peculiar phenomenon that, *where diphtheria is endemic*, tonsillectomy greatly increases the rate at which natural Schick immunity develops. This observation can only mean that tonsillectomised and Schick-positive boys were getting more intense antigenic stimuli than similar boys with tonsils living under exactly the same conditions. The mutation theory can explain how this is possible. The tonsils of subjects, without antitoxin, can in some way defend them from the action of diphtheria toxin, perhaps by forcing mutation on the attacking T.C.D. Tonsillectomised subjects, therefore, are not protected from diphtheria toxin to the same extent as those Schick-susceptible boys with tonsils, in whom mutation to A.C.D. most commonly occurs. Consistent with this hypothesis is the observation that at G.H.S. the carrier rate for T.C.D. was a little higher, and for A.C.D. much lower, in the tonsillectomised than in the control groups (see Dudley, 1931).

XI. One probable meaning of virulence.

The ecology of diphtheria, that is, the study of the mutual relations of the two species *H. sapiens* and *C. diphtheriae*, shows that the host herd adapts itself to the bacterial herd by the development of diphtheria antitoxin in the blood and tissue. This immunity is an acquired character which is stimulated by infection with T.C.D. But further experience shows that it is probable, in certain cases, adjustment between the two species is reached by some of the toxigenic members of the C.D. herd acquiring avirulence once they have established themselves in human beings who have no antitoxic defence. Such plasticity, or adaptive variation, under certain circumstances would be a valuable asset to the parasite species, since it enables a greater number of hosts to be colonised without damage, and without inducing a durable host resistance. And, other things being equal, it is obvious, the less harm done to the host, and the less antagonism aroused, the greater the *ultimate* benefit to the parasitic species. If this be true it may well be asked why has the avirulent variety not supplanted the virulent variety with the passage of time? The experience at G.H.S., when A.C.D. infection disappeared as herd immunity increased, suggests part of the answer. Topley and his co-workers (1928) have noted that in experimental epidemics virulence and infectiousness often vary together in the same species of bacterium (*B. aertrycke*). In the human field one of the best contrasts in this respect is the lower apparent infectiousness, as well as virulence, of *Variola minor* compared with *Variola major*. Infectiousness means power of dispersal to fresh hosts. A parasitic species depends for its existence on its power of dispersal, as much as on the closeness of its adaptation to the host habitat. Thus, during a set period, virulence, when reflecting power of dispersal, and avirulence, representing closeness of adaptation, will tend to be balanced according to the environmental conditions, and the type of resistance in the host herd.

"Smooth" virulent, and "rough" avirulent, phases are seen in many bacterial species. The "rough" phases are sometimes found in human and experimental animals who are recovering from an infection with the "smooth" phase. These facts taken with such experimental work as Wilson's (1928) on *discontinuous* variation in *B. aertrycke*, make one speculate whether the faculty of throwing off avirulent mutants is part of the general mechanism which has been evolved in bacterial herds, in order that they may adapt themselves more closely to their host herds under changing conditions. Thus, avirulent mutation in bacteria may be the complement of latent immunisation in the host.

The statement that a diphtheria bacillus develops avirulence, and a man antitoxin, because it suits their purpose to do so, would be pure nonsense unless one believes in, and understands the implications of, "The Origin of Species by means of Natural Selection" (or one of the newer modifications of this theory). Nevertheless, the statement is consistent with the tenets of one of the most widely held scientific "creeds," as well as with the hypothesis that T.C.D.

often mutates to A.C.D. in Schick-susceptible individuals. As Boycott (1929) says: "I do not think we can express the results more fairly or honestly than by saying an animal (bacterium), or man, grows blood, liver, or claws (avirulence or antitoxin), because in the prevailing circumstances it needs them—its actions in short are determined by its requirements."

XII. SUMMARY.

In a semi-closed institution, Greenwich Hospital School:

1. There was no constant relation between the prevalence of cases and carriers of virulent, or avirulent, diphtheria bacilli.

2. Carrier rates showed a seasonal periodicity with a maximum in the autumn and a minimum in the winter school term.

3. High rates for carriers of virulent bacilli were found in the absence of clinical diphtheria and presence of numerous Schick susceptibles.

4. As much as 40 per cent. of a random sample of healthy boys, none of whom contracted diphtheria, were discovered to harbour diphtheria bacilli (M.D.)[1] within 1 year, during which the average carrier rate in the sample was 6·8 per cent.

5. The carrier rate for avirulent diphtheria bacilli, among boys recently infected with virulent, was three times the expected rate.

6. The carrier rate for virulent diphtheria bacilli, among boys recently infected with avirulent, was no higher than the expected rate.

7. The frequency of avirulent diphtheria bacilli infections was twice as great among Schick susceptibles as among Schick immunes.

8. Carriers of virulent bacilli (with five exceptions) were always found to have Schick-negative reactions, in spite of their being more frequent among the junior members of the institution.

9. Carriers of avirulent diphtheria bacilli were as common among the senior as the junior *Schick-immune* members of the school.

10. Carriers of avirulent bacilli were less frequently found in those Schick-susceptible members of the community who were most quickly immunised by artificial diphtheria antigens.

11. The hypothesis that, when a Schick susceptible is infected with virulent diphtheria bacilli, the latter often acquire avirulence, could satisfactorily explain many anomalies in the distribution of latent infection with diphtheria bacilli and Schick immunity.

[Since this article went to press I have received a reprint from A. Garrido-Morales and O. Costa ("The Mechanism of Natural Immunity to Diphtheria," *Amer. J. Hyg.* (1931), **14**, 89), who describe the distribution of diphtheria carrier infection and Schick immunity in a school at San Juan, Porto Rico. In the main their results parallel those obtained at G.H.S. The most important difference between the tropical and G.H.S. observations was found in a sample

[1] See p. 193 for explanation.

of children, who were Schick tested and swabbed synchronously, in which the frequency of virulent carrier infections was greater among Schick susceptibles than Schick immunes. (However, only 9 T.C.D. infections were found among 642 children, but 5 of these were discovered among the 194 Schick susceptibles in this group.)—S. F. D.]

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