CONCENTRATION OF DIPHTHERIA ANTITOXIN IN THE BLOOD OF YOUNG ADULTS

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(With 9 Figures in the Text)

During the past six years an investigation has been carried out at the Wellcome Research Laboratories upon the diphtheria antitoxin titres of 3600 samples of blood from the umbilical cord. Barr, Glenny & Randall (1949) showed that the antitoxin titres of most mothers averaged 60 % of that in the cord blood, and was seldom below 50 % or above 100 %. The distribution of antitoxin titres in specimens of cord blood can be taken as an indication of the immunity state of the section of community from which they come. In the present investigation 75 % of the mothers were between 20 and 32 years old; few would have been artificially immunized within the previous 5 years and in the absence of natural stimulation their antitoxin titres would have reached a constant level. It is possible that there is still in this country a considerable amount of subclinical diphtheria, although carriers are rare, but it is probable that such infection is mostly encountered well before school-leaving age.

The first 200 specimens of cord blood were obtained from Guy's Hospital and from St Alfege's Hospital, Greenwich, by Dr K. J. Randall who was collaborating with two of the present authors in the immunization of babies. Barr, Glenny & Randall (1949, 1950) reported the results obtained, the relation between the antitoxin titres in the mother's blood and in cord blood, the rate of loss of passive immunity in babies, and the lowest concentration of inherited antitoxin that would interfere with successful immunization with alum-precipitated toxoid (A.P.T.). This work suggested the need for a large-scale survey of the distribution of antitoxin titres in samples of cord blood from a number of districts in the British Isles. This was carried out by Barr, Glenny & Parish (1951) who compared the results from 100 samples taken during 1949 from each of thirteen districts. A second batch of 100 was obtained from one town; these, together with the previous 200 taken by Dr Randall in 1947, made a total of 1600 divided into groups of 100. In 1100 instances the ages and immunization histories of the mothers were known. Results were analysed by two of the present authors, but publication was delayed until an opportunity arose to strengthen the conclusions by increasing the number of figures available. This occurred towards the end of 1951 when work was started at University College Hospital, London, involving immunization of babies on a large scale. As it was essential that the passive antitoxin titres of the babies should be

known, samples of cord blood were tested for diphtheria antitoxin content. The first thousand samples were collected between the end of September 1951 and early October 1952, with a gap of 7 weeks in June and July. The second thousand was completed at the end of August 1953. Throughout the whole of this work N.R.B. has been responsible for the clinical side and for the arrangements for the collection of the cord blood, K.B. for obtaining the immunization histories of the mothers and M.B. and A.T.G. for the immunological side, testing antitoxin titres, compiling and analysing results.

The information obtained from the mothers was similar to that collected for the earlier report (Barr, *et al.* 1949), with an additional item suggested by the previous results; each mother was asked whether most of her life had been spent in towns or in country districts. This is admittedly a vague classification but it provided some information.

Three series will be considered; the first, designated *49*, comprised the sixteen groups of 100 samples reported by Barr *et al.* (1951), fourteen of which were collected in 1949 and two in 1947. The other two series called *52* and *53* respectively were made up of successive groups of 100 from University College Hospital (U.C.H.), the first ten groups taken mostly in 1952 and the second ten mostly in 1953.

TITRATION OF ANTITOXIN

All tests were made by the guinea-pig intracutaneous method following the procedure described by Glenny & Llewellyn-Jones (1931) and the measurements involved were made by experienced technicians who carried out daily control tests to check the accuracy of their work and the stability of the toxin used. Examination of the lesions produced in guinea-pigs by the test mixtures were made almost invariably by M.B.

In the type of work under review it is not necessary to measure antitoxin titres at close intervals; tests made at approximately twofold differences are sufficient. In place of a scale in which all values are positive or negative powers of 2, three steps within tenfold differences were used involving simple figures, e.g. 0·001, 0·002, 0·004, 0·01, etc. The choice of a scale is important. There is a custom, somewhat widespread, for which no excuse or reason can be found, of using a scale of 1, 5, 10, 50, 100, etc., thus alternating two- and fivefold differences. There is still less excuse for the use of an arithmetical scale. Ramon & Richou (1950) recommended a range of tests for 1, 2, 3, etc., to 8, 9, 10 units continued illogically by jumping to 20 and starting the arithmetical scale again with increases of 10 units. The use of an arithmetical scale implies that close testing is both possible and desirable for titres approaching some simple negative or positive power of 10 such as 0·1, 1·0, 10 or 100, but either impossible or unnecessary for titres just above these figures. Thus at one end the test determines the difference between one and two units and at the other end between nine and ten. The test animals are unaffected by the notation used and die or live or exhibit reactions or not according to the amount of uncombined toxin in the mixture injected. When considering the distribution of antitoxin titres in any population it is essential to use a roughly geometric scale

because the logarithms of the titres and not the titres themselves are normally distributed in a population of individuals similarly treated.

Few samples of cord blood have been found to contain a detectable amount of antitoxin less than 0·001 unit per ml. so it has been considered unnecesary to test for smaller amounts. The titres recorded in this paper are the highest values at which no reaction was obtained in the test; the true titre lies between this recorded value and the next higher one in the scale. In calculation of geometric means the titre of each sample has been taken as the geometric mean of the two figures between which the titre lay.

DISTRIBUTION OF ANTITOXIN TITRES

Table 1 gives the distribution of diphtheria antitoxin titres in 20 groups of 100 samples of cord blood from U.C.H. without separating the two series *52* and *53*. When these figures are compared with those of the earlier series, *49*, recorded by Barr *et al.* (1951) the following points emerge:

(1) The number of specimens with less than 0·001 unit of antitoxin per ml. ranged from 21 to 59 % in series *49*, from 36 to 49 % in series *52* (numbers 1 to 10) and from 33 to 49 % in series *53* (numbers 11 to 20).

(2) The average number without this minimum amount of antitoxin in the 16 groups of series *49* was 34·6 %, a figure which is less than that in 19 out of 20 groups in the *52*, *53* series.

(3) The corresponding average for series *52* and *53* was 41·7 %, a figure greater than that in 13 of 16 groups in series *49*.

Three London hospitals were included in series *49*, two of these, St Alfege's (*k*) and Guy's (*o*) serve districts in the south-east of London near the Thames; the population might be expected to have a high natural immunity rate and relatively few samples had less than 0·001 unit/ml. (29 % from *k* and 21 % from *o*). The third London hospital, Queen Charlotte's (*g*) does not serve such crowded districts; the distribution of antitoxin titres from this hospital resembled the average distribution for the whole country and indicated a much higher immunity rate than that found in the population served by University College Hospital. It is difficult to account for the contrast between the mean figure of 41·7 % with titres below 0·001 from U.C.H. and 34 % from Queen Charlotte's.

A comparison of the distribution of antitoxin titres in groups of 100 samples shows that there was a strong tendency for the proportion with medium titres to increase as the total number with antitoxin increased. Thus in the country district *a* in series *49* with 41 % with titres of 0·001 unit or more, 16 had titres between 0·04 and 1·0 unit, while in Guy's Hospital, with 79 % with 0·001 unit or more, 57 had titres within these limits. Thus the total number with antitoxin was mostly made up of those with medium titres, and in addition 25 and 22 with titres outside this range. A further illustration of this is given in Fig. 1. In the form of diagram presented the vertical distance between any two horizontal lines shows the percentage of specimens containing quantities of antitoxin between the titres indicated by these lines; the height of any such line from the base shows the percentage of all

Table 1. *Distribution of diphtheria antitoxin titres among* 20 *groups of* 100 *cord blood samples taken at University College Hospital, London*

(The numbers at the head of each column refer to the order of collection.)

Antitoxin titres (units/ml.)	Number of samples in each group of 100																				Total	Percentage
	3	12	18	11	7	15	6	19	2	9	17	20	1	10	5	16	8	4	14	13		
Under 0·001	49	49	49	46	45	45	44	44	43	43	41	40	39	39	38	38	37	36	36	33	834	41·7
0·001	0	2	3	5	1	0	0	3	4	3	1	5	1	1	2	4	0	3	2	2	42	2·1
0·002	4	3	1	6	4	1	3	3	6	3	2	1	3	4	1	2	6	1	4	5	63	3·15
0·004	4	7	2	7	5	2	3	8	2	5	3	3	5	7	4	1	5	4	7	6	90	4·5
0·01	6	2	5	5	5	6	8	2	7	6	3	3	5	7	2	7	6	4	3	3	95	4·75
0·02	4	5	3	8	4	6	6	6	4	6	4	5	4	5	6	4	5	4	9	7	105	5·25
0·04	4	6	8	7	8	6	3	7	7	4	5	11	8	9	15	10	7	9	5	12	151	7·55
0·1	11	1	13	3	7	10	5	4	8	7	9	11	8	6	8	8	10	11	14	12	166	8·3
0·2	5	12	8	5	7	13	15	10	8	8	15	8	14	8	13	11	10	9	10	12	201	10·05
0·5	5	6	3	5	7	3	5	8	7	6	10	8	3	6	6	10	9	9	4	2	122	6·1
1·0	5	3	3	2	3	2	4	3	3	7	5	5	7	6	4	4	5	5	6	4	86	4·3
2·0	2	4	2	0	2	5	4	0	1	2	1	0	3	1	1	1	0	4	0	2	35	1·75
5·0	0	0	0	1	2	1	0	2	0	0	1	0	0	1	0	0	0	1	0	0	9	0·45
10·0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0·05

samples containing the indicated titre or more. In order to assist the comparison of results shown in different columns lines have been drawn connecting the horizontal lines representing 0·001, 0·01, 0·1 and 1·0 unit. Successive lines represent titres of 2·0 or 2·5 times those of the lines immediately above; thus as a general rule there are three areas between lines for tenfold differences in titre. Occasionally no samples were found with one or other of the usual titres, and a tenfold difference has only two subdivisions in which case one of the intermediate lines is so marked that full information is available. The highest line in any column, unless

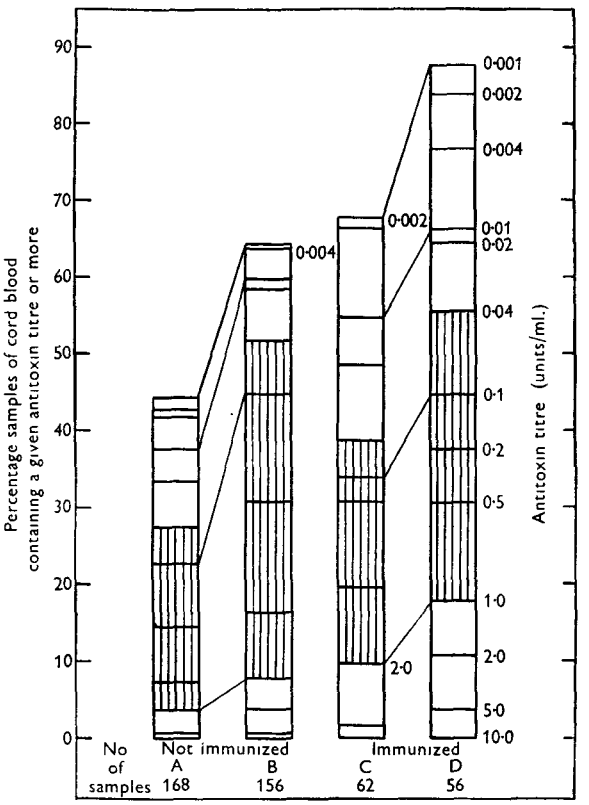

Fig. 1. Distribution of diphtheria antitoxin titres in samples of cord blood from unimmunized and immunized mothers from those districts with the least number with detectable antitoxin (columns A and C) and from those with the largest number (columns B and D).

otherwise marked, represents the lowest titre for which tests were made, that is 0·001. If, as in column C, this line is marked 0·002 it means that there were no specimens which passed the test for 0·001 unit but failed at 0·002, 1·6 % passed at 0·002 but failed at 0·004, and 11·3 % passed at 0·004 but failed at 0·01. At the top of column B there are only two subdivisions between 0·001 and 0·01; as the middle line is marked 0·004 and the tenfold lines represent 0·001 and 0·01 the subdivisions are 0·6 % passing at 0·001 and failing at 0·002 and 3·8 % passing at 0·004 and failing at 0·01.

A comparison is made in Fig. 1 of the distribution of antitoxin titres in two

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groups of 300 samples of cord blood from six districts from which immunization histories for most of the mothers were available. Columns A and C represent the districts with the lowest proportion of samples containing any antitoxin and B and D those with the highest proportion. Among the 300 samples, from the first group of districts 158 and from the second group 221 contained some antitoxin. In the first group there were 168 mothers (column A) who had no history of immunization or of clinical diphtheria and 156 (column B) in the second group. The proportions with antitoxin over 0·001 unit were 44·0 and 64·1 %; the difference between these two figures is practically the same as that between 23·8 % in column A and 44·2 % in column B for titres over 0·04 and under 1·0 unit/ml. There was, in addition, some difference in the number with lower titres (under 0·04), 16·7 % in A and 12·2 % in B; at the other end of the scale there were 3·6 % in A and 7·7 % in B with titres of 1·0 unit or more per ml. The main difference, however, lay in the number with medium values shown by the shaded areas in the diagram.

Among the immunized mothers (without history of clinical diphtheria) the medium range of titres did not have the same significance. In the first group (column C) there were 67·8 % with some antitoxin out of a total of 62, and 87·5 % of 56 in the other group (column D). The difference of 19·7 % was made up by an increase of 3·1 % in those with low titres, of 8·4 % in those with medium titres and of 8·2 % in those with titres of 1·0 unit per ml.

Table 2 gives further evidence of the change in distribution of titres accompanying the increase in total number with antitoxin. The 36 groups of 10 specimens of cord blood have been arranged in sets of 6 groups in order of the number with detectable antitoxin irrespective of the series to which they belonged. This number increased from 297 to 443 in successive batches of 600, the number with titres between 0·04 and 1·0 unit increased from 144 to 298, those with less than 0·04 decreased from 120 to 88 and those with titres above 1·0 unit varied irregularly between 33 and 57. The figures relate to all mothers irrespective of their immunization history; in the first five groups the number who reported that they had been immunized lay between 29·3 and 31·9 %.

Throughout the whole series of 36 groups of 100 samples of cord blood gathered from London and twelve other towns including one in Eire, one in Wales, three in Scotland and the rest from the Midlands and the industrial north of England, the variation in the total number with any antitoxin was approximately equal to the variation in the number with medium titres, that is between 0·04 and 1·0 unit per ml. Among the 3600 samples the average number with any detectable antitoxin was 61·4 %, made up of 36·3 % within the middle range of titres and 25·1 % outside this range—that is either below 0·04 or above 1·0 unit/ml. If in any group of 100 the number with titres outside the middle range is constant then in any population the proportion (y) within that range will be 25 % less than the proportion (x) with any detectable antitoxin, or $y = x - 25$. The values for x and y for each of the 36 groups have been plotted in Fig. 2. The divergent lines show the limiting values of y if these were 10 % or 25 % above or below $x - 25$. Of the 36 points 23 are within 10 %, 10 between 10 and 25 % away and 3 are slightly more than 25 % from the calculated figure. Thus it would appear that the extent to which any

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Table 2. *Summary of the diphtheria antitoxin titres of* 3600 *samples of cord blood divided into six equal sets arranged in order of the number with detectable antitoxin in each group*

Groups		Immunization history*				No. of samples with antitoxin titres				No. with detectable antitoxin divided into percentage with titres		
		Percentage of mothers with histories of			No. with known histories	>0·001 <0·04	>0·04 <1·0	>1·0 —	Total >0·001 —	>0·001 <0·04	>0·04 <1·0	>1·0 —
Series *49*	Series *52, 53*	α	β	γ								
a, b	3, 11, 12, 18	31·7	4·8	2·0	460	120	144	33	297	40·4	48·5	11·1
—	2, 6, 7, 9, 15, 19	30·0	4·8	3·2	563	122	173	41	336	36·3	51·5	12·2
c, d_1	1, 10, 17, 20	31·9	3·8	4·3	546	112	199	47	358	31·3	55·6	13·1
e, f	5, 8, 14, 16	29·3	6·8	2·3	571	109	236	33	378	28·8	62·4	8·7
g, h, d_2, *i*	4, 13	29·7	5·3	3·4	417	106	256	38	400	26·5	64·0	9·5
j, k, l, m, n, o	—	23·6	7·6	3·0	237	88	298	57	443	19·9	67·3	12·9

* α, immunized, had not had diphtheria; β, not immunized, had had diphtheria; γ, immunized, had had diphtheria.

population had been exposed to subclinical infection affected equally, or almost equally, the number with any detectable antitoxin and the number within a middle range and had little or no effect upon the number with low or high titres outside this range.

UNIMMUNIZED MOTHERS

The presence of diphtheria antitoxin in the blood of mothers who have neither been artificially immunized nor had diphtheria presumably must be due to subclinical infection. The extent to which this state occurs must be dependent upon the opportunity of contact with suitable carriers. This is well illustrated by the history of natural immunity in horses described by Glenny (1925). In the early years of

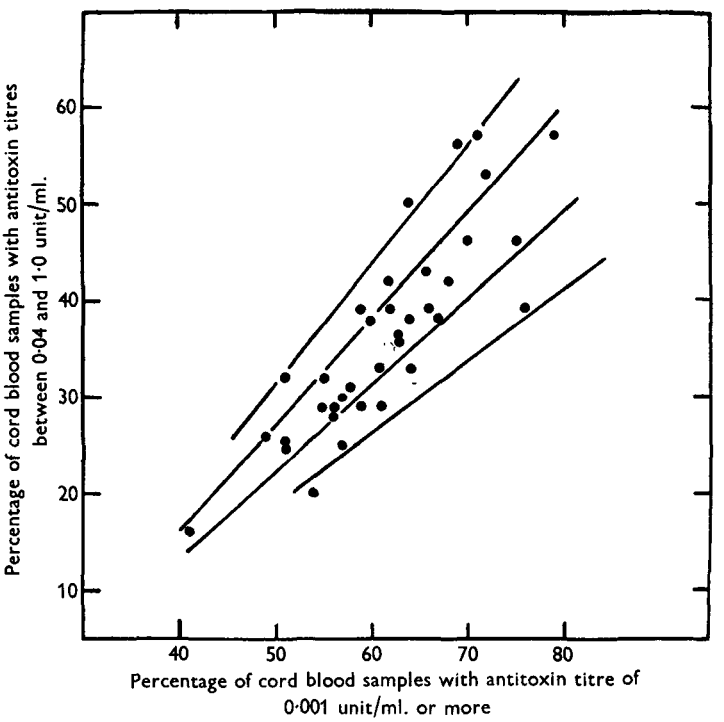

Fig. 2. Comparison between the number of cord blood samples containing between 0·04 and 1·0 unit of antitoxin per ml. and the number with 0·001 or more unit/ml. in each of 36 groups of 100. The divergent lines show the limits of 10 and 25 % accuracy of $y = x - 25$.

this century considerable numbers of horses were kept in large stables where many stablemen were employed and the majority of horses were groomed by many different men. As motors replaced horses, every omnibus replaced meant about twelve fewer horses, so the number of different men attending any horse fell and the number of horses with antitoxin in their blood declined until it became difficult for serum laboratories to find enough horses suitable for diphtheria immunization. At the end of the recent war there was a great increase in the proportion of suitable horses among those discharged from the army. It has been well established in the

past that the number of Schick negative children was much higher in crowded areas than in isolated communities.

A rough division has been made among the mothers at U.C.H. according to whether they had lived in town or in the country. A third group consisted of those with foreign surnames, most of whom had English christian names; some of the districts, including Soho, served by the hospital are very congested and contain a number of foreign colonies. Table 3 and Fig. 3 show the distribution of antitoxin titres among the unimmunized and immunized mothers in the three types of

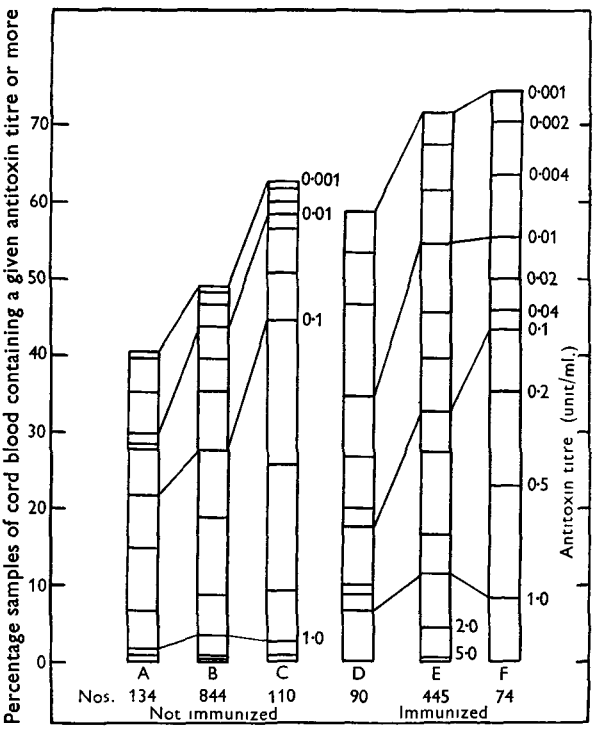

Fig. 3. Distribution of diphtheria antitoxin titres in samples of cord blood from unimmunized and immunized mothers at University College Hospital, divided according to their opportunity of acquiring subclinical infection through living in country districts (columns A and D) in towns (columns B and E) and in congested areas (columns C and F).

environment; columns A and D represent those from the country, B and E from town and C and F from congested areas. It is evident that the unimmunized mothers from the country had far less antitoxin than those from town and still less than those from congested areas. The proportion of the latter (column C) with 0·1 unit/ml. in the cord blood was approximately the same as that of the town dwellers, B, who had 0·01 unit and was greater than that of the country population, A, with 0·001 unit. The majority of the mothers with foreign surnames were born in England and much of the increase in antitoxin amoung them can be attributed to life in congested areas; columns A, B and C can therefore be regarded as the results of different degrees of opportunity for subclinical infection. It should

be noted that almost all the increase in numbers with antitoxin was provided by those with the middle range of titres as shown in Table 3. Of the 36 groups of 100 samples of cord blood dealt with in this paper the immunization histories of most of the mothers were known in all but five groups. No histories were obtained for districts *b*, *i*, *k*, *l* and *o* reported by Barr *et al.* (1951). The remaining 31 groups were divided into 6 sets of 4, 5 or 6 groups of 100 according to the numbers with less than 0·001 unit of antitoxin per ml.

Table 3. *The effect of environment upon the distribution of diphtheria antitoxin titres in cord blood*

History	District	Column in Fig. 3	Total tested	Percentage of all samples in each group: Low titres >0·001 <0·04	Medium titres >0·04 <1·0	High titres >1·0 —	Total >0·001 —
Unimmunized	Country	A	134	12·7	26·1	1·5	40·3
Unimmunized	Town	B	844	13·9	31·9	3·2	49·0
Unimmunized	Congested area	C	110	10·9	49·1	2·7	62·7
Immunized	Country	D	90	38·9	13·3	6·7	58·9
Immunized	Town	E	445	31·7	28·5	11·2	71·4
Immunized	Congested area	F	74	28·4	37·8	8·1	74·3

Columns A to F in Fig. 4 show the distribution of antitoxin titres in cord blood samples from unimmunized mothers and columns G to M from immunized: the latter will be considered in a later section. As in Fig. 1 the shaded areas show the proportions with medium titres; these increased in successive columns from 23·6 to 44·1 % while the total proportion with any antitoxin increased from 36·1 to 62·3 %. The remainder consisting of those with titres of 0·02 or less together with those of 1·0 unit or more fluctuated between 12·5 and 18·9 % (see Table 4).

Table 4. *Distribution of diphtheria antitoxin titres in cord blood samples from unimmunized mothers divided into sets of* 400, 500, 600 *arranged according to the total numbers with any antitoxin in each group of* 100

Nos. with antitoxin in each group of 100: Range	Average	No. of groups	Column in Fig. 4	No. of samples	Percentage of unimmunized mothers with: Low titres	Medium titres	High titres	Total
41–51	48·5	4	A	216	10·2	23·6	2·3	36·1
54–56	55·2	5	B	303	15·8	29·4	3·0	48·2
57–60	58·3	6	C	333	13·8	32·7	3·0	49·5
61–63	62·0	6	D	349	14·6	34·1	4·3	53·0
64–66	64·8	5	E	279	12·9	44·1	2·9	59·9
67–76	71·2	5	F	247	11·7	44·1	6·5	62·3

It has already been shown in Fig. 2 that among 36 groups not divided according to histories the proportion with medium titres could be calculated reasonably well by subtracting 25 from the proportion with any antitoxin. In each of the 31 groups

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with known histories the average number who reported that they had neither been immunized nor had had diphtheria was 52. Notwithstanding the small groupings 14 of the 31 individual groups showed the percentage with medium titres to be within 5 of that found by subtracting 15 from the total with any antitoxin. In 9 other groups the proportion with medium titres so calculated was within 15% of that actually observed. In these 23 groups which gave consistent results the proportion with titres outside the medium range averaged 15·2%. In 7 other

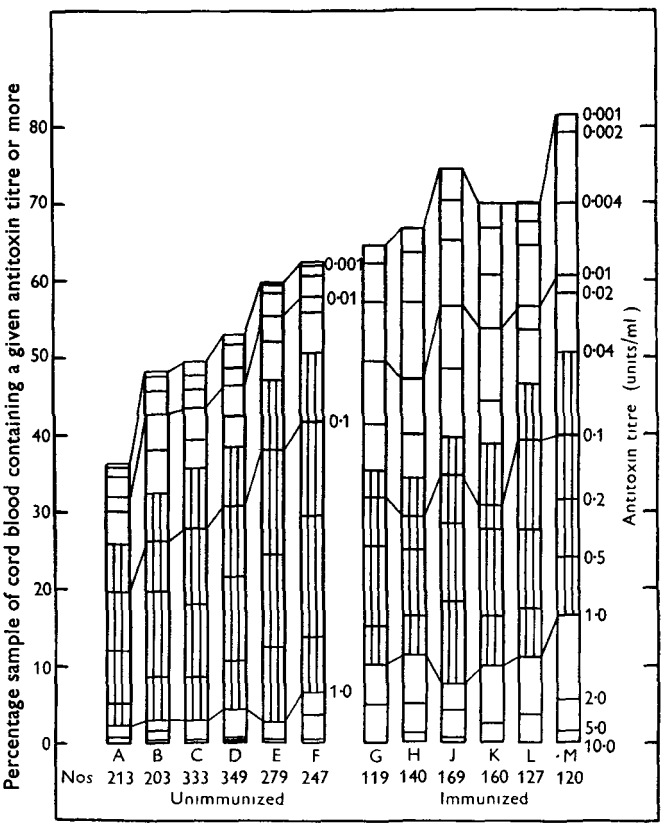

Fig. 4. Distribution of diphtheria antitoxin titres in samples of cord blood from unimmunized and immunized mothers showing the relation between the numbers with titres between 0·04 and 1·0 unit/ml. and those with titres of 0·001 unit or more per ml. See Table 4.

groups this proportion varied from 20·9 to 31·4, averaging 24·7%. There is a strong suggestion that the immunization histories of these groups were incorrect, and it is probable that more mothers had been immunized than were recorded. Of those with no history of clinical diphtheria the proportion reported as immunized was 27·9% in the abnormal groups and 34·0% in the remainder.

In the next section it is shown that artificially immunized mothers have a higher proportion of low titres (0·02 unit or less) than unimmunized mothers. The proportion of these titres among the seven abnormal groups was 19·8% compared with 11·2% among the rest of the unimmunized mothers. No explanation is

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offered for the eighth abnormal group in which 94·3 % of the samples with any antitoxin fell within the medium range.

Fig. 5 shows the distribution of antitoxin titres in samples of cord blood of unimmunized mothers divided according to the year of birth. The largest number with antitoxin were in the youngest division, those born in 1930 or later. It is probable that subclinical infections are encountered mostly during early school life and young mothers may not have lost so much of the resultant antitoxin as older ones but almost all loss in titre would have occurred within the first 2 or 3 years after infection. Mass evacuation of children during the early days of the war may have resulted in a great increase in opportunity of acquiring subclinical infection by children of all ages. The first four columns of Fig. 5 represent mothers who might have been involved in this evacuation. Of those mothers born in 1923 or later, 50·2 % had some antitoxin with a geometric mean of 0·113 unit/ml. with none of the four groups below 0·106; of those born in 1922 or earlier 49·0 % had anti-

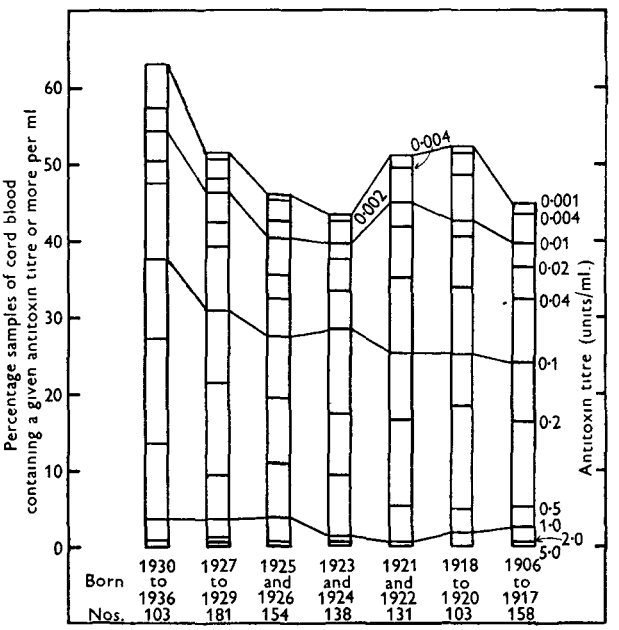

Fig. 5. Distribution of diphtheria antitoxin titres in samples of 968 unimmunized mothers at University College Hospital divided according to the year of their birth.

toxin with a mean titre of 0·083 with no group above 0·094. The results from the 103 samples of cord blood from mothers born in 1930 or later can be further divided into those from 59 mothers born in 1930 or 1931, and 44 born in 1932 or later; of the former 62·7 % had some antitoxin (47·4 % over 0·04 unit/ml.), and of the latter 63·6 % had antitoxin (47·7 % over 0·04). Of these younger mothers 14 out of 20 born in 1933 or later had some antitoxin and 13 (65·0 %) had 0·04 unit or more. Further confirmation of natural immunity in young mothers is given from results obtained since the completion of the 1953 series. Among the next thousand mothers there were 18 who were born between 1935 and 1937; of these only 5 had

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less than 0·001 unit of antitoxin per ml., the other 13 (72·2%) had 0·01 unit or more and 10 of them (55·5%) had 0·04 unit or more.

Unless the changed conditions in the lives of children during the war years increased their chances of infection it would have been expected that the mothers born between 1935 and 1937 would have had a lower immunity rate than those born earlier. The incidence of diphtheria has declined since 1941, but it was not until 1945 that the number of cases fell to one-half of the number previously

Table 5. *The number of mothers who reported that they had been immunized divided according to the year of their birth*

Date of birth	No. with known history	No. immunized	Percentage immunized
1912 or earlier	101	6	5·9
1913 to 1917	287	29	10·1
1918 to 1920	259	51	19·7
1921, 1922	273	49	17·9
1923, 1924	331	70	21·1
1925, 1926	352	104	29·6
1927	196	76	38·1
1928	187	90	48·1
1929	148	98	66·2
1930	139	91	65·5
1931	111	82	73·9
1932 or later	164	108	65·9
All	2548	854	33·5

notified annually; after 1945 the number fell rapidly each year. During the last 4 or 5 years of their school life children born round 1936 would have had fewer chances of contracting subclinical infection if this is dependent upon diphtheria incidence and carrier rates. It is possible, however, that infection still occurs, but the prevailing organism has lost virulence though still capable of producing some toxin.

There is another factor which must be taken into account. Some of the 'unimmunized' mothers had probably been rejected from immunization schemes because they were Schick negative. The test injection would in many cases have acted as a secondary stimulus and increased the antitoxin content. The number of such mothers, unintentionally boosted, must bear some relation to the number officially recognized as immunized. This number increased rapidly in successive age groups from 29·6% of those born in 1925 or 1926 to 66·2% of those born in 1929 and has remained round this figure for all the younger mothers. Although the proportion Schick tested before immunization may have been very small, the unimmunized group cannot be regarded as entirely representative; an unknown number of the younger mothers should be classified as 'not needing immunization'.

IMMUNIZED MOTHERS

The proportion of young mothers who had been artificially immunized was much greater than that of the older ones. Table 5 shows the numbers immunized divided according to the year of birth. Approximately 15% of all born in 1922 or earlier

had been immunized; this figure increased to about 20 % of those born in 1923 or 1924 and continued to rise each year until two-thirds of those born in 1929 had been immunized. After this date there was little change. Of the 561 mothers born after 1928, 379 or 67·6 % had had at least one course of immunization at some period or other. Scarcely a single person claimed to have received any boosting dose. Among the three series *49*, *52* and *53*, the proportion of all the mothers who

Table 6. *Age distribution among* 2548 *mothers in the three series*

Age	No. per 1000 mothers in 1949	1952	1953	Average
16	1	1	1	1
17	6	4	2	4
18	19	13	10	14
19	29	18	10	19
20	44	30	29	35
21	68	54	67	63
22	69	60	58	62
23	66	62	75	68
24	68	68	69	68
25	82	79	84	82
26	68	76	82	76
27	71	69	62	68
28	53	58	74	62
29	47	67	59	57
30	60	58	57	58
31	29	57	43	42
32	37	45	41	41
33	19	31	32	27
34	24	25	18	22
35	36	23	18	26
36	28	25	28	27
37	16	21	16	18
38	24	20	23	22
39	18	10	20	16
40	9	11	7	9
41 or more	9	15	15	13

had been immunized were 26·0, 34·1 and 38·1 % respectively. It is possible to forecast the numbers to be expected in the future by making use of the distribution of ages shown in Table 6. The figures so obtained would be misleading if the average age of the mothers changed or if the proportion immunized increased, or was not maintained.

Fig. 6 shows the distribution of antitoxin among cord blood samples from all mothers whose history was known. Column A gives the results from 1727 mothers who stated that they had not been immunized and had not had diphtheria; column B gives results from 837 mothers who claimed to have been immunized but had not had diphtheria, C represents 147 who had diphtheria without immunization, and D 82 who had had experience of both clinical diphtheria and of artificial immunization. Table 7 gives a summary of the proportion without antitoxin or within each of the classifications of low, medium or high titres. There were 19·9 %

more immunized than unimmunized mothers with any antitoxin in the cord blood due almost entirely to an additional 17·6% with low titres. As a result of this excess of low titres the geometric mean of the 71·5% with antitoxin was 0·072 unit/ml. compared with 0·111 unit amoung the 51·6% with antitoxin in the unimmunized group.

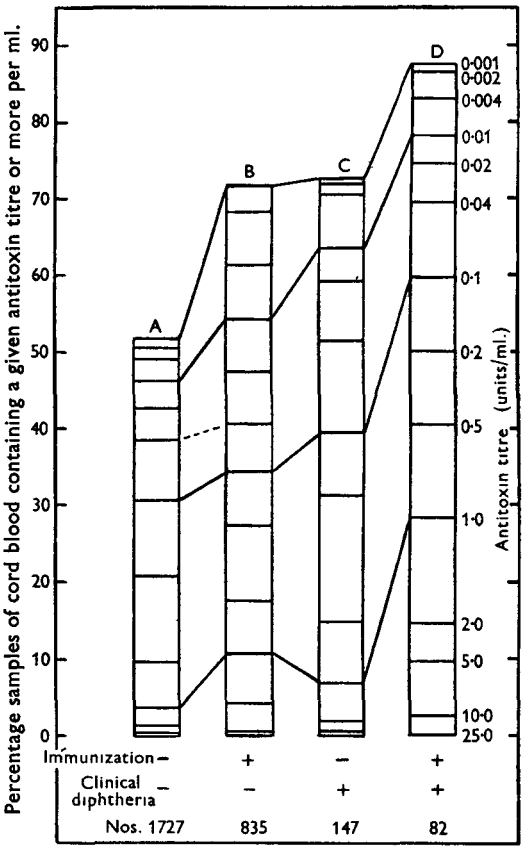

Fig. 6. Distribution of diphtheria antitoxin titres in samples of cord blood divided according to the histories of the mothers.

The figures given in Tables 5 and 6 show that columns A and B in Fig. 6 do not represent the antitoxin distribution in two populations differing only in relation to artificial immunization. The average ages were different, 28·9 years for the unimmunized and 24·5 for the immunized. In order to measure more exactly the effect of artificial immunization a comparison has been limited to mothers born in the years 1926 to 1931 inclusive, who formed a group in which the number of unimmunized and of immunized was almost equal, 118 of the former to 115 of the latter were found in the *49* series, 143 to 147 in *52* and 164 to 192 in the *53* series. The distributions of the cord blood titres are shown in Fig. 7, diagram A depicts those of the unimmunized and B the immunized. For convenience in arranging the scales the numbers with less than 0·001 unit of antitoxin have been omitted from A and B but are shown in the left-hand column of the upper composite diagrams (C).

The unimmunized can be divided into three populations: (*a*) a total of 46·3 % with no antitoxin, (*b*) a relatively small group with low titres overlapped to a slight extent by (*c*) a total of 40·9 % with medium and high titres of whom the great majority fall into the medium group with titres from 0·04 to 0·5 inclusive. In comparison, the immunized had a large proportion with low titres and two overlapping populations, one of medium and one of high titres. The effect of immunization is shown in the upper diagram of Fig. 7. If it is assumed that only a small proportion of mothers had been Schick tested, and that the distribution shown in A is typical of the unimmunized among the age group concerned, then any alteration from A to B can be regarded as due to artificial immunization. The reduction (due to

Table 7. *The effect of immunization and of clinical infection of mothers upon the distribution of diphtheria antitoxin titres in cord blood*

			Percentage of all samples in each group				Nos.		Percentage of those with antitoxin			
Immunization	Attack of diphtheria	Column in Fig. 6	No antitoxin — <0·001	Low titre >0·001 <0·04	Medium titre >0·04 <1·0	High titre >1·0 —	Tested	With antitoxin	Low titre >0·001 <0·04	Medium titre >0·04 <1·0	High titre >1·0 —	Geometric mean of titres
No	No	A	48·4	13·3	34·7	3·6	1727	892	25·8	67·1	7·1	0·111
Yes	No	B	28·5	30·9	29·8	10·8	835	597	43·2	42·7	15·1	0·072
No	Yes	C	27·2	21·1	44·9	6·8	147	107	29·0	61·7	9·3	0·110
Yes	Yes	D	12·2	18·3	41·4	28·1	82	72	20·8	47·2	31·9	0·252

immunization) in the number of those without 0·001 unit or more of antitoxin was 21·2 % of the total and practically the same as the increase, 21·0 %, in numbers with low titres among the immunized. Similarly, the reduction, 8·2 %, in the proportion of those with medium titres was almost the same as the increase, 8·5 %, of those with high titres. The effect of immunization can also be shown by assuming that if the 53·7 % with antitoxin in A had been immunized artificially they would have produced the highest titres found in B, and the additional 21·2 % in B with some antitoxin would have had none apart from artificial immunization.

Table 8 shows that the geometric mean of the antitoxin titres of all those with any antitoxin among the unimmunized was 0·129 unit/ml., while a similar proportion of the immunized had a mean titre of 0·227, and in addition there was a further group with lower titres averaging 0·0039. Similar results are given for the much larger numbers recorded in Fig. 6. The mean titre of the 51·6 % of unimmunized with any antitoxin was 0·111 unit/ml. compared with 0·214 for a similar proportion of the immunized with an additional 19·9 % with a mean titre of 0·0043.

A comparison of columns G to M with A to F in Fig. 4 and Table 4 shows that the increase in low titres after immunization was independent of the extent of natural immunity in the population. Although the proportion with antitoxin among the unimmunized increased from 36·1 to 62·3 % in columns A to F and the medium titre similarly increased from 23·6 to 44·1 %, the low titres among the immunized fluctuated between 23·6 % in column L and 33·6 % in H and 34·9 % in J. The proportions in the other three columns were between 29·4 and 31·2 %.

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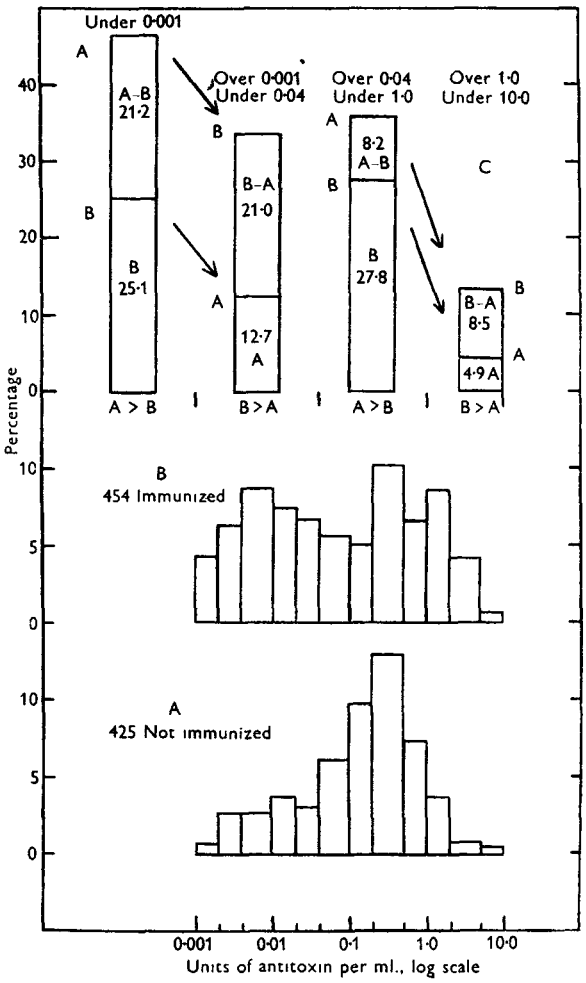

Fig. 7. The relative distribution of diphtheria antitoxin titres in samples of cord blood from mothers born in 1926 to 1931 inclusive of whom 425 (A) had not and 454 (B) had been immunized.

Table 8. *Comparison between the geometric means of the diphtheria antitoxin titres in cord blood samples from unimmunized and immunized mothers*

Calculation from	No. of unimmunized mothers	Percentage of total	No. of immunized mothers	Mean antitoxin titres: Unimmunized	Mean antitoxin titres: Immunized
Fig. 6	891 with antitoxin	51·6	431 with highest titre	0·111	0·214
	344 without antitoxin	19·9	166 with lowest titre	<0·001	0·0043
	492 without antitoxin	28·5	238 without antitoxin	<0·001	<0·001
	1727		835		
Fig. 7	228 with antitoxin	53·7	244 with highest titre	0·129	0·227
	90 without antitoxin	21·1	96 with lowest titre	<0·001	0·0039
	107 without antitoxin	25·1	114 without antitoxin	<0·001	<0·001
	425		454		

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All mothers with known histories were included in Fig. 6 and Table 7, and different subdivisions have been considered in Figs. 1, 3, 4 and 7. From these it is possible to obtain additional information upon the effect of immunization by subtracting the proportions in the various ranges of titres among the unimmunized from those among the immunized in the same population. This has been done in Table 9. Among the thirteen subdivisions recorded there were from 10·2 to 28·6% more of the immunized than of the unimmunized with any antitoxin in the cord blood. In all instances the proportions with low titres and also those with high titres were greater among the immunized; the excess with low titres varied from

Table 9. *The effect of artificial immunization of mothers upon the distribution of diphtheria antitoxin in cord blood*

Calculations made from		No. of mothers		Difference between the percentage samples of cord blood from unimmunized and immunized mothers with antitoxin titres			
Figure	Columns	Un-immunized	Immunized	All over 0·001	Low	Medium	High
1	A to C	168	62	+23·7	+12·3	+ 5·2	+ 6·1
	B to D	156	56	+23·4	+20·0	− 6·7	+10·1
3	A to D	134	90	+18·6	+26·2	−12·8	+ 5·2
	B to E	844	445	+22·4	+17·8	− 3·4	+ 8·0
	C to F	110	74	+11·6	+17·5	−11·3	+ 5·4
4	A to G	216	119	+28·6	+19·2	+ 1·6	+ 7·8
	B to H	303	140	+19·7	+17·7	− 6·5	+ 8·5
	C to J	333	169	+25·0	+21·1	− 0·8	+ 4·7
	D to K	349	160	+17·0	+16·6	− 5·3	+ 5·7
	E to L	379	127	+10·2	+10·7	− 8·7	+ 8·2
	F to M	247	120	+19·3	+19·1	−10·0	+10·2
6	A to B	1727	835	+19·9	+17·6	− 4·8	+ 7·2
7	A to B	425	454	+21·2	+21·0	− 8·2	+ 8·5

10·7 to 26·2% and the excess with high titres varied from 4·7 to 10·2%. In all but two subdivisions the proportion with medium titres was lower among the immunized. The two exceptions were from populations with low natural immunity rates. The proportion of low titres among the injected mothers was from 1·8 to 2·9 (average 2·3) times that among the uninjected; similar figures from high titres were from 2·3 to 4·3 (average 2·9). Low and high titres form very definite groups. There was a higher proportion among the immunized of each of the five individual values comprising the low titres in 39 out of 40 comparisons made from the six divisions shown in Fig. 4, the complete population in Fig. 6 and the equal age groups in Fig. 7. Similarly, there were among the immunized 15 out of 16 higher proportions with titres of 1·0, 2·0 and 5·0 units/ml. The medium titres were not so well defined; values of 0·5 unit appeared to belong to a neutral zone between medium and high titres.

Many of the mothers were able to say when they had received injections of prophylactic, but the time of subclinical infection can only be a matter of con-

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jecture. A number of the mothers were indefinite in their replies, and times of immunization were recorded vaguely as 'when a baby', 'as a child' or 'at school'. Some immunized as babies may have responded badly owing to residual passive immunity at the time of injection so none of this group was included in the analysis; 'at school' may have been any age from 5 to 18, and these results have also been ignored. The majority of those recorded as immunized 'as a child' were probably injected between the ages of 1 and 5. Fig. 8 shows that there is a tendency for

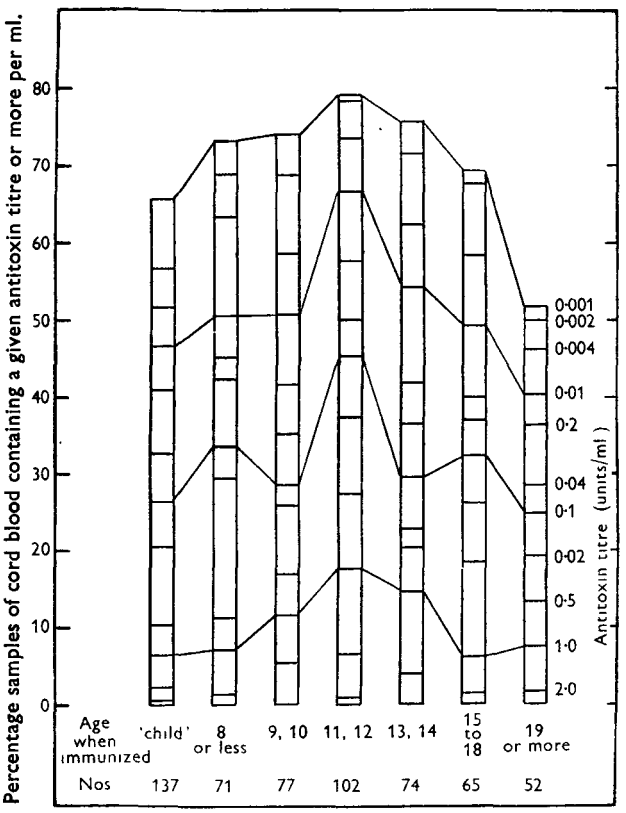

Fig. 8. Distribution of diphtheria antitoxin titres in samples of cord blood from immunized mothers divided according to the age of immunization.

more women to have detectable antitoxin and particularly to have high titres in their cord blood if they had received their course of immunization around the age of 11 or 12. Of 102 mothers immunized when 11 or 12 years old, 18 had one or more units of antitoxin per ml. compared with 9 of 137 injected 'when a child' ($\chi^2 = 6{\cdot}080$, $P = 0{\cdot}013$). If the next group is also included there were 14 of 208 immunized at 8 years old or younger with high titres; this again was significantly less than the number among the 11- or 12-year group ($\chi^2 = 7{\cdot}667$, $P = < 0{\cdot}01$). The numbers in the 8 years or younger group, excluding those injected 'when a child', with titres of one or more units were too small for a proper comparison to be made; if the numbers with titres of 0·5 are included there were 8 of 71 in this young group,

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this proportion being significantly less than the proportion, 28 of 102, within the same range of titres among the 11 or 12 groups ($\chi^2 = 5{\cdot}76$, $P = 0{\cdot}017$).

Of 52 mothers immunized when 19 or over, 25 had no detectable antitoxin compared with 21 of 102 in the 11 or 12 group ($\chi^2 = 11{\cdot}24$, $P = < 0{\cdot}001$) or with 59 of 253 in the three groups from 9 to 14 years ($\chi^2 = 12{\cdot}03$, $P = < 0{\cdot}001$). The proportion of mothers with high titres, one unit or more, in the 11 or 12 group was definitely greater than 8 of 117 found in the two last groups, 15 years or more ($\chi^2 = 5{\cdot}11$, $P = 0{\cdot}024$).

RECOVERED CASES

It is difficult to decide whether clinical diphtheria has any immunizing effect. A comparison between the first three columns in Fig. 6 shows that the proportion of mothers who maintained some antitoxin in their blood was practically the same whether they had been artificially immunized (B) or had had diphtheria (C) and in both groups the proportion was considerably higher than that among those who had relied upon subclinical infection only (A). Although there was little difference between the proportions with any antitoxin in groups B and C the distribution of titres was very different. Fig. 9 shows the distribution in terms of the totals with any antitoxin in the four groups. The columns representing the results from mothers who had had diphtheria (C) and those who had been immunized (B) have been transposed and the areas representing medium values have been shaded to show more easily the similarity of effect of subclinical (A) and of clinical (C) diphtheria. Many unimmunized may have escaped subclinical infection; only 51·6 % had any antitoxin in their cord blood. Depending upon the accuracy of the histories given, all or almost all in the other group had diphtheria with the result that 72·8 % had some antitoxin. The close similarity of the distributions of the titres in the two groups shows either (i) that the immunizing effect of subclinical and clinical diphtheria were almost identical, or (ii) that clinical diphtheria had little or no immunizing effect, but recovered cases were almost invariably subjected to subclinical infections owing to the many temporary carriers in their immediate surroundings. For every overt case there would probably have been many latent infections. This increased number of contacts could account for the increase of just over 20 % in the samples of cord blood containing any antitoxin; a similar increase, 21·2 %, is shown in Fig. 1 between 44·1 % among unimmunized mothers relying upon subclinical infection in districts with the least amount of natural immunization (column A) and 64·1 % from districts with a high natural immunity rate. The distribution of titres is however quite different. In Fig. 1 the increase in the total number with any antitoxin was accompanied by a similar increase (20·4 %) in the medium titres and a fall of 4·5 % in low titres, while the recovered cases in Fig. 6 showed an increase over the naturally immune of only 10·2 % among the medium titres and a considerable increase (7·8 %) among the low titres. This strongly suggests that the differences between the results from recovered cases and from unimmunized mothers were not due to an increased number of naturally immune among the former but to some immunizing effect of subclinical diphtheria.

The high titres found in blood from recovered cases subsequently immunized

shows that clinical diphtheria considerably enhanced their response to immunization. Column D, in Fig. 6, shows that about 90 % of such mothers had antitoxin in the cord blood and 50 % had titres of 0·2 unit or more. Fig. 9 shows that the proportion of titres above one unit among those with any antitoxin was over 30 % compared with 15 % among the artificially immunized and less than 10 % among

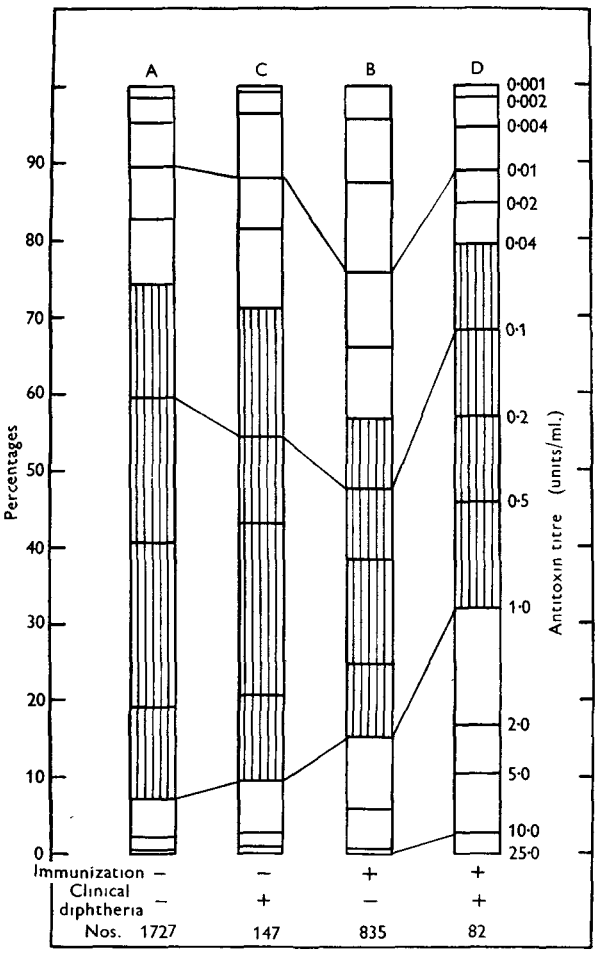

Fig. 9. Distribution of diphtheria antitoxin titres in samples of cord blood from those mothers shown in Fig. 6 who maintained some antitoxin in their circulation.

the other two groups. Fig. 8 shows that the proportion of titres over one unit was highest (22·2 %) among mothers immunized at the age of 11 or 12; the proportion was high for all immunized between the ages of 9 and 14, for in these groups 38 of 194 with any antitoxin (19·6 %) had over one unit compared with 22 of 214 (10·3 %) for those immunized earlier or later. These results suggest that clinical diphtheria establishes stronger potential immunity than subclinical infections do even under optimal conditions.

Relatively few mothers gave exact ages or dates for both attack and immuniza-

tion, seventeen reported that the two events occurred in the same year and several of them referred to immunization 'at the same time' or 'soon after' the attack. Among these 17 there were 5 with no antitoxin in their cord blood; this might be expected if immunization had started before the elimination of passive immunity conferred by antitoxin treatment. Other cases of failure due to the same cause may have occurred among those who gave no dates; it thus appears probable that immunization carried out at a reasonable interval from recovery produced lasting immunity in all cases.

DISCUSSION

Although the concentration of antitoxins in cord blood is usually slightly greater than that in the mother's circulation, it can be used, nevertheless, as a measure of her immunity. This immunity may have been produced by subclinical or clinical infection or by artificial immunization many years earlier. At the time the tests were made the antitoxin content must in most cases have reached an almost constant level maintained by 'continued production', the extent of which depended upon the degree of response to earlier stimulation and not upon the time elapsing since that stimulation. Barr & Glenny (1952) stated that the fall in antitoxin titre after the peak of response 'continues until all the antitoxin is lost that is in excess of the amount that can be maintained by the new steady rate of production'. Barr & Glenny (1949) found that the level subsequently maintained by hyperimmunized horses was usually 'about a tenth of the value at the end of immunization'. Barr & Cunliffe (1954) found that the diphtheria antitoxin titres of nurses about 20 months after a boosting dose were also about one-tenth of the titres shortly after the injection. Without continued production, actively produced antitoxin would be lost constantly at the same rate as homologous passive antitoxin in babies, which according to Barr *et al.* (1949) is 'a steady logarithmic rate of loss of 13·9 % per week'. Barr, Glenny & Howie (1953), referring to this statement, said that 'if the growth of the babies is taken into account the rate of loss of total antitoxin is 11·8 % per week'. At this rate 90 % of the antitoxin would be lost in 18 weeks and about 99 % in 36 weeks. The antitoxin content of cord blood from mothers some years after any specific stimulation must therefore be a measure of the rate of 'continued production' which can be regarded as the most important result of any form of prophylactic treatment.

The effect of subclinical infection is mainly to produce that degree of continued production of antitoxin that would maintain antitoxin titres between 0·04 and 1·0 unit/ml. The proportion of cord blood samples with titres within this medium range depends upon the opportunities that any given population had of becoming infected. Once such natural immunization had occurred the antitoxin produced was sufficient to protect against subsequent infection, and the majority of titres would not rise above this level. If titres below 0·04 had been produced by poor responders or by encounters with mild infections the numbers with low titres in any population would have been related to the number of cord blood samples with medium titres. The number of low titres was found to be quite independent of the number with medium titres and was almost constant for every population tested.

It is possible that among the denser populations the immunizing power of the infecting organism was greater than that met in country districts, and this would have reduced the proportions of relative failures among the poor responders. If this were so there would have been a larger proportion of high titres. The numbers with high titres were very small, but when sufficiently large groups were taken the numbers were found to be approximately one-tenth of those with medium titres suggesting that they were produced by particularly good responders.

Although the immunity induced by subclinical infection was mostly sufficient to protect against clinical diphtheria at any subsequent time, the resulting antitoxin was not always sufficiently high to swamp out the dose of prophylactic used in artificial immunization. Some neutralization must have taken place if the first injection had been given soon after natural stimulation of all but the very poor responders or even when given long afterwards to very good responders. The success of artificial immunization between the ages of 9 and 14 and particularly between 11 and 12 was in all probability due to the time elapsing since the majority had been naturally immunized. The optimum time would have been after antitoxin titres had fallen and before potential immunity had waned. It seems probable that most of the natural stimulation had occurred by the time the children were 7 or 8 years old.

The aim of any scheme of immunization should be to produce life-long immunity. The methods practised in the past do not appear to have achieved this. The number of women with little or no antitoxin in the cord blood was almost as high among the immunized as among the rest of the population, unless immunization had been preceded by clinical diphtheria or by considerable subclinical infection. The rapid reduction of these aids to immunization emphasize the need for at least one reinforcing dose to be regarded as forming part of the main course of immunization. Although there was no difference between the immunizing effects of clinical and subclinical diphtheria so far as antitoxin production was concerned, the many titres of one or more units per ml. among recovered cases later artificially immunized suggests that greater potential immunity is established by a clinical attack. Any marked difference in the size or the duration of antigenic stimulus in either form of infection would have resulted in a difference in the amounts of antitoxin produced; the difference in potential immunity resulting suggests a difference in the quality of the antigen produced. The great reduction in incidence of diphtheria in the presence of many immune hosts during recent years may have changed the metabolism of the invading organisms so that some modification of toxin is produced, or some auxilliary component is absent. It is possible that carriers of organisms of this form of modified virulence may avoid detection.

SUMMARY

1. The diphtheria antitoxin titres of several thousand samples of cord blood have been analysed with relation to the histories of the mothers.

2. Among mothers without histories of diphtheria or of immunization those born in 1933 or later showed a higher degree of natural immunity.

3. The distribution of antitoxin titres among mothers who had suffered from diphtheria was similar to the average distribution among the unimmunized.

4. The differences in proportions with any antitoxin in different groups of unimmunized mothers were almost entirely made up of those with titres above 0·04 and below 1·0 unit per ml.

5. Among the artificially immunized mothers there were many more with low titres, i.e. below 0·04, and a few more with high titres, i.e. over 1·0, than among the unimmunized.

6. Mothers immunized between the ages of 9 and 14 with the peak at 11 or 12 years showed the highest immunity rate.

7. The greatest number of high titres occurred among recovered cases subsequently immunized.

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