GROUP D STREPTOCOCCI IN THE FAECES OF HEALTHY INFANTS AND OF INFANTS WITH NEONATAL DIARRHOEA

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(With 3 Figures in the Text)

CONTENTS

I. INTRODUCTION

Although several different organisms have been suspected as the cause of neonatal diarrhoea, the aetiology of this syndrome still remains obscure. Among others who have suggested that streptococci may be concerned Cron, Shutter & Lahmann (1940) reported a typical epidemic of neonatal diarrhoea with a case mortality of 82% in which a haemolytic streptococcus was isolated from the faeces and the throats of all infants examined, but unfortunately the organism was not further identified. Wheeler & Foley (1945) isolated group D streptococci belonging to one serological type from an outbreak of diarrhoea among infants in the nurseries of a maternity hospital.

Gale (1940) has shown that strains of *Streptococcus faecalis* produce an enzyme, tyrosine decarboxylase, which specifically decarboxylates tyrosine to tyramine. The appearance in the faeces of affected infants in two outbreaks of neonatal diarrhoea of large numbers of β-haemolytic group D streptococci with an unusually high tyrosine decarboxylase activity, led Gale (1944) to suggest that such organisms might play a part in the aetiology of neonatal diarrhoea.

The introduction of *Str. faecalis* into the food of infant rats was followed by a fatal diarrhoea, the severity of which depended on the tyrosine decarboxylase activity of the organism and the addition of tyrosine to the diet (Gale, 1941). Diarrhoea could also be produced in young rats by feeding with tyramine, but

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older rats were not susceptible to the action of the organisms or the tyramine because the adult animal is protected against toxic amines by the action of amine oxidase. Epps (1945) found that the activity of this enzyme was significantly low in the liver, duodenum and kidney of human infants for the first 2 weeks of life, but that the activity developed to the adult level by the end of 3 months.

Serological typing by Shattock (1946) of Gale's strains of group D streptococci from two epidemics indicated that all strains isolated from one outbreak belonged to one serological type, and all strains from the other belonged to another type.

The purpose of the work reported here was to investigate the group D streptococcal flora of the faeces of healthy infants and of cases of neonatal diarrhoea, with special reference to the incidence of these organisms, to classify by serological typing the strains isolated and to determine their tyrosine decarboxylase activities. Ten outbreaks diagnosed by clinicians and paediatricians as neonatal diarrhoea, which occurred in widely distributed parts of the country over a period of 18 months from December 1946 to June 1948, provided the material for this work. Faeces from infants in maternity units where no such outbreaks had been diagnosed were also examined for comparison. The investigation also included an examination of the general aerobic flora of the faeces of a number of healthy breast- and bottle-fed infants.

II. MATERIALS AND METHODS

Streptococci were usually isolated from samples of infants' stools or, for convenience in outbreaks, from rectal swabs. In a few of the outbreaks, organisms were also isolated from other sources such as the throats of sick infants, faeces of mothers of infants, milk powders, or at post-mortem from the heart blood, intestinal or stomach contents.

(*a*) *Isolation of organisms from infants' faeces.* Samples of stools, collected aseptically, were suspended in $\frac{1}{4}$ strength Ringer's solution to a standard opacity of Brown's tube no. 8 (Burroughs Wellcome Ltd.). This suspension, and a 1 in 10 dilution of it, were streaked with a loop (4 mm. diameter) on to yeast dextrose agar. The undiluted suspension was also plated on yeast dextrose agar to which had been added 0·05% w/v thallous acetate to inhibit the growth of Gram-negative bacteria. Rectal swabs were streaked directly on to yeast dextrose agar with and without thallous acetate. After aerobic incubation for 24 hr. at 37° C. three or six typical streptococcal colonies were usually picked from each plate for further identification and examination.

(*b*) *Estimations of numbers and percentages of streptococci and other aerobic organisms in the faeces.* The colonies present after plating stools and swabs as described were counted using the most convenient dilution plate. Identification was by colony appearance confirmed microscopically when necessary. Total numbers of all organisms isolated on a plate were estimated for the stool specimens, but only the proportionate numbers of the different organisms were recorded for the rectal swabs. Proportionate numbers were approximately determined by marking a square inch on the part of the plate with the most suitable number of colonies on it and counting the colonies of different species in this area.

Preliminary experiments showed that a delay of 24 or 48 hr. in transit through the post did not affect the proportion of streptococci present in the samples.

(*c*) *Physiological and serological tests.* Methods used for these are described in a previous paper (Sharpe & Shattock, 1952).

III. SOURCES OF MATERIAL

Healthy infants

Seventy-eight specimens of faeces from sixty-five healthy infants were examined. Twenty-four specimens came from a Reading hospital, two from a private house in Reading, eighteen from a maternity unit in Cambridge and thirty-four from a London maternity hospital. Seventy-two specimens were from infants aged 5–14 days and six from infants from 14 days to 1 month. From seven infants a second specimen was taken 5 or more days later. Sixty-three specimens were from breast-fed infants, six from breast plus supplementary feeding, seven from bottle-fed infants and two from infants whose mode of feeding was unknown. An infant receiving expressed breast milk was classified with those who were artificially fed.

Infants involved in outbreaks of neonatal diarrhoea

Faeca lmaterial was obtained from infants involved in ten outbreaks of neonatal diarrhoea. Descriptions of outbreaks A, B, C, D, E and L are based on joint reports of clinicians, epidemiologists and paediatricians who investigated them. Few details are known of the cases included under F, G, H and K, but they were diagnosed as typical neonatal diarrhoea.

In all outbreaks stools of affected and contact infants were examined for shigella and salmonella organisms with consistently negative results.

Outbreak A. From November 1946 to January 1947 inclusive, loose green stools were noted in fifty-seven of sixty-eight full-term infants in a municipal maternity home. No infants were seriously ill and there were no deaths. Most of the infants were breast fed and few complementary feeds were given. All infants had received glucose water in the first few days of life.

Rectal swabs were taken on five different occasions, and in all twenty-five infants were swabbed, of whom fourteen were suffering from diarrhoea, three developed diarrhoea shortly (1–2 days) after the swabs were taken and eight were not affected although they were contacts. Six of the affected infants who were transferred to an isolation hospital were swabbed again there, one week after the removal.

Outbreak B. Starting in August 1946, a few cases of mild diarrhoea occurred sporadically among infants in the maternity department of an infirmary. Three infants died from diarrhoea in November, and three more in December. Two babies developed loose stools but were not really ill. No details of the type of feeding were available.

One of the fatal cases was swabbed at post-mortem, and the two infants who developed mild diarrhoea, twenty healthy contact infants and fifteen of the mothers were also swabbed.

Outbreak C. An outbreak of diarrhoea affecting fifteen out of sixteen infants in the maternity ward of an E.M.S. hospital, occurred in January 1947. Fourteen of

the infants were not seriously ill as there was little loss of weight, although the stools were green and relaxed. One infant was more seriously ill, but recovered.

Most of the infants were breast fed, but three of the sixteen infants were artificially fed and two received supplementary feeds. Glucose water was given to all infants in the first few days of life. Two sets of rectal swabs were taken from affected infants.

Outbreak D. An outbreak of epidemic neonatal diarrhoea occurred in February and March 1947 in the maternity unit of a hospital. Twenty infants became ill and fourteen died. The outstanding symptoms of the disease were diarrhoea with watery green or yellow stools, preceded in some cases by loss of weight with severe dehydration. All infants had received glucose feeds during the first few days of life. One infant was breast fed, fifteen were bottle fed and four were partially breast fed.

Rectal swabs and, in some cases, swabs from the upper respiratory tract were taken from five sick infants, and from eleven contacts. Post-mortem material was also examined from three fatal cases. All these specimens were plated at another laboratory, and strains of those streptococci which were able to grow on 8% bile blood agar were sent here, together with similarly isolated streptococci from opened and unopened dried milk powders which were being used in the maternity unit.

Further details of this outbreak have been given by Taylor, Powell & Wright (1949), who investigated it in detail and isolated the same serological α-type of *Bact. coli* from all cases examined.

Outbreak E. During October and November 1947, an outbreak of neonatal diarrhoea occurred in which thirty-five infants became ill with frequent motions and some vomiting, and eight died.

Fifteen of the infants affected were breast fed, sixteen bottle fed and four were partly breast fed.

Eleven strains of *Str. faecalis* obtained from six sick infants, three infants with relaxed stools, and two healthy contacts were isolated at another laboratory and sent here for examination.

Outbreak F. A mild outbreak occurred during January 1947, with green stools but little general upset. Swabs were taken from ten affected infants.

Outbreak G. Specimens from an outbreak occurring in January 1947 were plated at another laboratory, and streptococci isolated from three cases were sent to us here. No details of the severity of the outbreak are known.

Outbreak H. A serious outbreak with several deaths occurred during December 1946. Unfortunately, few details and little material were made available. Post-mortem samples from one fatal case and stools from three sick infants were examined.

Outbreak K. In June 1948 cultures of streptococci isolated from post-mortem material of two infants were received. Both cases had been diagnosed as probable neonatal diarrhoea.

Outbreak L. Sporadic cases of enteritis had occurred among infants in the maternity department of a hospital from September until December 1946, when an explosive outbreak started among the premature infants and spread rapidly. Twenty-five infants became ill and six died.

The infants had been fed on pooled scalded expressed breast milk, samples of which were found to be sterile.

Faecal specimens or rectal swabs were taken from seventeen cases, four of which died, two of them within a few hours of the samples being taken. Faeces of three healthy contacts, all of whom later became ill, and of eight of the 'isolated' contacts in another ward, five of whom later developed mild diarrhoea, were also examined. Although these latter infants were supposedly isolated, several possible routes of cross-infection were present.

Cases of mild diarrhoea had been occurring among the mothers since September. Some of the infants of these mothers were affected, others were healthy, whilst some healthy mothers had affected infants.

The explosive nature of the outbreak among premature infants, after the preliminary cases, together with the clinical picture, were very suggestive of neonatal diarrhoea, and, although complicated by the history of mild diarrhoea in the mothers, it was considered to be a true outbreak of neonatal diarrhoea.

IV. AEROBIC FAECAL FLORA OF HEALTHY INFANTS WHO WERE NOT CONTACTS OF NEONATAL DIARRHOEA

The aerobic flora of the faeces observed on yeast dextrose agar was found to comprise coliforms, streptococci, micrococci (this term includes staphylococci, micrococci, sarcina and rhodococci), yeasts and, very occasionally, a spore-forming organism (Table 1). This varied flora was present within 5 days of birth, and the flora of specimens from 5-day-old infants was found to be similar to that of infants aged 7–14 days and to that of infants between 14 days and 1 month old.

Table 1. *The aerobic faecal flora of healthy breast- and bottle-fed infants*

Organisms isolated	No. of specimens: Breast feeding	Bottle, breast and supplementary feeding	Type of feeding unknown	Total no. of specimens
None	4	—	—	4
Streptococci	49 (77·8 %)*	12 (92·3 %)	2	63 (80·8 %)
Coliforms	54 (85·7 %)	13 (100·0 %)	2	69 (88·5 %)
Micrococci	37 (58·7 %)	4 (30·8 %)	—	41 (52·6 %)
Yeasts	7	1	—	8
Total no. of specimens	63	13	2	78

* Percentage figures in brackets represent the number of specimens from which a particular organism was isolated, expressed as a percentage of the total number of specimens examined.

Table 1 shows the incidence of the organisms isolated. Although the number of specimens from breast-fed infants was much higher than from artificially and supplementary-fed infants, a comparison is given of the two groups.

Single species were isolated alone from only three specimens, and consisted of

coliforms, streptococci and micrococci respectively. Streptococci were found in 80·0 %, coliforms in 88·5 % and micrococci in 52·6 % of all samples examined. Yeasts were relatively infrequent. Streptococci and coliforms occurred more frequently in specimens from artificially fed infants, whilst micrococci were found more frequently in those from breast-fed infants; Olsen (1949) made similar observations, whilst McFarlan, Crone & Tee (1949), using blood agar and incubating anaerobically, isolated streptococci from 86 % and coliforms from 61 % of the stools of breast-fed infants, from 82 and 86 % respectively of breast- and complementary-fed infants and from 92 and 100 % respectively of artificially fed infants.

The aerobic conditions and media employed were unsuitable for the isolation of lactobacilli, particularly *Lactobacillus bifidus*, which has been reported as predominating in the intestinal flora of breast-fed infants.

The number of streptococci isolated and the streptococcal percentage of the total flora varied greatly from specimen to specimen, the latter from less than 1 to 100 %. The most commonly found ranges were 100–1000 streptococcal colonies per plate and 1–25 % streptococci. Scatter diagrams showed that there was no correlation between the numbers of streptococci and the numbers of other organisms isolated from the samples.

Incidence of group D streptococci. HCl extracts of 191 cultures of streptococci isolated from sixty-three specimens of faeces (fifty-two infants) were tested serologically by the precipitin test against specific group D serum. Strains not reacting as group D were tested against group N serum. 170 of the strains were classified serologically as group D (89·0 %), and three as group N. Of the eighteen which could not be grouped serologically, nine were classified as *Streptococcus salivarius* by physiological tests. Fifty-nine of the sixty-three specimens contained group D streptococci (93·7 %).

Thus streptococci are frequently found in the faeces of healthy infants and group D is by far the most common group of streptococcus present.

V. COMPARISON OF GROUP D STREPTOCOCCI ISOLATED FROM HEALTHY INFANTS AND FROM INFANTS INVOLVED IN OUTBREAKS OF NEONATAL DIARRHOEA

For convenience, infants associated with outbreaks of diarrhoea were divided into two groups:

(*i*) sick infants, convalescents and those swabbed 1–2 days before the onset of illness.

(*ii*) healthy contacts who did not develop the illness.

(*a*) *Percentage of group D streptococci in the total aerobic flora of infants' faeces*

As it was found in the healthy infants that 93·7 % of the specimens containing streptococci contained group D streptococci, and as 89·0 % of all streptococci isolated proved to be group D, it was considered that a fair estimate of the numbers of group D streptococci present would be obtained by taking the total streptococcal

counts as the group D streptococcal counts, although this method disregarded the occasional colony of streptococci other than group D found in a few specimens.

Whenever possible the percentage of group D streptococci in the faeces was estimated, but in a number of the outbreaks (D, E, G and K) the cultures of streptococci were isolated in other laboratories and this information was not obtained.

Table 2. *Percentage of streptococci in the faeces of healthy, sick and healthy contact infants*

% streptococci of the total aerobic flora	Nos. of specimens of infants' faeces containing streptococci		
	Healthy infants	Sick infants	Healthy contact infants
<1	6	—	—
1–10	14	10	3
11–20	8	10	3
21–30	2	3	—
31–40	5	5	—
41–50	2	6	—
51–60	2	2	1
61–70	2	5	—
71–80	1	5	1
81–90	3	12	2
91–100	4	28	5
Total no. of specimens	49	86	15

Table 2 shows the percentages of streptococcal colonies in all colonies of the total flora of forty-nine specimens of faecal material from healthy infants, eighty-six specimens from sick infants from outbreaks A, B, C, F, H and L and fifteen specimens from healthy contacts from outbreaks A, B and L. The percentage of group D streptococci present in the faeces of sick infants is markedly greater than in healthy infants, the modes being 90–100 and 1–10 % respectively. The data are too limited to draw conclusions about the healthy contacts.

(*b*) *Species and varieties of group D streptococci isolated*

The species and varieties of group D streptococci isolated were classified by their physiological characteristics as in the scheme used by Shattock (1949). The distribution of these species amongst 172 infants is given in Table 3. *Str. faecalis* was isolated most frequently from all three groups of infants, the incidence being higher in healthy and sick infants than in healthy contacts, in whom *Str. liquefaciens* and *zymogenes* were more prevalent. *Str. durans* was seldom isolated from any group of infants.

(*c*) *Serological types of group D streptococci present in infants' faeces*

A description of the serological typing of group D streptococci has been given elsewhere (Sharpe & Shattock, 1952). Strains of group D streptococci isolated from 186 sources were typed serologically by slide agglutination tests, using twenty-four

type-specific absorbed sera prepared for this purpose. Table 4 shows the distribution of these serological types.

Table 3. *Numbers of healthy, sick and healthy contact infants from whom the different species and varieties of group D streptococci were isolated*

	No. of infants in each class from whom group D streptococci were isolated					
	52 Healthy infants		84 Sick infants		36 Healthy contact infants	
Species of group D streptococci	No. of infants from whom species were isolated	% infants from whom species were isolated	No. of infants from whom species were isolated	% infants from whom species were isolated	No. of infants from whom species were isolated	% infants from whom species were isolated
Str. faecalis	41	78·8	65	77·4	20	55·6
Str. faecalis var. *zymogenes*	2	3·8	11	13·0	7	19·4
Str. faecalis var. *liquefaciens*	13	25·0	15	17·9	12	33·3
Str. bovis	8	15·4	18	21·4	8	22·2
Str. durans	1	1·9	3	3·6	0	—
Unclassified	12	23·0	20	23·8	3	8·3

Total number of infants from whom faecal group D streptococci were isolated and identified = 172.

Healthy infants

The serological types of sixty-six of eighty strains of group D streptococci isolated from forty-six healthy infants were identified; the other fourteen strains did not agglutinate with the type sera available. A diversity of types, fourteen in all, was encountered. Certain types appeared to be established in the different nurseries and continued to occur in specimens taken from different infants at different times. In the Cambridge series type 8 was found in ten and type 14 in seven out of fourteen infants, four infants having both types. These two types were isolated at intervals over the sampling period of 7 weeks. Thirteen of these infants were breast fed, the other received supplementary feeding.

In the specimens from a London hospital two types, 9 and 5, occurred most frequently, being found in seven and six respectively out of eighteen infants. Both types were isolated at intervals during the 4 weeks that specimens from this source were examined. Seventeen of these infants were breast fed, the other received supplementary feeding.

Of the organisms in the Reading specimens, taken over a period of 12 weeks, types 1 and 16 were isolated from four and five infants respectively out of fourteen; a greater variety of types was noted in this series. Seven of the infants were breast fed, three had supplementary breast feeding and four were bottle fed.

It seems possible, particularly in the Cambridge maternity unit, that there was

Table 4. *Distribution of serological types of group D streptococci isolated from the faeces of healthy, sick, healthy contact infants and from other sources, from thirteen different maternity units*

	No. of infants from whom serological types were isolated																			No. of sources other than infants' faeces from which serological types were isolated					Total no. of infants and other sources from which each type was isolated
	No. of healthy infants				No. of sick infants Outbreak										No. of healthy contacts Outbreak						D				
Serological type	Cambridge	Reading	London	Total	A	B	C	D	E	F	G	H	L	Total	A	B	D†	E	L	Total	B Mothers	Throats, Milk powders, P.M.* material	H P.M.* material	K	Total	
	14	14	18	46	15	3	13	5	9	10	3	3	23	84	3	19	11	2	1	36	5	12	1	2	20	
1	3	4	3	10	13	.	4	1	.	4	.	1	12	35	3	3	4	.	1	11	3	1	.	2	6	62
2	.	.	.	.	3	.	.	.	.	.	.	.	1	4	.	2	.	.	.	2	.	.	.	.	.	6
3	.	2	.	2	3	.	1	1	.	.	.	.	1	6	.	.	.	.	.	.	.	1	.	1	2	10
4	.	.	.	.	.	1	.	.	.	.	.	.	.	1	.	5	.	.	.	5	.	.	.	.	.	6
5	.	1	6	7	1	2	3	.	4	1	.	1	.	12	.	7	1	.	.	8	.	.	1	.	1	28
6	1	2	4	7	.	1	1	.	2	.	.	.	2	6	2	2	3	1	.	8	.	.	.	.	.	21
7	.	2	.	2	.	1	.	.	.	.	.	.	.	1	.	5	.	.	.	5	.	.	.	.	.	8
8	10	2	.	12	.	1	.	.	.	2	3	.	.	6	.	1	.	.	.	1	.	2	.	.	2	21
9	.	.	7	7	1	.	6	3	.	.	.	2	1	13	.	2	1	1	.	4	.	1	1	.	2	26
10	1	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1	2
11	1	1	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
12	.	.	.	.	2	.	1	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	3
13	.	1	.	1	3	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	4
14	7	.	.	7	.	.	.	.	1	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	8
15	.	.	.	.	.	1	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
16	.	5	.	5	.	.	.	.	.	1	.	.	.	1	.	.	.	.	.	.	.	3	.	.	3	9
17	.	.	.	.	.	1	.	.	.	1	.	.	10	12	.	.	.	.	.	.	.	.	.	.	.	12
18	.	2	.	2	1	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	3
19	.	.	.	.	.	.	.	1	.	.	.	.	.	1	.	.	.	.	.	.	.	2	.	.	2	3
20	.	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
21	.	.	.	.	.	.	.	.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	.	.	.	1
22	.	.	.	.	.	.	.	.	.	2	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	2
23	.	.	.	.	.	.	.	.	.	1	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
24	.	.	.	.	.	.	.	.	.	1	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
u	2	4	8	14	.	2	7	4	2	2	.	2	12	31	.	5	4	.	.	9	2	4	1	.	7	61

* P.M. = post-mortem material, including intestinal contents, stomach contents or heart blood.
† Three infants suffering from mild digestive upsets included.
u = unidentified strain

a source of infection in the nursery itself which infected the infants as they entered the nursery, so that the same streptococcal types kept recurring. In the London series this recurrence of streptococcal types was not so marked. In each of these series, however, there was a distinct tendency for specimens taken concurrently to contain the same serological types. In the Reading specimens, a greater diversity of serological types of group D streptococci was found, and different streptococcal types were usually found in different specimens. Bottle-fed infants tended to carry a greater diversity of serological types than breast-fed infants; usually two or three different types were isolated from the same specimen in the former, but this also occurred in a few of the breast-fed infants.

Three pairs of breast-fed twins were included, one from each locality. The types isolated were never identical, although in two pairs each infant had one type in common. In the third pair, different types were found in each infant's faeces. It is thus indicated that the mother is not the only source of infection.

Out of seven infants from whom more than one specimen of faeces was taken, three still carried the same serological type, whilst with the other four the types found in the second specimen differed from those in the first.

In general, as might be expected, different serological types predominated in the different maternity units. Types 1 and 6 were the only types common to all three maternity units, but neither occurred frequently.

Infants involved in outbreaks of neonatal diarrhoea

Outbreak A. As several swabs were taken from some of the infants it was sometimes possible to investigate the organisms in the faeces before the onset of diarrhoea, during illness, and also during convalescence. Large numbers and high percentages of streptococci were isolated from these infants. From fourteen of the forty-seven rectal swabs examined, streptococci were isolated as the only aerobic organisms present in the faeces. In nine other rectal swabs streptococci constituted over 80 % of the flora. Many colonies of streptococci were picked from the plates and all were found to be group D.

No streptococci were isolated from twelve infants who were under 3 days old. From four, no organisms at all were isolated, and from the other seven only micrococci were isolated. In the 1-day-old infants these micrococci were present in very small numbers, and may have been contaminants acquired during swabbing. When five of these infants later developed diarrhoea further swabs were taken from three of them which on culturing gave rise to profuse growths of group D streptococci.

The prevalence of serological type 1 was very marked, for it was isolated from thirty out of thirty-five faecal specimens containing group D streptococci, and in nineteen of them was the only type isolated. It was also recovered from thirteen of the fifteen infants who were ill or became ill within the next 2 days. It was also recovered from three contact infants who were not affected but of whom two had an aerobic flora at one time consisting solely of type 1. It appears that, whether or not type 1 had any aetiological significance, this organism had spread to nearly every infant in the nursery.

Outbreak B. Little material was obtained for examination from sick infants. Types 6 and 15 were isolated from the infant who died, types 4, 5 and an unidentified type from one infant with loose stools, and types 5, 7, 8 and an unidentified type from the third infant. Group D streptococci were isolated from nineteen of the twenty contact infants. Here again diverse types were met with, eight different types being identified and five strains remaining unidentified. Where a second swab was taken from an infant after an interval of 4 days, the types present in the second sample were usually different from those previously isolated. A different serological type was isolated from each of a pair of twins swabbed on the same date.

The proportion of streptococci to other organisms varied, the distribution being somewhat like that in healthy infants. Only once did the aerobic flora of the faeces consist solely of group D streptococci, and in only one sick infant were large numbers of group D streptococci observed.

Group D streptococci were less frequently recovered from mothers of infants, being isolated from only five out of fifteen mothers. Type 1 was found in three instances and two unidentified types in the other two. Type 1 was not isolated from the infants of the three mothers carrying type 1.

Outbreak C. In some infants large numbers of coliform organisms were present which, on plating, overgrew everything else, even when the inhibitory medium was used. The streptococci isolated from two specimens were not group D, but later specimens from the same infants included group D in their flora. When group D streptococci were found they were present in large numbers, and as a high proportion of the total flora. In thirteen out of the nineteen specimens from which they were isolated they consisted of more than 80 % of the total flora. Type 9 was the most common serological type, then type 1, being found in six and four of the fifteen infants respectively.

Outbreak D. A heterogeneous collection of types was isolated from the five sick infants, type 9 from three, including twins, type 1 from one, type 3 from one and type 19 from one infant. From the post-mortem material type 8 was isolated from two infants and type 19 from the third. Serological types from eleven contact infants who did not contract the illness, three of whom were suffering from other slight upsets, were also studied. Type 1 was the most common type isolated (four infants) and types 5, 6 and 9 were also found. Examination of group D streptococci from opened and unopened tins of dried milk powders from the nursery showed that types 9, 10 and 16 (from two tins) had been isolated respectively from four previously unopened tins, and type 16 and unidentified types respectively from two opened tins. Although type 16 appeared to be the most common type from this particular series of tins of dried milk powders, it did not appear to have become established in the intestine as it was not recovered from the faeces. This type was, however, isolated from several of the healthy bottle-fed infants (Table 4). In the three instances in which group D streptococci were obtained from the throat or nasopharynx, the same serological type was also found on the rectal swab.

Outbreaks E, F, G, H and K. Typing of group D streptococci from outbreaks E, F, G, H and K did not reveal any types common to all, nor any types predominating greatly in any one outbreak. In E type 5 was the most common and

occurred in four out of nine sick infants. In F a diversity of types was found, type 1 being the most common. High percentages of streptococci (97–100 % of the total flora) were found in the faeces of four of these infants. From two of these type 1 was isolated and from the other two types 23 and 24 respectively.

From three infants involved in outbreak G type 8 was isolated, and from three infants and one post-mortem sample from H, types 9 and 5 were most frequently isolated. From post-mortem material obtained from K types 1 and 3 were recovered.

Outbreak L. Group D streptococci occurred in twenty-six of the thirty-one specimens examined. Four specimens from which group D were absent were from infants under 3 days old. Two serological types were prevalent, types 1 and 17. Type 1 occurred in ten out of seventeen ill babies, in two out of six who later became ill and in one 'isolated' infant who remained healthy. Type 17 occurred in nine sick infants, and in one who later became ill. This type was otherwise uncommon, only two other strains being isolated during the whole course of this investigation, and its high incidence among these infants again postulates a common source from which it was spread. From the four infants who died, type 1, types 1 and 17, type 6 and types 6 and 21 were isolated respectively. In none of these cases were streptococci present as a high percentage of the aerobic flora, although swabs were taken from two infants shortly before death. Thus, at a time when large numbers of the causal organism might be expected to be present in the faeces, only small numbers of group D streptococci were present.

(*d*) *Incidence of prevalent serological types of group D streptococci present in infants' faeces*

The serological types of streptococci most frequently found in healthy infants were 8, 1, 5, 6, 9 and 14, in sick infants 1, 9, 5, 17, 8, 6 and 3, and in healthy contact infants 1, 5, 6, 4, 7 and 9 (Table 4). The high incidence of type 17 in the sick infants is due to its frequent occurrence in one outbreak only and is not representative of its general distribution. This also applies to type 14 in the healthy infants' series.

Table 5 gives the percentage of infants in each group from whom each of these more commonly occurring streptococcal types was isolated. A statistical comparison between the number of healthy infants and sick infants and between the number of healthy infants and healthy contact infants from whom type 1 was isolated showed that the difference between healthy and sick infants was significant, whereas the difference between healthy and healthy contact infants was not. When similar comparisons were made with type 8 it was found that the differences between both groups were significant (Table 5). Further comparisons were not made.

Distribution of group D streptococci among thirteen maternity units

Table 6 shows the distribution of types amongst thirteen maternity units from which the streptococci were obtained. Type 1 was the most common, occurring in eleven localities. Next came types 5, 6, 9, 3 and 8. Other types were isolated much less frequently; none occurred in more than three maternity units, and nine were

found in only one. Type 3 was curious in that, although it was never isolated from many infants in one place, it was quite widespread throughout the country. Possibly this is a type which does not persist long. The American workers Wheeler & Foley (1945), however, found that their serological type H 69 D 5, which in this

Table 5. *Incidence of the most commonly occurring types of group D streptococci in the faeces of healthy, sick and healthy contact infants*

Serological type	Percentage of infants in each group from whom each serological type was isolated: Healthy infants	Sick infants	Healthy contact infants
1	21·7	41·7	30·6
3	4·3	7·1	—
4	—	1·2	13·9
5	15·2	14·3	22·2
6	15·2	7·1	22·2
7	4·3	1·2	13·9
8	26·1	7·1	2·8
9	15·2	15·5	11·1
14	15·2	1·2	—
17	—	14·3	—

Statistical comparisons:

Type 1. Healthy infants compared with sick infants $\chi^2 = 4{\cdot}37^*$.
Healthy infants compared with healthy contact infants $\chi^2 = 0{\cdot}43$.

Type 8. Healthy infants compared with sick infants $\chi^2 = 7{\cdot}42^{***}$.
Healthy infants compared with healthy contact infants $\chi^2 = 6{\cdot}57^{**}$.

χ^2 has been calculated in the normal way using Yates's correction for continuity (Fisher, 1941).

* $0{\cdot}05 > P > 0{\cdot}02$; ** $0{\cdot}02 > P > 0{\cdot}01$; *** $0{\cdot}01 > P > 0{\cdot}001$.

Table 6. *Distribution of serological types of group D streptococci amongst thirteen maternity units in different localities in Great Britain*

Serological type	No. of localities found	Serological type	No. of localities found
1	11	13	1
2	3	14	2
3	6	15	1
4	1	16	3
5	9	17	3
6	9	18	2
7	2	19	1
8	6	20	1
9	8	21	1
10	2	22	1
11	2	23	1
12	2	24	1

Localities of maternity units from which streptococci were isolated:

Ashton-under-Lyne
Birmingham
Blackburn
Cambridge
Cardiff
Derby
Hull
Leicester, Hospital A
Leicester, Hospital B
London
Nuneaton
Preston
Reading

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laboratory was found to agglutinate with type-specific serum type 3, was the most common type of group D isolated, occurring in the faeces of eight out of ten healthy infants, in the throats of nine out of ninety-seven healthy infants, in the throats of ninety-two out of 114 infants with respiratory diseases, in post-mortem material from twenty-seven out of sixty-one infants and children under 11 years old who died from different causes, and was isolated from a number of adults suffering from various diseases. Two strains of group D isolated by Gale from outbreaks and typed by Shattock, were found to be type 4 and an unknown type respectively.

(e) *Tyrosine decarboxylase activities*

In Figs. 1, 2 and 3 the tyrosine decarboxylase activities of strains of streptococci derived from healthy, ill and contact infants are shown. A similar picture is obtained in each group of infants. Strains derived from all sources have activities which give histograms of bimodal form and which show two groups of active producers and non-producers of tyrosine decarboxylase. The same modes of 0·0–0·2 and 1·4–1·6 pH units are found in all groups of infants.

Table 7. *Tyrosine decarboxylase activities of serological types of group D streptococci*

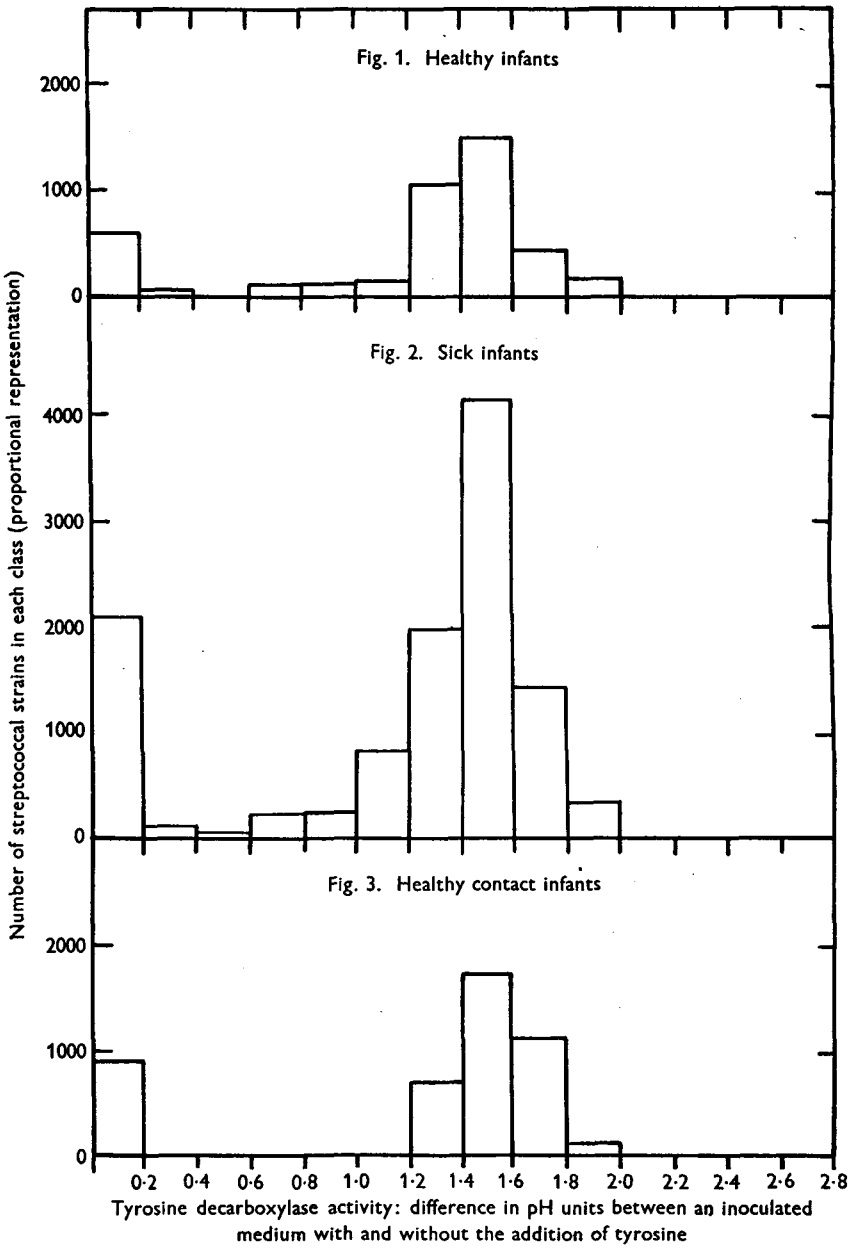
Active producers of tyrosine decarboxylase activity = 1·0–2·0 pH units		Intermediate producers of tyrosine decarboxylase activity = 0·4–1·0 pH unit		Non-producers of tyrosine decarboxylase activity = 0·0–0·4 pH unit		Active or non-producers of tyrosine decarboxylase	
Serological type	No. of strains	Serological type	No. of strains	Serological type	No. of strains	Serological type	No. of strains
1*	83	14†	8	2	7	16	2+ 6−
3	11	24	1	7	8		
4	7	*u*	3	10	2	18	3+ 1−
5*	28	—	—	12	3		
6	24	—	—	13	4	—	—
8	23	—	—	17	12	—	—
9	33	—	—	15	1	—	—
11	2	—	—	21	1	—	—
19	7	—	—	23	1	—	—
20	1	—	—	*u*	30	—	—
22	2	—	—	—	—	—	—
u	39	—	—	—	—	—	—
Totals	260	—	12	—	69	—	12

* One strain of intermediate activity encountered.
† One strain of no activity encountered.
u = unidentified strains.

In Table 7, 353 strains of group D streptococci (several from the same infant) have been divided into four categories according to their tyrosine decarboxylase production. The ability to produce this enzyme and the extent to which the organisms produced it were in general found to be associated with serological types. Highly active strains were the most prevalent (eleven types) and the most commonly found types, 1, 5, 6, 8 and 9, were all in this category. Strains with high tyrosine decarboxylase activities occurred as frequently in sick as in healthy infants.

VI. DISCUSSION

A comparison between strains of group D streptococci isolated from healthy infants and those isolated from infants associated with outbreaks of neonatal diarrhoea shows that these organisms appear to have little significance in outbreaks of neonatal diarrhoea. The fact that the numbers of group D streptococci in the faeces of sick infants are markedly increased may be due to conditions in the



Figs. 1–3. Histograms of the tyrosine decarboxylase activities of group D streptococci isolated from healthy, sick and healthy contact infants' faeces.

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gut in diarrhoea favouring their growth, so that the increased numbers are a result and not a cause of the condition.

Serological typing shows that several different types occurred among group D streptococci isolated from the faeces of both healthy and sick infants. Usually in an outbreak one or two types were prevalent, but, apart from outbreak A, they were never common to enough affected infants in a community to suggest that they occurred more frequently than the types found in healthy infants. In both healthy and sick infants, one or two types were found to predominate in individual maternity units. But although one particular type usually predominated in an outbreak, it was not always the same one. In outbreaks A and F, type 1 was most frequently found, in B and D a diversity of types occurred, in C types 1 and 9 predominated, in H types 5 and 9, in E type 5 and in G type 8 were the most common, whilst in L types 1 and 17 were prevalent.

Thus the most frequently occurring types in the different outbreaks were 1, 5, 8, 9 and 17, which were, apart from type 17, the predominant types in the groups of healthy infants. No single type was found in all outbreaks and, although types 1 and 5 were isolated from six outbreaks, type 1 was also isolated from three and type 5 from two of the three groups of healthy infants. There is thus no evidence that group D or any particular serological type in this group is the general cause of neonatal diarrhoea.

From the observations on healthy infants one would expect to find particular types predominating in one hospital, and that type doing so over a considerable period of time. The prevalence of the uncommon type 17 in outbreak L, whilst epidemiologically not significant, shows the likelihood of a strain being disseminated to a large number of infants, if conditions are suitable.

Sharpe (1948) has already shown that tyrosine decarboxylase activities of group D streptococci appear to be associated with the other physiological characteristics of these organisms on which the species and varieties of this group are classified. The present work indicates that tyrosine decarboxylase production was also usually associated with serological type, since only occasionally were an active and inactive strain found in the same type. As all the more common serological types were found to be distributed among both sick and healthy infants, no particular types being restricted to one or the other, the tyrosine decarboxylase activities were similarly distributed. There was, in this survey, no tendency for highly active strains to occur in sick infants or inactive strains in healthy ones. The predominant types in healthy and sick infants, 1, 5, 8 and 9, were all active producers of tyrosine decarboxylase.

Recent work by Ross (1951) has shown the absence of tyrosine in the amino-acids excreted in the faeces by healthy breast- and bottle-fed infants and by infants suffering from gastritis. It was demonstrated however, that tyrosine and three other amino-acids which also could not be detected in the faeces were present in the intestinal secretions of infants suffering from gastritis, and that the intestinal organisms were responsible for their disappearance. It is suggested that 'at least in infants with diarrhoea and vomiting, the intestinal organisms preferentially use these amino-acids'.

Recently, other workers have found a particular serological type of *Bact. coli* to be closely associated with outbreaks of infantile diarrhoea and vomiting (Bray, 1945; Giles & Sangster, 1948). Taylor *et al.* (1949) found this serological type, which they designated D433, present in all cases examined in an outbreak of neonatal diarrhoea, and Kirby, Hall & Coackley (1950) and Magnusson, Laurell, Frisell & Werner (1950) have described outbreaks of neonatal diarrhoea in which this organism was isolated from all sick infants. The evidence presented by these workers suggests that this type strain, and possibly other serological types of *Bact. coli* (Smith, Galloway & Speirs, 1950) have an aetiological significance in certain types of outbreaks of gastritis and neonatal diarrhoea.

It is now becoming recognized, however, that the term neonatal diarrhoea has been applied to a number of syndromes. Laurent (1944) suggested that neonatal diarrhoea consisted of at least two diseases of different aetiology, one being much more serious than the other. Lembecke, Quinlivan & Orchard (1943) described two outbreaks of neonatal diarrhoea in the same hospital; the first they considered a severe one with a significantly higher case rate amongst artificially fed than breast-fed or partly breast-fed infants; the second consisted of mild cases with no deaths and was limited almost exclusively to breast-fed infants. Kirby *et al.* (1950) considered that the term covered at least six main groups, differing clinically and aetiologically. These included outbreaks due to accepted pathogens, epidemics of diarrhoea and vomiting with a high mortality rate and a very constant clinical picture, mild outbreaks in which diarrhoea was the dominant feature in infants who were not very ill, outbreaks in which adults were also affected, epidemics of diarrhoea and vomiting associated with an influenza-like illness in adults, and outbreaks of diarrhoea with stomatitis possibly due to a virus. These authors investigated a serious outbreak of diarrhoea and vomiting in infants which caused a mortality of 43 % and isolated *Bact. coli* D433 from rectal swabs of all thirty cases. Of the breast-fed infants only 4·5 % became ill, and of those not receiving milk directly from their mothers, including those having expressed breast milk, 68 % became ill. Six months later another outbreak of neonatal diarrhoea of a much milder nature occurred in which there were sixteen cases and no deaths. In contrast to the first epidemic, *Bact. coli* D433 was never isolated from the stools, and breast-fed infants were affected as frequently as those artificially fed.

The outbreaks A, C, D, E and L reported here can be divided in a similar way into two groups—either outbreaks with high case mortality rates, mainly affecting bottle-fed infants and those having expressed breast milk, or mild outbreaks with no deaths affecting both breast- and bottle-fed infants.

Outbreaks D, E and L were of a serious nature, with vomiting and severe dehydration and several deaths. In outbreak D, with a mortality rate of 70 %, only one infant was exclusively breast fed; in outbreak E, with a mortality rate of 23 %, fifteen out of thirty-five of the infants were exclusively breast fed; in outbreak L, with a mortality rate of 23 %, all infants received expressed breast milk. In these outbreaks relatively few group D streptococci were found, and in outbreaks D and E many diverse serological types occurred. In outbreak D *Bact. coli* D433 was isolated

by other workers from all sick infants examined. Strains of *Bact. coli* from E and L were not examined serologically.

In outbreaks A and C the infants were not usually seriously ill, and the chief symptom was relaxed green stools. Most of the infants were breast fed. The high incidence and constant presence of group D streptococci type 1 in outbreak A has already been emphasized. In outbreak C, although large numbers of group D streptococci were isolated from some infants, no one serological type predominated extensively, and *Bact. coli* was also present in large numbers but was not investigated serologically.

In outbreak A, *Bact. coli* was often absent from the plates, a pure culture of group D streptococci type 1 being obtained from some infants. The picture was somewhat similar to that reported by Gale (1944), except that the organisms were not β-haemolytic, although the outbreaks reported by Gale were serious. Only outbreak A investigated here presented the marked prevalence of group D streptococci with a single type predominating. Once again, successive infants may have been infected from a common source in the nursery. The glucose water was suspected but was not examined bacteriologically. In healthy infants streptococci usually constituted less than 50 % of the total aerobic flora of the faeces, and in only one sample were streptococci alone isolated. High percentages of streptococci were never found in a whole series of specimens taken concurrently. It might be expected, if this were a transient infection with type 1, that the sick infants who were transferred to another hospital and swabbed 7 days after this transfer would have acquired a fresh streptococcal flora in their new surroundings. However, type 1 was isolated from all six convalescent infants, in three of whom the aerobic flora consisted solely of type 1. Diarrhoeal conditions may have favoured the establishment of this type, already present in small numbers in the intestine, or alternatively unfavourable factors in infant management may have rendered the gut particularly susceptible to these organisms, and their increased numbers have caused the diarrhoea, possibly by means of the increased amount of tyramine produced by this almost purely streptococcal flora. Three unaffected infants were found to be excreting this organism in large numbers, but symptomless carriers are frequently met with (Rubbo, 1948; Smith *et al.* 1950). The tyrosine decarboxylase activities of the streptococci in the two mild outbreaks were found to be strikingly similar to those in the three serious ones.

As the clinical evidence shows a distinction between the various neonatal diarrhoea syndromes and the bacteriological evidence bears this out (Kirby *et al.* 1950), organisms other than *Bact. coli* cannot be completely eliminated from consideration in the milder outbreaks of neonatal diarrhoea. There is insufficient evidence to suggest that group D streptococcus type 1 was the aetiological agent in outbreak A, and in fact this serological type 1 was found to be the most widely distributed type in infants' faeces in this country and to occur in both sick and healthy infants. Attention is, however, drawn to this as an outbreak of neonatal diarrhoea of a mild nature where *Bact. coli* was isolated in small numbers or not at all, and where a single serological type of group D streptococci of a high tyrosine decarboxylase activity was present in unusually high numbers in the faeces of affected infants.

SUMMARY

1. The aerobic faecal flora of healthy infants was found to comprise coliform organisms, streptococci and occasionally yeast and spore-bearing organisms. Coliforms and streptococci occurred more frequently in the stools of artificially fed than of breast-fed infants in whom micrococci occurred more frequently.

2. Of 191 strains of streptococci isolated from healthy infants' faeces and tested serologically, 89·0 % fell into Lancefield's group D. Of the stools examined 93·7 % contained group D streptococci.

3. A comparison of the group D streptococcal flora of the faeces of healthy infants and of infants associated with outbreaks of neonatal diarrhoea showed that the percentage of these organisms found in the latter was much higher than in healthy infants, the modes being 90–100 and 10–20 % respectively.

4. The same species and varieties of group D streptococci were found in both healthy and sick infants. *Streptococcus faecalis* was the predominant species.

5. Serological typing of strains of group D streptococci from healthy infants from three maternity hospitals showed that particular types tended to predominate in each maternity unit and to recur over a considerable period of time, in each instance suggesting a reservoir of dissemination to successive infants as they arrived. With artificial feeding an increased diversity of serological types was found.

6. Serological types of group D streptococci isolated from sick infants during ten outbreaks of neonatal diarrhoea showed a considerable variety. Apart from one outbreak (A), where type 1 was isolated with great frequency, one or two types tended to predominate in each outbreak, but not to any great extent, and not always the same one.

7. Types 1, 5, 8 and 9 were the types of group D streptococci most commonly found in both healthy and sick infants, but type 1 occurred in a significantly greater percentage of sick than of healthy infants.

8. Tyrosine decarboxylase activities of group D streptococci were usually associated with serological types. The distribution of activities of these organisms was similar in healthy and sick infants.

9. The possibility that neonatal diarrhoea may consist of more than one syndrome is discussed, and several of the outbreaks described here are classified into two groups.

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