PAPERS AND ORIGINALS

Screening Methods for Covert Bacteriuria in Schoolgirls

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Summary

Screening tests for bacteriuria based on two different principles were evaluated in 1582 schoolgirls aged 5-11 years, and in 26 girls aged 3-16 years attending hospital with symptomatic urinary tract infection. Tests for hypoglycosuria, performed by a semi-automated fluorometric method and with Uriglox strips on early-morning urine samples voided after overnight fasting, gave unacceptably high false-negative rates (16.7% and 20.8% respectively).

Oxoid and Uricult dipslides were immersed in fresh midstream specimens of urine obtained at school and read after overnight incubation at 37°C. Both gave comparable results, with low false-positive rates and no false-negative responses. The higher cost of screening by dipslides was halved by using the "dipstream" technique, which also gave no false-negative results. Its false-positive rate of 13.5% could be reduced to 1.8% by disregarding colony counts of 10^8 non-faecal organisms and over per litre, which appear unimportant in school-children.

Bacteriuria was found in 2.3% of the schoolgirls; 39% of them had symptoms, compared with 7.2% of the healthy girls, and 25% showed vesicoureteric reflux, which in 17% was associated with renal scarring. Since the natural history of covert bacteriuria and its relation-

ship with reflux and scarring remain undetermined further research is required. The dipstream technique offers a simple, reliable, and comparatively cheap screening method which could also be applied in general practice.

Introduction

Bacteriuria is prevalent in schoolgirls but its long-term health hazards will be determined only by prospective studies. An essential prerequisite is a simple, reliable, and inexpensive screening test. Though several methods have been evaluated individually they have not been subjected to a controlled comparative trial under field conditions. Bacteriological methods have the advantage of identifying the infecting organism but entail great care when collecting the urine specimens to avoid gross contamination by vaginal commensals. Of the available techniques the dipslide^{1,2} appeared to afford the nearest approach to a fully quantitative method.

Urinary glucose concentrations under 0.1 mmol/l (0.002 g/100 ml) occur in the presence of significant bacteriuria provided that the patient is fasting and the urine has remained in the bladder for four to six hours.³ Low urinary glucose concentrations can be determined by a semi-automated fluorometric method⁴ or detected by a commercially available paper-strip test.⁵ The theoretical advantage of these techniques is that they depend on alterations which have already occurred in the bladder urine as a result of the action of pathogenic bacteria, provided that contamination of the sample when voiding is eliminated by a bacteriostatic agent.

In a project undertaken at the suggestion of a Medical Research Council working party, set up under the chairmanship of Professor (now Sir) Douglas Black to consider the methodology of screening for bacteriuria, we compared two makes of dipslide (Oxoid and Uricult) with the fluorometric and Uriglox hypoglycosuria tests under field conditions in schoolgirls aged 5-11 years. The dipslide was found to be more reliable but was more expensive. In a second survey we therefore retested it using the "dipstream" technique with a large reduction in cost. We here present our combined results together with some clinical and radiological observations made in the bacteriuric schoolgirls.

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First Survey

This survey was conducted at four Birmingham primary schools, at which all 970 girls aged 5-11 years participated. After preliminary visits to each school to explain to the staff the nature of the investigation, letters were sent to parents inviting their co-operation and, with the agreement of local general practitioners, offering treatment for confirmed cases of urinary tract infection. Schools were then visited two mornings weekly during four successive terms, some 20 girls being screened at each session.

Twenty-six girls aged 3-16 years attending hospital with untreated symptomatic urinary tract infection were also studied. None had a previously diagnosed abnormality of the urinary tract. The same procedures were followed as with the schoolgirls.

COLLECTION OF SPECIMENS

Two urine specimens were obtained from each girl the same morning—an early-morning specimen (E.M.U.) collected at home for glucose determination and a Uriglox test, and a midstream specimen (M.S.U.) collected at school under nursing supervision for the inoculation of dipslides and a plate colony count.

On rising, the child voided the E.M.U. into a sterile container to which 0.1 ml 10% merthiolate had been added to prevent the utilization of glucose by contaminant bacteria. Containers were issued together with cyclostyled instructions that no food or drink should be taken from 22.00 hours until the E.M.U. had been collected next morning. Each mother was also asked to complete a questionnaire to indicate whether her daughter suffered from frequency, dysuria, or enuresis or had a past history of urinary tract infection, and to record the time the E.M.U. was collected and time of the last voiding the previous night. This enabled us to determine approximately how long the urine had been in the bladder before collection, specimens being recorded as *satisfactory* if this was more than six hours and *unsatisfactory* if less.

The M.S.U. was collected without vulval cleansing, the omission of which does not increase the risk of contamination.^{6,7} Specimens were collected in an urinal designed by Huttunen *et al.*,⁷ which holds two sterile disposable gallipots. One of these is positioned anteriorly in line with the urethra to collect the M.S.U., which is voided under pressure, and the other is placed on the bottom of the urinal and collects urine voided under low pressure at the beginning and end of micturition. This method succeeds provided that the bladder is reasonably full and the child is free from anxiety. Specimens collected in the anterior gallipot were recorded as *satisfactory* and those in the other gallipot as *unsatisfactory*.

LABORATORY METHODS

Fluorometric Determination.—The method was based on that of Schersten and Tibbling.⁴ Analyses were usually carried out within 48 hours of receipt of the E.M.U., though the use of a preservative permitted delay of up to a week,⁸ and could be performed at a rate of 25 an hour. Specimens with a glucose concentration of 0.1 mmol/l (0.002 g/100 ml) or more were recorded as *negative*, and those with a concentration of less than 0.1 mmol/l as *positive*. Urinary urea and creatinine concentrations were also measured on an AutoAnalyzer to see whether hypoglycosuria in the absence of bacteriuria could be explained by dilute urine.

Uriglox Test.—This was performed on the E.M.U. by the nurse in attendance and repeated independently by the technician in order to assess observer error. The test was carried out according to the manufacturer's instructions. A *negative* result is shown by the development of a definite blue colour within 10 minutes, and a *positive* result, equivalent to a urinary glucose concentration under 0.1 mmol/l, by the absence of any such change. An occasional faint blue colour, indicating a borderline glucose level, was recorded as a *trace*.

Oxoid and Uricult Dipslides.—Both dipslides were of similar design, with MacConkey's agar on one side. On the reverse, Oxoid had cystine-lactose-electrolyte-deficient medium (CLED), whereas Uricult was supplied with nutrient agar, though it is now available with CLED. Both dipslides were inoculated immediately after the M.S.U. was collected and incubated overnight at 37°C. Bacterial counts on most of the dipslides were estimated by comparison with the manufacturers' charts. Counts of 10⁷-10⁸/l, however, especially with mixed growth, were more accurately estimated by counting the colonies on the dipslide and converting to bacteria per litre from the manufacturers' graphs.

Surface-viable Bacterial Colony Counts.—Blood and MacConkey's agar plates were inoculated with 0.02-ml aliquots of undiluted urine, taken with sterile haemoglobin pipettes direct from the gallipots, within minutes of voiding. Plates were incubated overnight at 37°C, the blood agar in increased carbon dioxide tension, and colony counts made the next day. When repeating initial bacterial counts over 10⁷/l the urine was diluted × 100 and × 1000 and 0.02-ml aliquots were explanted as before. With both dipslides and plates all colony counts of 10⁷/l or under were recorded as *negative*, as were most higher counts of non-pathogenic vaginal commensals such as diphtheroids and lactobacilli, especially when in mixed culture. Counts of 10⁸ or more of a recognized urinary pathogen per litre were recorded as *positive*, and intermediate counts (10⁷-10⁸) as *borderline*. When the results of the dipslides and surface colony count did not agree the colony count was assumed to be correct in reaching a final bacteriological diagnosis.

FOLLOW-UP AND FINAL DIAGNOSIS

Further action in each case depended on the initial results and is indicated in the fig.

Flow diagram showing procedure for evaluating screening methods.

Bacteriuria was diagnosed when a colony count of 10⁸ or more of a recognized urinary pathogen per litre was obtained by the plate method in at least two M.S.U.s voided on separate days.

When the results of the initial tests failed to agree with the final diagnosis as defined above they were described as either *false-positive* or *false-negative*.

RESULTS

Only one parent refused to participate, and all but five girls (99.5%) returned E.M.U.s with completed questionnaires. Eight per cent. of both E.M.U.s and M.S.U.s were recorded as unsatisfactory. Sixty-eight girls (7%) were unable to provide an M.S.U. at school on the required morning and were excluded from analysis. Of the 902 children remaining in the survey, 24 (2.7%) were found to have significant bacteriuria. Analyses of the performance of each screening test are given below and include the results obtained from the 26 hospital patients with bacteriuria.

Fluorometric Determination.—The results are given in table I. Four of the 24 bacteriuric schoolgirls (16.7%) and 11 of the 26 hospital patients (42.3%) gave false-negative results. Unsatisfactory E.M.U. collection occurred in only three of the 15 false-negative cases. All but three of the 17 specimens yielding false-positive results had low urea and creatinine concentrations, indicating dilute urine.

Uriglox Test.—Discrepancies in results recorded independently by the nurse and technician (table II) were mainly due to a higher incidence of trace readings by the technician, which reduced the frequency with which he predicted the correct bacteriological diagnosis. E.M.U.s, however, gave negative results for both observers in five bacteriuric schoolgirls and 10 hospital patients, yielding false-negative rates of 20.8% and 38.5% respectively. Moreover, discrepancies between the Uriglox readings and fluorometric determinations were observed in 16 instances.

Oxoid and Uricult Dipslides.—Both makes of dipslide were simple to use and interpret and yielded similar results. All schoolgirls and hospital patients with persistent bacteriuria had positive results on both initial and repeat testing (table III). The false-positive rates

TABLE I—First Survey. Results of Initial Fluorometric Urinary Glucose Determinations Related to Final Diagnosis

Glucose Concentration (mmol/l)	Schoolgirls			Hospital Bacteriuric Patients
	Total	Uninfected	Bacteriuric	
Negative (≥ 0.1) ..	860	856	4*	11*
Positive (< 0.1) ..	37	17†	20	15
No specimen ..	5	5		
Total	902	878	24	26

* False-negative rates: schoolgirls 16.7%; hospital patients 42.3%.

† False-positive rate 1.9%.

Conversion: SI to Traditional Units

Urinary glucose: 1 mmol/l $\approx$ 0.018 g/100 ml.

TABLE II—First Survey. Results of Initial Uriglox Tests, Read Independently by Nurse (N) and Technician (T), Related to Final Diagnosis

Test Reading	Schoolgirls						Hospital Bacteriuric Patients	
	Total		Uninfected		Bacteriuric		N	T
	N	T	N	T	N	T		
Negative (blue) ..	865	859	860	854	5*	5	10*	11
Trace ..	3	12	3	9		3	1	5
Positive (no colour) ..	29	26	10†	10	19	16	15	10
No specimen ..	5	5	5	5				
Total	902	902	878	878	24	24	26	26

* False-negative rates: schoolgirls 20.8%; hospital patients 38.5%.

† False-positive rate 1.1%.

TABLE III—First Survey. Results of Initial Oxoid and Uricult Dipslide Tests Related to Final Diagnosis

Dipslide	Colony Count/l	Schoolgirls			Hospital Bacteriuric Patients
		Total	Uninfected	Bacteriuric	
Oxoid	$\leq 10^7$	835	835		
	10^7-10^8	26	26		
	$\geq 10^8$	41	17*	24	26
	Total	902	878	24	26
Uricult	$\leq 10^7$	840	840		
	10^7-10^8	19	19		
	$\geq 10^8$	43	19†	24	26
	Total	902	878	24	26

* False-positive rate 1.9%.

† False-positive rate 2.2%.

were 1.9% for Oxoid and 2.2% for Uricult. Borderline counts, however, were obtained in a further 2.9% and 2.2% of non-bacteriuric girls, necessitating repeat tests. M.S.U.s were recorded as unsatisfactory for 29.6% of the false-positive and borderline results compared with 7.7% of the remainder.

Surface-viable Bacterial Colony Counts.—Colony counts performed on the initial M.S.U.s collected at school yielded many more borderline results than either dipslide, mainly owing to the enhanced growth of vaginal commensals on blood agar. *Escherichia coli* was isolated from 22 of the bacteriuric schoolgirls, klebsiella from one, and faecal streptococci from one. These organisms were also isolated from nearly half of the M.S.U.s giving borderline or false-positive results, the remainder consisting of vaginal commensals such as diphtheroids and staphylococci.

Second Survey

In view of the clear-cut superiority of the dipslide over the hypoglycosuria methods the second survey was concerned with further simplifying the technique and reducing its cost. By using the "dipstream" technique⁸ the collection of an M.S.U. was unnecessary and nursing procedures were eliminated. Moreover, it appeared that the 7% failure rate associated with voiding an M.S.U. at school could be substantially reduced if the child performed the test at home. The survey was carried out in five primary schools during three consecutive school terms, a total of 775 girls aged 5-11 years participating. The organization was similar to that of the first survey.

METHODS

Each girl was supplied with an Oxoid dipslide and written instructions to void an M.S.U. direct on to both sides of the dipslide, with care to avoid skin contact, on waking the next morning. The dipslides were brought to school the same morning, where a further M.S.U. was obtained as in the first survey. This second specimen was used for immersion of a second Oxoid dipslide and a plate colony count. Since during our first survey, as in the studies of Walten and Kunin,¹⁰ no girl with persistent bacteriuria gave a borderline result (table III) we chose for the second phase a cut-off point of 50×10^6 /l, below which the results were recorded as *negative*. Counts of $50 \times 10^6-10^8$ were regarded as *borderline*, and those of 10^8 and over as *positive*. When one or more borderline or positive results were obtained from the dipslides and plate culture all three tests were repeated (see fig.), and when similar results were again obtained the child was referred to hospital, where a third M.S.U. was collected for plate colony count.

RESULTS

Only one parent refused to participate, and a further 10 children were unable to provide adequately inoculated dipslides; these 11 (1.4%) were excluded from analysis. A few partially inoculated dipslides were, nevertheless, interpretable and are included for analysis. On the other hand, 84 girls (10.8%) were unable to provide M.S.U.s at school, despite having inoculated the dipslides satisfactorily at home, and had to be excluded from the study. Bacteriuria was confirmed in 12 (1.8%) of the remaining 680 children.

The dipstream method yielded positive results on both initial and repeat testing in all 12 bacteriuric girls (table IV). The organisms

TABLE IV—Second Survey. Results of Initial Oxoid Dipslide Tests, by Dipstream and Immersion Techniques, Related to Final Diagnosis

Colony Count/l	Dipstream			Immersion		
	Total	Uninfected	Bacteriuric	Total	Uninfected	Bacteriuric
$\leq 50 \times 10^6$	567	567		633	633	
$50 \times 10^6-10^8$	11	11		8	8	
$\geq 10^8$	102	90*	12	39	27*	12
Total	680	668	12	680	668	12

* False-positive rates: dipstream 13.5%; immersion 4.0%.

isolated were *E. coli* in 11 cases and klebsiella in one, and they grew well on both the MacConkey and CLED media. One girl was not included among the 12 bacteriuric girls because though all three initial procedures gave positive results repeat tests gave negative results. When she was subsequently referred by her general practitioner with symptomatic urinary infection it was disclosed that she had received ampicillin for otitis media after the first screening tests, and it is almost certain that she would otherwise have qualified as a bacteriuric.

Ninety girls who subsequently proved to be uninfected also gave dipstream colony counts of 10^8 /l or over—a false-positive rate of 13.5%. Detailed bacteriological records, however, disclosed that in 59 of them contaminant organisms were suppressed by the MacConkey medium, growing only on the CLED medium, whereas the pathogens isolated from all 12 bacteriuric girls grew equally well on both media. Moreover, 78 of the 90 false-positive results were due to non-faecal organisms, especially *Staphylococcus albus*, or included faecal organisms in mixed culture and were presumably vulvovaginal contaminants. Thus it appears possible to reduce the false-positive rate to

TABLE V—Comparison of Results and Costs of the Five Screening Methods

Method	False-negative Results (%)	False-positive Results (%)	Costs* (£ per 100 Tests)
Fluorometric glucose ..	16.7	1.9	12.22
Uriglox ..	20.8	1.1	16.04
Uricult (immersion) ..	0	2.1	20.90
Oxoid:			
Immersion ..	0	1.9	20.88
Dipstream, all organisms	0	13.5	10.40
Dipstream, faecal organisms only	0	1.8	10.40

* Determined in January 1972.

1.8% by disregarding counts of 10^8 and over due to non-faecal organisms or mixed cultures.

Comparative Results and Costs

The rates of false-negative and false-positive results and the costs of the five methods are given in table V. The high false-negative rates obtained with the hypoglycosuria methods contrast with the complete reliability of both makes of dipslide.

A comprehensive cost analysis of each test showed that using the dipstream technique in the child's home costs less than half as much as the conventional dipslide method carried out at school, without impairment of reliability. The cost per bacteriuric child detected by the Oxoid dipstream technique, based on our overall prevalence rate of 2.3%, was estimated (in January 1972) as £5.24 if all false-positive tests were repeated, and £4.71 if only those due to faecal organisms were repeated. We emphasize, however, that this is merely the cost of detecting bacteriuria.

HEMA-COMBISTIX TESTS

In both phases of the study M.S.U.s were also tested with Hema-combistix and checked the following week if abnormal. None of the bacteriuric schoolgirls, two of the hospital patients, and 34 non-bacteriuric schoolgirls were found to have proteinuria of ++ or more. Out of 20 retested, only three showed persistent proteinuria, which proved to be postural in each instance. No child had haematuria but one 7-year-old girl was found to have glycosuria, which on investigation proved to be due to diabetes mellitus at a presymptomatic stage.

CLINICAL AND RADIOLOGICAL FINDINGS

The questionnaires indicated that 39% of the bacteriuric girls exhibited symptoms, notably enuresis, compared with only 7.2% of normal girls; a previous history of urinary tract infection was recorded in 14% and 3.9% respectively. These differences are highly significant ($P < 0.0001$). Thirteen children (36%) had radiological abnormalities, including vesicoureteric reflux in 9 (25%), associated with renal scarring in six (17%). Three showing persistent gross reflux¹¹ and poor renal growth on repeat radiological examination subsequently underwent surgical reimplantation of the affected ureters. Three other girls showed non-progressive scarring without demonstrable reflux. Only four of the 13 girls with radiological abnormalities exhibited symptoms, including only one of those with gross reflux. Twenty-seven (75%) of the 36 bacteriuric girls, including all those with radiological abnormalities, relapsed within two years of initial treatment.

Discussion

Of the screening tests investigated, both hypoglycosuria methods gave unacceptably high numbers of false-negative results. These findings are at variance with those of Köhler *et al.*¹² in 4-year-old children and those of Emmerson¹³ in pregnant women, but are similar to the results obtained by Dosa *et al.*¹⁴ in infants and by McKinnon *et al.*¹⁵ in adults. Since the fluorometric method accurately determines the urinary glucose concentration it must be concluded that the principle on which the test is based may not always apply in children, even when the urine has remained in the bladder for more than six hours. Moreover, the discrepancies noted between the fluorometric and Uriglox results are a further indication of the latter's unreliability.

On the other hand, both makes of dipslide proved completely reliable. Interpretation is rapidly carried out by matching with the manufacturers' charts and does not demand advanced technical skill. All urinary pathogens grew well on both sides of the dipslides but the CLED medium of Oxoid provided colonial differentiation, which was lacking on the nutrient agar of Uricult. An advantage of the dipslide over the hypoglycosuria tests is that it provides a precise bacteriological diagnosis at the time of screening. Our results with the dipstream technique are

comparable to those obtained by McAllister *et al.*⁹ in hospital patients, and we agree with them that the test should be applied in general practice before antibiotics are prescribed, since a negative result would cost less than a course of treatment. Nevertheless, we believe that performing the test in the privacy of the toilet is less likely to cause inhibition than their method with the child standing in a bath.

All bacteriuric children in our study gave colony counts of 10^8 or more per litre, and it therefore appears unnecessary in practice to repeat the tests for lower counts. Nevertheless, a high proportion of colony counts of 10^8 or more, especially with the dipstream method, occurred in girls who subsequently proved to be uninfected. Since these false-positive results necessitated repetition of the tests, often causing needless parental anxiety as well as increasing the cost of screening, any means of reducing their number without impairing the reliability of the method is worth considering. The evidence from this study and from published data on schoolgirls¹⁶⁻¹⁷ and paediatric patients⁹⁻¹⁸ is that enterobacteria account for the overwhelming majority of urinary tract infections in childhood. For population screening it therefore appears safe to disregard non-faecal organisms, even at counts of 10^8 or more per litre, with negligible risk of overlooking genuine bacteriuria. In this manner the false-positive rate of the dipstream technique could be reduced to as little as 1.8% with further saving in cost.

The 2.3% prevalence of bacteriuria in this survey was somewhat higher than observed elsewhere.¹⁹⁻²¹ This may reflect the small sample which we tested, as well as the exclusion of secondary school girls, who have a lower prevalence of bacteriuria.¹⁶⁻²⁶ The finding of symptoms in almost two-fifths of the bacteriuric schoolgirls is in accord with the observations of Savage *et al.*²⁰ who suggested the term "covert" bacteriuria. In most instances these symptoms improved with treatment. It is noteworthy, however, that eight of the 13 children with radiological abnormalities of the urinary tract had neither symptoms nor a past history of urinary tract infection. Since they are the children most likely to benefit from screening, questionnaire techniques²² would not be an effective method of detection. Radiological procedures are far too complex and costly for screening, as well as difficult to justify ethically.

"Pyelonephritic" scarring is probably non-progressive unless associated with severe vesicoureteric reflux.¹¹⁻²³ The most vulnerable children are those under 5 years old, in whom intrarenal reflux may be found.²⁴ Since bacteriuria may be intermittent or even absent in some cases, however, screening may fail to detect a minority of children with potentially serious renal disease. Ideally, screening should be carried out in pre-school children but, though technically feasible,²⁵ the administrative difficulties at this age¹⁶ remain an obstacle. The fact that three of the 36 bacteriuric girls in our series showed progressive renal scarring and required ureteric reimplantation suggests that some benefit might accrue, even at school age. We have costed only the detection of bacteriuria, however, and the real cost of a screening programme must take into account the much greater expenditure incurred in investigating and treating it. Until the natural history of untreated bacteriuria and the relative roles of bacteriuria and reflux in the aetiology of renal scarring have been elucidated the extensive application of prescriptive screening, at public expense, must be regarded as premature. Nevertheless, continuing research in this field will necessitate screening, for which the dipstream technique offers a simple and reliable test at comparatively low cost.

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Immune Status of Children with and without Severe Infection during Remission of Malignant Disease

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Summary

The immune status of children with malignant disease in remission was assessed using various immune function tests. Children with infections had significantly more neutropenia, hypogammaglobulinaemia, and impaired cell-mediated immune responses than those without. These two groups combined had much more absolute lymphopenia and impairment of both cell-mediated immunity and antibody-producing capacity than control children with non-malignant conditions. Regular immunological evaluation is recommended for children with malignant disease when new intensive treatment schedules are under trial and for individual patients particularly prone to develop infections during treatment.

Introduction

In the last 10 years an aggressive therapeutic approach has radically altered the prognosis for children with acute leukaemia and other malignant diseases.^{1,2} Though such treatment produces long remissions its immunosuppressive effects make children susceptible to opportunistic infections with organisms of low pathogenicity.^{3,4} Such infections endanger life as much as the severe bacterial infections which occur in relapses but are associated with different patterns of immunological disturbance. The malignant process also may impair a child's immunity. Leucopenia,⁵ diminished antibody production,⁶ and impaired cell-mediated responses^{7,8} all increase the child's susceptibility to infection.

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We investigated the immune status of children receiving cytotoxic treatment during remission of malignancy and compared them with normal children and children with malignant disease before treatment or after completing protracted treatment. We aimed to define the type and extent of immune defect and how it differs during infections and what tests might predict those at particular risk of infection.

Patients

The 56 children studied included 36 with leukaemia and other malignant diseases and 20 controls most of whom were poorly thriving and had had recurrent pneumonia, sepsis, or enteric infections, for which no underlying cause was apparent (table I). The children with malignancies suffered the following 14 infections: bronchopneumonia (probably non-bacterial) (3 cases), pneumocystis pneumonia (3), herpes zoster (3), measles and pneumonia (2), candidiasis (2), and cytomegalovirus infection (1). Some children were tested more than once during the study and all the results were analysed.

Methods

We assessed cell-mediated immunity by peripheral blood lymphocyte counts, tuberculin 1/100 or 1/1000 skin tests, and lymphocyte culture with non-specific mitogens and specific antigens. Neutrophil production was measured by neutrophil count. Humoral immunity was measured by serum immunoglobulins and titres of several anti-bacterial and antiviral antibodies. The percentage of non-thymic dependent B-lymphocytes was measured in some patients. Many children had low neutrophil and lymphocyte counts, which made certain tests impossible.

For white blood counts 1 ml blood was anticoagulated with EDTA. Total white cell counts were performed with a Coulter counter and differential counts of 100 cells with Leishman's stained smears. The rest of the blood was separated by centrifuge over a gradient of 24 parts 9% w/v Ficoll (Pharmacia) to 10 parts 34% w/v Triosil 440 (Nyegard and Co., Oslo). It was layered over the solution after sterilizing by millipore filtration in a 3:2 Ficoll: blood ratio. The gradient was spun at 400 g at 4°C and lymphocytes aspirated from the plasma/Triosil interface. The plasma was assayed for (a) IgG, IgA, and IgM by single radial immunodiffusion, using Hoechst tripartigen plates and immunoglobulin standard; (b) bacterial agglutinins with disposable microtitre trays (Flow Labs), using bacteroides, proteus, and *Escherichia coli* isolated in pure culture from human faecal and