# Raised urinary secretory IgA in chronic diarrhoea

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## Abstract
Secretory IgA outputs in urine have been measured in 24 malnourished Gambian children who had been admitted to hospital with chronic diarrhoea and in 43 children from a rural Gambian village. Village children of poor nutritional state (≤74% weight for age compared with the National Center for Health Statistics reference curve) had secretory IgA outputs that were only one third of those of better nourished individuals. In contrast, the patients with chronic diarrhoea had secretory IgA outputs that were significantly raised compared with village children, regardless of nutritional state. These results demonstrate that secretory IgA production in the urinary tract can be stimulated by intestinal disease, suggesting that malnourished children are able to mount a response to mucosal infection and supporting the hypothesis of a common secretory immune system.

Secretory IgA, the principal immunoglobulin of mucosal defence in the human, is found in substantial quantities in most external fluids. Secretory IgA concentrations in the secretions of the eye, nasopharynx, mouth, and duodenum have been shown to be significantly reduced in children with moderate and severe malnutrition.[1-6] An appreciable decrease in IgA containing cells has been noted in jejunal mucosae of severely malnourished children.[7] These studies suggest that mucosal defence is compromised by malnutrition and that this may be a contributory factor in the high incidence of infection common in malnourished children. Intestinal lymphocytes from malnourished children, however, have been shown to have enhanced abilities to synthesise secretory IgA in vitro.[8] Raised concentrations of secretory IgA have been observed in the duodenal fluid of malnourished children with concomitant enteric infection.[9] This suggests that the capacity to increase secretory IgA production in response to local infection may be retained in malnutrition.

The study of Gambian children, reported here, extends these findings by demonstrating that secretory IgA production into urine is lower in malnourished children but that it is raised in those with chronic diarrhoea. This observation indicates that malnourished children are able to mount a mucosal immune response to infection. In addition, it suggests that secretory IgA production at one location can be stimulated in response to infection at another mucosal site, lending support to the concept of a common secretory immune system.

## Subjects and methods

### SUBJECTS
Twenty four children (11 boys and 13 girls) who had been admitted with malnutrition and chronic diarrhoea (three or more loose stools/day for at least two weeks) to the children's ward of the Medical Research Council (MRC) Laboratories, Fajara, The Gambia, West Africa, were recruited into the study. The children were 10–31 months' old and had been suffering from diarrhoea for a median duration of 14 weeks (range 2–52). Twenty three of the 24 patients had weight for height relative to the National Center for Health Statistics (NCHS) reference[10] of less than ≤74%; seven children had marasmic kwashiorkor and one kwashiorkor. The mean (SD) weight for age of these patients was 58 (6)% (range 49–73). One patient was undernourished with a weight for age of 87% and weight for height 88%. Microscopy of stools and small bowel mucosae together with serological tests showed that 15 patients were infected with *Giardia lamblia*, two of whom also had *Strongyloides stercoralis* infection, and two patients were infected with *Ascaris lumbricoides*.

Two urine collections were made, the first at the beginning of the study (week 1), the second three to four weeks later immediately before discharge (week 3). Microbiological analysis of urine collected at the beginning of the study showed that one patient had a klebsiella urinary tract infection, 18 had sterile urine, and five were untested. Blood from each patient was collected at week 1 and analysed for serum IgA using an automated turbometric technique on a centrifugal analyser (Cobas-Bio, Roche).

To assess urinary IgA production in Gambian children without chronic diarrhoea, urine samples were collected from 43 boys aged 9–25 months who were living in the rural villages of Keneba and Manduar during November and December 1987. The children had a mean (SD) weight for age of 80 (12)% (range 55–121) and a mean (SD) weight for height of 87 (7)% (range 75–108). For data analysis the group was divided into quartiles by weight; the most poorly nourished group having weight for age ≤74% (n=11).

The urine samples were collected as part of studies on intestinal function which had been approved by the MRC Gambia ethical committee.

### URINE COLLECTION AND ANALYSIS
Urine samples in both groups of children were collected over a timed period of five to six hours using disposable, self adhesive urine bags (Hol-

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lister) to which one drop of 0·2 g/ml chlorhexidine gluconate had been added as preservative. At the end of the collection period, the volume of urine passed was recorded and samples were stored at −20°C.

Aliquots of urine samples were diluted 10–80 times and analysed for IgA using two enzyme linked immunosorbent assay (ELISA) methods. One method is specific for molecules containing both α chain and secretory component determinants (secretory IgA) and the other detects any molecule containing α chain determinants (secretory IgA, serum IgA, IgA fragments).[11] Purified human secretory IgA (Sigma) was used as standard. When secretory IgA is the only IgA species present the ratio of results obtained with the two methods (total IgA: secretory IgA) is unity. Between batch precision, measured using a pooled diluted breast milk sample analysed on each plate during the series, was 5% for both assays. The lower limit of measurement of IgA in urine was 30 μg/l. Creatinine was measured in urine diluted 1:10 with 0·1 mmol/l hydrochloric acid using the Jaffe method on a centrifugal analyser (Cobas-Bio, Roche).

There was no evidence of any age trends in the data set and consequently the results from all subjects within each group have been analysed together. Similarly, within the group of patients with diarrhoea, no significant differences were observed between boys and girls and the results for both sexes have been combined. The data were transformed to logarithms to normalise positively skewed distributions. Urine volumes, secretory IgA outputs and creatinine outputs were standardised to amount produced per six hours. Student's $t$ test for independent samples was used to compare groups, paired $t$ test to compare the urines of patients collected at weeks 1 and 3. Geometric means (antilog (mean of logged values)) and, as a measure of variance, a value termed the geometric standard error (defined as [antilog(mean+standard error of logged values)–geometric mean]) are reported.

## Results

The results of the urine analyses are given in the table. For the subjects living in the rural community, secretory IgA outputs were significantly lower, by a factor of three (p<0·01), in the children with weight for age ≤74% compared with those who were better nourished.
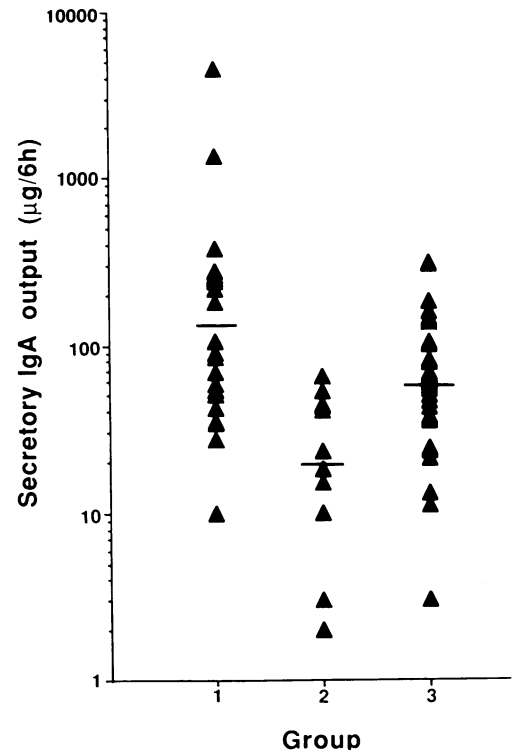


Urinary secretory IgA output of Gambian children. The groups of children are as follows: (1) malnourished with chronic diarrhoea, n=24, (2) controls without diarrhoea, weight for age ≤74%, n=11, (3) controls without diarrhoea, weight for age ≥75%, n=32. The horizontal bar across each group denotes the geometric mean.

No further effect of anthropometric status was visible in the children with weight for age ≥75%. In contrast, the secretory IgA outputs of the patients with chronic diarrhoea were significantly raised compared with the village children. The difference in mean IgA outputs was considerable: 6·8 times higher than the outputs of poorly nourished children (p<0·001) and 2·3 times higher than well nourished children (p<0·01). In the children with chronic diarrhoea, the higher outputs of secretory IgA were not related to anthropometric status and were still observed in those patients with severe malnutrition (geometric mean (+SE) secretory IgA output (μg/6h): weight for age ≤59%=127 (+33), n=12; weight for age ≥60%=120 (+75), n=12). These findings persisted when only the boys in the group with chronic diarrhoea were compared with the village boys (boys with

*Secretory IgA and creatinine in the urine of Gambian children with and without chronic diarrhoea*

| | Diarrhoea | Control | |
| --- | --- | --- | --- |
| | | ≤74% Weight for age | ≥75% Weight for age |
| Secretory IgA output (μg/6h) | 123 (+37) | 18 (+8)*§ | 54 (+10)† |
| Creatinine output (mg/6h) | 15 (+2) | 13 (+2)‖ | 19 (+2) |
| Secretory IgA concentration (μg/l) | 1340 (+426) | 433 (+190)‡ | 615 (+130)‡ |
| Creatinine concentration (mg/l) | 163 (+28) | 295 (+72)‡ | 252 (+33)‡ |
| Volume (ml/6h) | 95 (+15) | 43 (+13)† | 71 (+11) |
| Secretory IgA:creatinine (μg/mg) | 8·2 (+2·5) | 1·5 (+0·6)*‖ | 2·9 (+0·5)* |
| Total IgA: secretory IgA (μg:μg) | 1·3 (+0·1) | 1·4 (+0·2) | 1·2 (+0·1) |
| No of children | 24 | 11 | 32 |

Values are geometric mean (+geometric standard error) as defined in methods section. Control subjects are divided by weight for age relative to the NCHS reference.
Significance of $t$ test comparing diarrhoea and control children: *p<0·001, †p<0·01, ‡p<0·05.
Significance of $t$ test comparing control children with weight for age ≤74% and ≥75%: §p<0·01, ‖p<0·05.

diarrhoea, secretory IgA output (μg/6h): 91 (+32), n=11). No correlations were observed between secretory IgA output and weight for height in any group of children.

The raised secretory IgA outputs of the children with chronic diarrhoea were reflected in increased urinary concentrations of secretory IgA and secretory IgA: creatinine ratios (table). The ratio of total IgA: secretory IgA in the urines of both groups was similar at around 1·3, demonstrating that the majority of urinary IgA was in the secretory form, and that the proportion of species other than secretory IgA (fragments, serum IgA) were comparable in all groups. The similarities in total:secretory ratios and creatinine outputs between the patients and village children indicate that the observed differences between groups were unlikely to be artefacts caused by potential variations in the completeness of urine collections or in sample preservation.

No significant differences were observed in any parameter measured in the urines of the chronic diarrhoea patients at week 1 and week 3. The changes in values measured for each individual were not related to their clinical progress during the three week interval. No associations within this group were observed with anthropometric state or the presence of enteric parasites. The one subject with a urinary tract infection had secretory IgA outputs in the middle of the group range. No significant differences were noted in any parameter between boys and girls. The geometric mean (+SE) serum IgA concentration of the diarrhoea patients was 14·1 (+4·5) mg/l. No correlations were found between serum IgA concentrations and either urinary IgA concentrations or outputs at week 1.

## Discussion

This study has demonstrated that poorly nourished children living in rural West Africa have outputs of secretory IgA in their urine that are approximately one third of those of better nourished individuals. This finding is in agreement with reports from Thailand, India, and South America in which reductions of 35–50% have been noted in the secretory IgA concentrations of tears, saliva, nasopharyngeal fluid, and duodenal juice during moderate and severe malnutrition.[1-6] This study has also demonstrated, however, that malnourished children who are suffering from chronic diarrhoea have raised outputs of secretory IgA in their urine. Although previous studies have suggested that mucosal infection can stimulate local secretory IgA production in malnourished children,[9] this is the first report to suggest that infection of the gastrointestinal tract can elicit a response in the output of urinary IgA.

Secretory IgA is a large protein with a molecular weight of approximately 375 000 daltons. It is composed of dimeric IgA, produced by B lymphocytes resident in mucosal lymphoid tissue, which is coupled with a polypeptide, secretory component, in the local epithelial cells before secretion. In contrast, the IgA that circulates in blood is predominantly in a monomeric form with a small proportion present in polymers and complexes; none of these species contains secretory component. The use of two sensitive ELISA methods in the present study, one specific for secretory IgA, the other for any molecule containing regions recognisable by anti-IgA antibodies (secretory IgA, serum IgA, IgA fragments), established that the majority of IgA in the urine of Gambian children was secretory IgA and that it was the output of this molecule that was affected by malnutrition and infection. The observed rise in urinary IgA output during chronic diarrhoea cannot, therefore, be ascribed to an influx of small IgA species from the circulation. This view is further supported by the lack of any association between urinary IgA and serum IgA concentrations in the patients with chronic diarrhoea.

As the molecular size of secretory IgA is considerably above the upper limit for filtration through the kidneys, it is highly unlikely that secretory IgA in urine originates at a distant site and is transported into urine via the circulation. It is more probable that the secretory IgA is produced locally in the mucosal epithelium of the urinary tract. The low urinary IgA outputs of poorly nourished Gambian children noted in this study may have been the result of a reduction in the density of resident B lymphocytes as has been described in the gastrointestinal tract.[7] An increase in the population of these lymphocytes or stimulation of secretory IgA secretion could account for the observed rise in urinary IgA outputs by Gambian children with chronic diarrhoea.

The mechanism by which infection in the gastrointestinal tract could result in increased urinary IgA production is unknown. It is possible that lymphocytes from the lymphoid tissue of the gut could migrate through the lymphatic system to the urinary tract. This trafficking of lymphocytes between mucosal sites has been hypothesised to be responsible for the range of specificities observed in breast milk secretory IgA.[12] Alternatively, it is possible that some factor may be released into the circulation in response to the enteropathy that stimulates secretory IgA production in the urinary tract. This factor might possibly be a lymphokine released from activated T cells within the small intestinal mucosae. This same group of children was shown to have jejunal changes consistent with a cell mediated immune reaction to luminal antigens.[13] A factor with the ability to stimulate the differentiation of lymphocytes into IgA producing cells has been demonstrated in breast milk.[14] Whatever the mechanism involved, the results of this study indicate that there is communication between different secretory tissues of the body, a finding that is consistent with the hypothesis of a common secretory immune system.[15 16] They also demonstrate that, despite the reduction in secretory IgA production which accompanies poor nutritional state, malnourished children retain the capacity to mount a mucosal response to infection.

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## The *BMJ* and quack medicines

Sir William Osler considered the urge to take medicines to be one of the characteristics that define the human race. Useless cures have no doubt always been advocated, and not only by unqualified practitioners, and large fortunes have been made by the sellers of proprietory medicines of secret formula. In the September issue of *History Today* (1990;40:45–51) Peter Bartrip describes the heyday of patent medicines in the nineteenth and early twentieth centuries and the part taken by the *BMJ* in their control. Under two editors, Ernest Hart (1867–1898) and Dawson Williams (1898–1928) the journal mounted a campaign against proprietory medicines and Williams published analyses of various popular cures showing them to be made up of ingredients that were worthless in both a medical and a monetary sense. Publication of the exposés began in 1904 and a book, *Secret Remedies. What They Cost and What They Contain*, published in 1909 was a bestseller and followed two years later by *More Secret Remedies*. Largely as a result of the journal's campaigning, a parlimentary select committee was appointed 1912 to look into the problem and its report called for strict controls on the manufacture and advertising of patent medicines. Unfortunately the report was published in 1914 on the day the first world war began and the recommendations were ignored. Legislation in 1917, 1939, and 1941 prohibited advertising of cures for venereal diseases, cancer, and a variety of other conditions.

Despite its campaigning the *BMJ* continued, for financial reasons, to carry advertising for medicines of undisclosed composition and it was strongly criticised for doing so by the editor of the *Journal of the American Medical Association* in 1912 and by the Canadian Medical Association in 1924. Tobacco advertising caused a problem in the 1950s and was finally refused in 1957.

Peter Bartrip's book, *Mirror of Medicine. A History of the British Medical Journal*, was published by Oxford University Press on September 27th.

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