MATERIAL AND METHODS
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The present study was carried out in the Department of Paediatrics and Department of Biochemistry, M.L.B. Medical College, Jhansi.

Selection of cases:

The cases included in this study comprised of children presenting with protracted diarrhoea. Cases were picked-up from out-patient and admitted cases of the Department of Paediatrics. Diarrhoea was considered as protracted when it lasted for more than two weeks.

History and Clinical examination:

In each case a detailed history particularly with regard to diarrhoea, its duration and severity, nature of stool, colour of the motion, presence of mucous or blood was recorded. History of vomiting, abdominal distension, crampy abdominal pain, frothy stools, perianal excoriation, failure to thrive, fever, anorexia was noted. A detailed dietary history regarding the nature of feeds, date when artificial milk was started (including duration of feeds) was noted. Finally the
nutritional history, regarding the average amount of proteins and calories consumed was assessed.

In the physical examination main stress was laid on whether the child had signs of malnutrition. Besides, a general examination of other systems was done.

Investigations:

Stool examination: Macroscopic examination: Colour, odour, frothiness and presence of mucous or pus were noted. It was followed by microscopic examination of freshly passed specimen. Stool samples were examined for the presence of ova, parasites and cysts, particularly of giardia and Entamoeba histolytica. Presence of pus cells and red cells were also noted.

Stool culture: In every case stool culture was done at the time of admission.

Stool pH: estimation was done in all samples, immediately after collection. It was done using sensitive narrow range B.D.H. paper. pH estimation was done both at the time of admission and also at the time of discharge.

Stool for reducing substances:

All the samples within 1 hour of collection were tested for reducing substances by Benedict's test.
To 5 ml of Benedict’s reagent, 8 drops of liquid stool was added and boiled for about 2-3 mts. and the colour change, especially with precipitate formed, was noted. It ranged from Greenish yellow as 1+, yellow as 2+, orange as 3+ and Brick red as 4+.

In cases where sucrose was suspected as the offending sugar, acid hydrolysis were done. For hydrolysis, stool filtrate was boiled with equal amounts of N/10 HCl for 30 seconds, prior to testing with Benedict’s reagent. Presence of sugar in stool (＞5%) was taken as evidence of sugar intolerance.

Rubner’s test: All the samples were subjected to Rubner’s test which is yet another test for detecting the presence of reducing substances in the stool.

3-5 ml of liquid stool was taken in a glass tube. To this was added 0.3 - 0.5 g. of lead acetate. The solution was boiled for 2-4 min. and then cooled. Subsequently, 2-3 ml of strong liquid ammonia was added to above solution. It was again boiled for 2-4 min. and then allowed to stand for 5-10 mts. A pink or Brick red precipitate showed sugar in the stool while yellowish or dirty white precipitate showed negative results. If the test was negative, 2-3 ml of strong liquid ammonia solution was again added and the solution
was boiled for 2-4 minutes. After allowing resultant solution to stand for 5-10 minutes, colour of precipitate was again observed. The last procedure was done according to modified Rubner's test.

**Stool chromatography:** Initially the stool sample was prepared by suspending stool in distilled water, centrifuging and then filtering the supernatant. The filtrate was used directly for chromatography.

Ascending thin layer chromatography method was employed using silica gel as the medium, impregnated on glass slide. The solvent used was a mixture of N. Butanol, glacial acetic acid and distilled water in the ratio of 60 : 30 : 4. The stool sample along with pure standard solution of different sugars like lactose, sucrose, glucose and galactose were placed on the silica gel slide using fine capillary glass tubes. Then, the silica plate was kept in the glass chamber which contained the solvent. By capillary action, the solvent rose on the silica plate and in about 6 - 7 hours, solvent reached the top of plate. The plate was subsequently removed and dried in hot air oven at 110°C for about 15 minutes. The chromatograms was then stained using universal iodine dye.
The sugar present in each sample was identified by visual comparison of the sample spot with the spots of the standard sugar samples and a qualitative estimation was done (Stahl, 1969).