(1967) and (*d*) as (*b*) but with 10% NaCl added to the diluent reagent.

This finding is substantiated by the results from 67 different sera analysed in duplicate by the manual and the modified AutoAnalyzer methods (Fig. 1). Although there is good correlation between the two methods (correlation coefficient r = 0·995), the results given by the latter method are higher (regression coefficient b = 1·11).

An attempt to improve further the modified AutoAnalyzer method by increasing the concentration of NaCl from 5 to 10% was unsuccessful (Table). The use of sera with known iron content to replace the standard iron solutions is the ideal way of correcting the discrepancy. However, this is not always practicable and it is suggested that a correction factor (−10%) should be applied to results obtained by the AutoAnalyzer procedure.

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Technical methods

A simple concentration method for the detection of parasitic ova and cysts in faeces

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During the course of a survey of intestinal parasitic ova and cysts in recently arrived immigrant children carried out by this laboratory, (Thompson, Hutchison, and Johnston, 1972), it rapidly became obvious that a method was required which would fit easily into the routine of a busy laboratory. Such a method was required to yield as many positives for helminth ova and protozoal cysts as possible on the single specimen submitted, and to be technically simple and not time consuming. The method evolved makes use of concentration by centrifugation combined with the addition of a dye mixture: this gives (1) good contrast on microscopy of the wet preparations and (2) tolerates the addition of Lugol's iodine for the confirmation of protozoal cysts, since the combined dye mixture is stable.

Method

A portion of faeces about the size of a pea is emulsified with a wooden stick in a tube of peptone broth (5 ml). With a large-bore (approximately 2 mm external diameter) Pasteur pipette the supernatant is transferred to a centrifuge tube. Two drops (approximately 0·05 ml) of the stain mixture, consisting of equal parts of 10% (w/v) aqueous Nigrosin and 1% (w/v) aqueous Alcian Blue, is added, together with 4 to 5 drops of concentrated formalin solution (40 vol %). The tube is centrifuged at 3 000 revs/min for five minutes in the MSE Minor. Most of the supernatant is then discarded into Lysol, leaving a volume of fluid equal to that of the button of deposit. With a Pasteur pipette the mixture is gently homogenized and 'wet preparations' are made. Scanning for ova and cysts is carried out using the 16 mm objective with a daylight filter for three to five minutes.

To confirm the morphology of protozoal cysts,

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1 to 2 drops of Lugol's iodine is placed on a slide together with a drop of the stained faecal concentrate. Gently mixing with a pipette and the addition of a coverglass produces a suitable preparation.

Results

Single samples of faeces from each immigrant child were examined in the above way, and among 4 000 children a crude helminth infestation of 36·7% was found; of 2 709 samples examined *Giardia lamblia* was found in 9·2%.

By this method all the common ova, ie, hookworm, Enterobius, Ascaris, Trichuris, Hymenolepis, Schistosoma, and Taenia were easily found, as were the cysts of *Entamoeba coli*, *Iodamoeba bütschlii*, *Giardia lamblia*, and *Endolimax nana*.

Hymenolepis, hookworm, and Enterobius ova are brightly transparent, with a straw-coloured tinge and stand out against the grey-purple background. The black mammillated outer coat of the intact Ascaris ovum is accentuated. The characteristic orange-brown colour of Trichuris ova and the light brown colour of *Schistosoma mansoni* ova are easily seen against the background stain.

It should be noted that the amount of formalin added is critical; an increase causes shrinkage of the protozoal cyst contents, particularly with Giardia. Furthermore, if observations on the viability of *Schistosoma mansoni* ovum are required, eg, detection of mirocidial movement, ciliary action, or flickering of the flame cells, the formalin is omitted.

Occasionally the faeces were processed up to and including the addition of the dye mixture and left overnight at room temperature or in the cold room; completing the method the following day resulted in no appreciable deterioration in morphology or numbers of ova or cysts.

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New technique for the observation of sickling under known pO_2 and percentage oxgyen dissociation

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In the past, attempts to correlate pO_2 levels with the degree of sickling required the fixation of red cells before their examination. This was usually achieved by formalinization (Allison, 1965; Harris, Brewster, Ham, and Castle, 1965; Cerami and Manning, 1971).

The advent of potential therapeutic agents, capable of suppressing the sickling phenomenon, made a simpler and more direct technique for observation of sickled cells under known pO_2 tensions desirable.

Method

It is possible to correlate the oxygen dissociation curve of haemoglobin with pO_2 tensions, using the method of Bellingham and Huehns (1968). This has been conveniently achieved by suspending washed red cells in an isotonic phosphate buffered saline and measuring the absorption curves in a modified SP800 recording spectrophotometer (Unicam, Cambridge). The variable pO_2 was achieved by prior exhaustion of the cuvette under vacuum and subsequent controlled admission of air.

In order to view and photograph the cells in the cuvette, they must be immobilized during the exposure of the film. This can be achieved by boring a 2 mm diameter hole with a diamond drill through the centre face of a wall of the unassembled cuvette. The hole is then covered by annealing a 1 mm thick coverslip to the outer wall. All faces of the cuvette are optically planed and polished (Wesley Coe Ltd, Cambridge).

It is theoretically undesirable to begin the experiment by sickling all cells under study by total evacuation of the tonometer. Decreasing pO_2 tensions are obtained by blowing known mixtures of nitrogen and oxygen (Rotometers Ltd, Croydon) into the cuvette over the surface of the red cell suspension before equilibrating for 10 minutes at 37°C.

The thickness of the film of red cell suspension adherent to the under surface of the coverslip may be adjusted by gently tapping the cuvette.

After standard microphotography, the absorption curve is read. Finally, the pO_2 of an aliquot of/the

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