supernatant. The suspension is transferred to a bijou bottle connected to the Hemming's filter containing a Whatman no. 41 filter paper. The bottles with the filter are centrifuged at about 2,000 r.p.m. for five minutes. This retains particles of diameter greater than $5\ \mu$, including epithelial cells, but allows bacteria and smaller debris, *e.g.*, phosphates, to pass through.

Glycerin albumin is spread thickly on two 3×1 in. slides. The moist filter paper is removed from the Hemming's filter and an imprint made on the first slide. The imprinted material is relatively fluid and is mixed by means of a slide end with the glycerin albumin on the slide. The mixture is drawn down with the slide edge into several parallel thick transverse lines, to make scanning for cells easier. The filter paper is then placed firmly on the second slide and the imprinted area ringed on the reverse of the slide with a diamond. The filter paper is then discarded.

The slides are fixed in formalin vapour at 60°C . for one hour, washed in water, and stained at room temperature for 10 minutes with cresyl fast violet. The smears are finally washed in water and mounted in glycerine jelly.

This technique removes much of the debris normally found even in a fresh urine. It is possible to use a cellulose acetate filter in place of the Whatman no. 41, but being a finer filter much debris is retained.

This technique may have some value in the examination of cells in urine deposits for malignancy.

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Glove box system for micro-determination of protein-bound iodine in children's blood

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The use of blood samples from pricked fingers or heels is essential in paediatric practice. In central London, a commensurate reduction in the scale of quantities necessary for the well-established procedure for estimating protein-bound iodine (P.B.I.) of Foss, Hanks, and Van Slyke (1960) resulted only in excessive atmospheric contamination. To overcome this the procedure is now carried out in the glove box system to be described.

APPARATUS

The glove box, shown in the figure, consists of a Dexion-supported table and working chamber. The rear wall of the system is made of block-board to provide strength. The left hand wall of asbestos carries a central hole and short sheet iron tunnel connexion to the muffle furnace. The furnace door operates without hindrance and the electrical controls were detached and reinstalled below the table.

FIG. 1. The glove box is described in the text. Dimensions (cm.) are: table height 80; main chamber 90 wide, 55 deep, 80 high; air-lock $45 \times 45 \times 45$; glove holes 15 diam., 16 from table top, centres 45 apart. Perspex parts cut from 10 mm. sheet.

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The right hand wall of the main chamber is made of Perspex and carries a vertically operating sliding door connecting with the 'air-lock' chamber, and is operated by wires running over pulleys to a foot-operated treadle. The air-lock has a hand-controlled, vertically operating external sliding door through which samples and reagents are passed. Below are mounted the main electrical controls, the respirator canister¹, the flow meter and, to the rear and not visible in the figure, the pump².

A centrifuge (M.S.E. minor) is built into the floor of the main chamber with motor and controls outside the chamber. The floor space of the chamber is ample for the tube racks, reagent bottles, and small 37° operating water bath (Luckham, Ltd.) The perspex front panel of the main chamber carries the rubber gloves³ worn by the manipulator.

OPERATION

During operation purified air was continually supplied to ports at the rear of both chambers by the pump and respirator system. The procedure of Foss *et al.* (1960) requires the centrifuge for the Somogyi precipitation and washing of the protein, the muffle furnace for the incineration with alkali at 600° and 37° water bath for control of the catalysed ceric-arsenite and the brucine colour development reactions. The rack of tubes was passed to the outside through the air-lock for measurement of the extinctions due to the colours, using the micro cells (0.5 ml.) of a Spectrochem (Hilger and Watts) instrument.

CHEMICAL PROCEDURE

The procedure of Foss *et al.* (1960) was scaled down to use 0.2 ml. samples of serum. KOH instead of Na₂CO₃ was tried for the incineration (Vilkki, 1958; Malkin, 1965) but was found to be without significant effect. Likewise incorporation of KClO₃ (Marlow, 1958) was not adopted since variable recoveries were obtained. The brucine end point of Grossmann and Grossmann (1955) was found to increase sensitivity and was adopted.

Iodide standards were used at three concentrations

¹Puretha type CC, Siebe Gorman Ltd., Chessington, Surrey.

²RB4, Edwards High Vacuum Ltd.

³Veedip Protective Gauntlets, RL015/023.

with every batch of analyses, together with Versatol serum of known P.B.I. concentration to check recoveries. Control charts were plotted of calibration slope, Versatol result, and thyroxine recovery. Batches of results would be rejected if the standard readings were not colinear and not of usual slope or if the Versatol result deviated 5% from that expected.

COMMENT

Authors describing micro methods for the determination of P.B.I. (Sanz, Brechbühler, and Green, 1956; Malkin, 1965) do not state that special precautions are required to avoid atmospheric contamination. Since enclosing our procedure in the glove box the sporadic occurrence of spuriously high results has ceased and was presumably originally due to atmospheric contamination. The calibration points have remained colinear, the slope has not deviated from 0.29/100 mμg. iodine, and the Versatol standard has given an average value agreeing well with the makers' statement of serum P.B.I. concentration; for example, determined concentration 4.69 ± 0.21 (S.D.) μg./100 ml., stated concentration 4.7.

SUMMARY

A glove box system is described for the protection of the procedure for the determination of P.B.I. from interference by atmospheric contamination. Serum samples 0.2 ml., from finger or heel-prick blood are used.

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