Technical methods

Simple, inexpensive micro method for the estimation of T_3 uptake

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An inexpensive micro method for T_3 uptake has been devised. This makes use of only 0·1 ml of patient's serum, approximately 130 mg of Sephadex, and approximately 0·15 μCi of ^{125}I-liothyronine. After incubation the Sephadex is washed three times and the radioactivity is then measured. Centrifugation is not required during the washing process because the Sephadex beads settle rapidly.

Requirements

The following should be assembled: 15 ml polypropylene cylindrical centrifuge tubes (semi-transparent); Sephadex beads—G.25, coarse (Pharmacia); liothyronine labelled with ^{125}I (Radiochemical Centre, Amersham); isotope counting equipment incorporating a well-crystal scintillation counter; standard normal serum (pooled sera prepared from batches of 30, stored at −20°C and replaced every two months); 0·1 M citrate buffer *p*H 5·2; a small cup to measure around 130 mg of Sephadex with a reproducible accuracy of ±2%. This can be made of glass but must be filled from a metal spatula[1]; automatic pipette for delivering isotope solution and buffer, set at 0·75 ml; micropipette to deliver 0·1 ml of serum; 10 ml syringe with spring-return piston for washing Sephadex deposit.

Preparation

^{125}I-Liothyronine (200 μCi) is diluted to 10 ml with 50% aqueous propylene glycol and stored in the refrigerator. For two weeks' use it is further diluted to approximately 0·15 to 0·2 μCi per ml with 0·1 M citrate buffer, *p*H 5·2, and stored in the refrigerator. This quantity is equivalent to about 5 nanograms per test. Lots of 50 ml are convenient to do about 65 tests. The Sephadex is dispensed into the centrifuge tubes using the measuring cup, and 0·75 ml of citrate buffer is added to the Sephadex which is allowed to swell for a few minutes.

[1]I have made a cup, as follows: 7·5 mm (O.D.) glass tubing is drawn out to a tapered form, cut at the narrow part, and the narrow end closed in the flame. The wide end is cut forming a cup 1 cm long and with an internal diameter of 5 mm at the open end. This is then fused to a length of glass rod of 3·75 mm (D). This cup is filled with Sephadex to overflowing by means of a vibration spatula. The weight delivered varies within ±2%. The excess Sephadex is caught in a receptacle and returned to stock.

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The Test

This is carried out at room temperature (18°-26°C). Only small variations of uptake due to temperature are encountered in this range (Cavaliere, Castle, and Searle, 1969) affecting standard and unknowns equally.

1 Of test serum, 0·1 ml is added to the Sephadex-buffer mixture (in duplicate if preferred) and gently mixed.

2 Of diluted ^{125}I-liothyronine, 0·75 ml is added to each tube, using the automatic pipette, and mixed by gentle tapping. The mixing is repeated occasionally until at least 60 min has elapsed (Fig. 1). A permanent mark is made on the tubes some 2 mm above

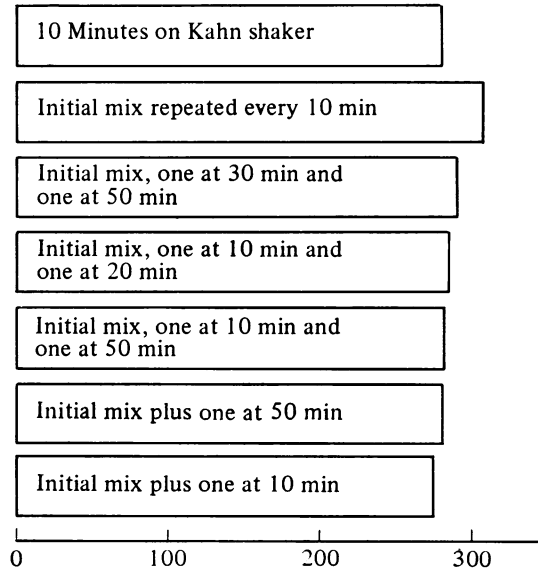

Fig. 1 *Uptake counts obtained with thyrotoxic serum after one hour at room temperature comparing different mixing procedures.*

the level of the Sephadex. This represents about 1 ml of free fluid. At this stage the radioactivity of the tubes may be counted by timing 10,000 counts to ensure that each has received the same dose of liothyronine.

3 WASHING

The spring-return syringe is filled to 9·0 ml with tap water, a Teflon catheter needle, 6 in. long, 0·05 in. I.D. attached to it, and the tip of the Teflon tube passed to the bottom of the centrifuge tube containing Sephadex etc. Pressure on the piston delivers a rapid flow of water which suspends the Sephadex. Frothing should be avoided as Sephadex granules may be trapped in the froth. The syringe is withdrawn, refilled, and the operation repeated on the next centrifuge tube. By the time 10 or 20 tubes have been filled in this way, the Sephadex in the first tube will have settled, and the supernatant fluid is removed from each tube in sequence down to the mark, with a Pasteur pipette attached to a filter pump. This leaves about 1 ml of fluid mixed with the Sephadex. The washing is repeated twice more, and the Sephadex precipitate is ready for radiation counting, when each sample is timed for 10,000 counts.

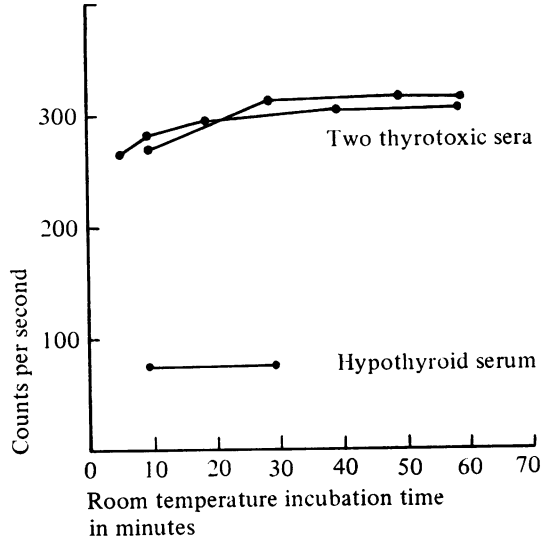

Fig. 2 *This shows that incubation must be continued for at least 40 minutes to ensure completion of uptake.*

Calculating Results

Assuming that all the tubes have received the same dose of liothyronine, there is no need to calculate the percentage of radioactivity taken up by the Sephadex in each tube. Instead, the count obtained in the tube containing the standard normal serum can be taken as unity and the tests recorded as a ratio to this (Sephadex uptake ratio).

Some Special Considerations

1 CHOICE OF SEPHADEX

(a) This material has the advantage of not binding free radioiodide (Clarke, 1967). Therefore, liberation of free radioiodide, consequent on dissociation of the liothyronine (which may occur in time), will not affect the uptake results. Resin, on the other hand, may bind free radioiodide.

(b) It has been found that Sephadex binding is completed with myxoedema sera in about 10 minutes at room temperature, but thyrotoxic sera may require 40 minutes before further uptake ceases. Therefore, provided at least 60 minutes is allowed for binding to the Sephadex no correction for time is required (Fig. 2).

2 CHOICE OF POLYPROPYLENE TUBES

(a) These tend to be of uniform dimensions and are more suitable for counting relatively soft isotopes such as ^{125}I.

(b) Using a buffer of *p*H 5·2, the tubes take very little part (approximately 10%) in the competitive absorption of the added liothyronine, and are very easily cleaned with Lab-brite or similar materials.

3 CHOICE OF BUFFER

0·1 M citrate buffer at *p*H 5·2 gives much higher Sephadex uptake than phosphate buffer *p*H 7·2 or *p*H 7·5 (personal observation).

4 CHOICE OF FLUID FOR WASHING SEPHADEX

Tap water gave nearly as good results as buffer for this purpose, and was more convenient. Binding of liothyronine to the polypropylene tubes accounted for up to 10% of the total binding. Washing with buffer (*p*H 5·2) reduced this to 2%. In either case the final result with the test sera was unaffected.

5 SERUM CONCENTRATION

Although Maclagan and Howorth (1969), using Amberlite resin, and Cavaliere *et al* (1969), using Sephadex, required a final serum concentration respectively of 1/50 and 1/32, the widest range in the method presented here was given by a final serum concentration of 1/16.

6 COST OF REAGENTS

Sephadex

Allowing some 6% for wastage, each test would cost approximately 1·5 pence (0·625 N.P.).

Liothyronine

Allowing 500-600 tests (out of a possible 13,00-1,600 tests) for the 200 μCi, cost per test would be 3p.

Buffer

Cost per test 0·01 pence (0·0042 N.P.).

Total cost per test is 8·71 pence (3·63 N.P.) which compares favourably with the resin sponge method costing 135 pence (56·3 N.P.) per test in kit form.

7 ACCURACY OF THE METHOD

One hundred and eight consecutive sera were tested in duplicate and the standard error of this method was found to be 2·221%.

8 AGREEMENT WITH RESIN SPONGE METHOD (TRIOSORB-125)

Figure 3 shows a comparison of results obtained by the Sephadex and resin-sponge methods. From this the normal limits of the Sephadex uptake ratio are 0·85 to 1·34 compared with 0·86 to 1·20 for the resin-sponge method.

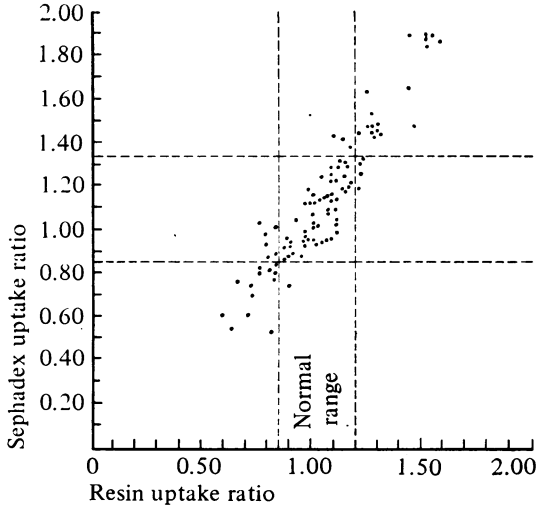

Fig. 3 *The results of duplicate estimations done by the Sephadex method compared with single estimations on the same sera done by the resin method. 'Uptake ratio' indicates the uptake of the unknown divided by the uptake of normal serum.*

Demonstration of sensitized lymphocytes in blood

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Under certain circumstances, both physiological and pathological, it becomes of interest to determine the occurrence of sensitized lymphocytes in blood. The macrophage migration inhibition test has been adapted for this purpose (Soborg and Bendixen, 1967) but presents certain technical features which make it, in the hands of some workers (Kaltreider, Soghor, Taylor, and Decker, 1969), unreliable and open to error. (This has been our own experience to date (Hughes, unpublished data).) A simple, reliable and reproducible method has now been developed both for human and animal work and offers quantitative results suitable for statistical analysis. In principle it depends upon the interaction of sensitized lymphocytes with their sensitizing antigen to produce a factor which causes inhibition of macrophage migration and forms the basis of the macrophage migration inhibition test (Bloom and Bennett, 1966). The same or a similar factor also reduces electrophoretic mobility of macrophages (Sundaram, Phondke, and Ambrose, 1967; Hughes, Caspary, and Field, 1970)—an action which can be blocked by antilymphocytic serum (Field, Hughes, and Caspary, 1969; Caspary, Hughes, and Field, 1970). In the reduction of macrophage mobility by specific antigen (Sundaram *et al*, 1967; Diengdoh and Turk, 1968; Field *et al*, 1969; Hughes *et al*, 1970) the latter interacts with those lymphocytes (10-15%) present in the peritoneal exudate of the sensitized animal. In the method described here, antigen is allowed to

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