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.

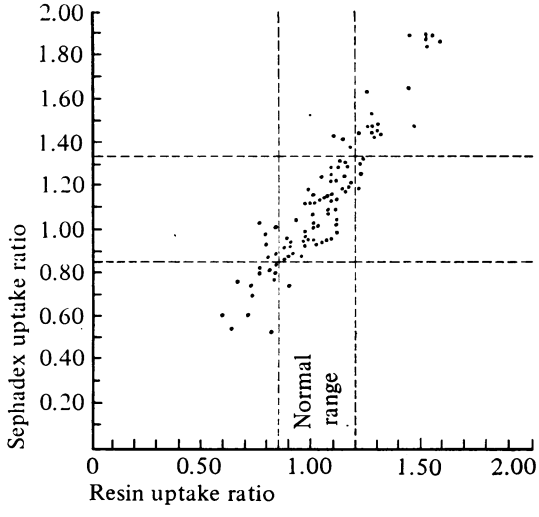

Fig. 3 *The results of duplicate estimations done by the Sephadex method compared with single estimations on the same sera done by the resin method. 'Uptake ratio' indicates the uptake of the unknown divided by the uptake of normal serum.*

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 (This has been our own experience to date (Hughes, unpublished data).)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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Simple, inexpensive micro method for the estimation of T_3 uptake—continued

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react with the blood lymphocytes under test and the effect upon guinea pig macrophage electrophoretic mobility measured, ie, the guinea pig macrophages are used purely as an indicator system of lymphocyte-antigen interaction. Preliminary experiment showed, as anticipated, that simple mixture of blood lymphocytes with guinea pig peritoneal exudate resulted in a mixed lymphocyte reaction. In order to eliminate this the guinea pig peritoneal exudate (with its 10-20% lymphocyte content) was irradiated. Trial showed that 100 rads (Cobalt 60γ rays, at 56 cm from a 4,000 Curie source, dose rate 100r in 54 sec) was adequate to incapacitate peritoneal lymphocytes from taking part in a mixed reaction although it did not interfere with their viability as measured by a dye exclusion test. Macrophage electrophoretic mobility was unaffected by this treatment. Irradiated guinea pig exudate was therefore usable as an indicator system for the detection of macrophage migration slowing factor resulting from interaction of blood lymphocytes and antigen to which they might be sensitized.

The exudate of guinea pig peritoneal macrophages was elicited by intraperitoneal injection of sterile liquid paraffin and washing out the peritoneal cavity with Hanks balanced salt solution containing 5 units of heparin/ml some eight to 12 days later. This suspension was washed twice (spinning at 250 *g* for 10 minutes) in heparinized balanced salt solution and finally suspended in TC 199 (without heparin) the volume being adjusted to yield 10^7 cells/ml. Usually 80-200 $\times$ 10^6 macrophages were obtained from a guinea pig of 400-600 g.

Blood lymphocytes were obtained by venepuncture (or cardiac puncture where appropriate in animals). Twenty ml blood was defibrinated with glass beads, followed by removal of polymorphs with saccharated iron as used by Hughes and Caspary (1970) in their modification of the method of Coulson and Chalmers (1964). Generally there was a yield of about 10^6 lymphocytes/ml blood.

In carrying out a test 0·5 $\times$ 10^6 blood lymphocytes (in 0·5 ml TC 199) were added to 1·0 ml of TC 199 containing 10^7 irradiated guinea pig macrophages and 1·5 ml TC 199 added to bring the total volume to 3 ml. To this the antigen to be tested was added in 0·1 ml of saline. The actual amount of antigen may have to be determined, but in our own experiments we have used 100 μg of encephalitogenic factor (Caspary and Field, 1970), or special aliquots of thyroid derived antigens (Field, Caspary, Hall, and Clark, 1970). This mixture was allowed to stand at laboratory temperature (18-20°C) for 90 minutes. Controls were set up with antigens not suspected, eg, kidney extract, zoster antigen. Electrophoretic mobility was measured in a Zeiss cytopherometer. All measurements were made in TC 199 medium and experience has shown that different batches of this medium might give slightly different actual migration speeds. However, differences between controls and positives were preserved exactly. It should be added that some experience with the cytopherometer is needed before consistent results can be expected and that it is useless to begin a set of observations unless the apparatus is 'behaving itself'. In our experience common sources of trouble are the draining stopcock or microleaks at the partitioning membranes. A cell was timed in both directions of the potential difference, care being taken that only macrophages (easily identified under phase contrast by their size and liquid paraffin content) are studied. Ten different cells were used for each specimen so that the mean of 20 readings could be computed. The ambient temperature of the electrophoretic cell was maintained at 23 $\pm$ 0·05°C. It has been our practice to number specimens and make all measurements 'blind' so that possible bias in observation is eliminated.

Migration speed in the presence of antigen is compared with that in its absence, ie, in the control mixture of irradiated macrophages and lymphocytes under test, and may be expressed as a percentage change. Results have been clear cut in neurological (Caspary and Field, 1970) and thyroid (Field *et al*, 1970) disease, but the method is applicable to a very wide spectrum of physiological and pathological conditions in which sensitization of circulating lymphocytes to possible antigens is suspected or is to to be studied. Such conditions range form autoimmune disease to sarcoidosis (where delayed sensitivity anergy would be of special interest), rheumatoid arthritis, sensitization to viral antigens, sensitization to placental tissue in unexplained repeated abortion, systemic lupus erythematosus, and many other conditions. In addition we have shown that the method can be used as a rapid means of LATS assay in serum; as an *in vitro* method of titrating antilymphocytic serum (since ALS has a blocking action in the test) and as a possible means of controlling the effects of therapy in multiple sclerosis or in graft rejection.

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