either beyond the termination of a contraction or in susceptible subjects to the end of a contraction, will result in loss of consciousness at the amnesic level. With 70% nitrous oxide unconsciousness will occur sooner.

Most mothers stated that the gas and oxygen machine helped them, and nearly three-quarters of them found that the help was complete or considerable; the replies of the midwives bear out these findings. Just over a quarter of the mothers, however, found the help inadequate, the figures again being confirmed by the midwives' replies, and there is clearly room for improvement—though it has already been pointed out that any analgesic for use in labour must not only relieve pain but leave unimpaired the mother's ability to co-operate and exclude the risk of fetal anoxia. Apparatus designed to supply a variable concentration of gas, to be adjusted according to the mother's response, makes it possible to reconcile the requirements of pain relief and of co-operation of the mother more satisfactorily than do machines delivering a fixed concentration; such apparatus is already in use under medical supervision in hospitals.

Many of the questions in this investigation were inevitably of a subjective nature. It may, however, be concluded that the 50% oxygen and 50% nitrous oxide mixture can safely be used by unsupervised midwives, both in hospitals and in domiciliary practice. Clearly there is a need for further re-

search to improve the standard of obstetric analgesia in Britain, and it is apparent that in any such research the many variables to be found in both the mother and the midwife must be taken into account.

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Evaluation of Thyopac-3 Test in the In-vitro Assessment of Thyroid Function

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Summary: Thyopac-3, a new commercial test kit for the in-vitro assessment of thyroid function, has been appraised in a group of 411 patients. The Thyopac-3 test was found to be simple and rapid to perform and accurate in diagnosis. Of 117 patients shown to be euthyroid by other criteria, 5% had Thyopac-3 values outside the normal range (derived from a group of 135 normal patients), and 10 of 79 thyrotoxic and 4 of 37 hypothyroid patients had values within the normal range.

In a direct comparison in 190 patients with the resin uptake test the Thyopac-3 test emerged very favourably in regard to diagnostic accuracy and was technically easier and quicker to carry out. There was a non-linear negative correlation between results from the two procedures.

Introduction

Numerous in-vitro techniques for assessing thyroid function and status have been developed since the introduction of the red blood cell (R.B.C.) uptake of $^{131}\text{T}_3$ (Hamolsky *et al.*, 1957, 1959). These include uptake of labelled thyroid hormones by anion exchange resin (Mitchell *et al.*, 1960; Sterling and Tabachnick, 1961; Clark, 1963), coated charcoal (Herbert *et al.*, 1965; Irvine and Standeven, 1968), and Sephadex (Hansen, 1966). The last material has been used in chromatographic systems (Shapiro and Rabinowitz, 1962; Cuarón and Fucugauchi, 1964), and dialytic (Cavalieri and Searle, 1965; Garnett *et al.*, 1965), and electrophoretic methods (Burke *et al.*, 1964) have also been described.

All these tests are based on the fact that thyroid hormones are transported in a reversible binding equilibrium by specific plasma proteins (Robbins and Rall, 1957). Consequently when exogenous radioactive labelled thyroid hormone is added to plasma (or serum) it will partition, depending on the previous degree of saturation of binding sites with endogenous hormone, between a bound and an unbound form, the latter being available for absorption to other sites (R.B.C., resin, etc.) or capable of diffusion through a dialysis membrane. Thus, when binding sites are relatively saturated owing to an excess of endogenous hormone, as in thyrotoxicosis, a greater proportion than normal of labelled hormone remains dissociated from the proteins (or, conversely, a decreased quantity is bound) and increased "uptake" values result. In hypothyroidism the reverse occurs.

Absolute changes in binding protein concentration without thyroid disease will in themselves cause changes in these uptake measurements—for example, in pregnancy, oestrogen therapy, or the nephrotic syndrome—as will competition for binding sites by drugs (salicylates, diphenylhydantoin). Such alterations may mislead the clinician but can be recognized if a serum protein-bound iodine (P.B.I.) is also performed (Clark and Horn, 1965).

The several procedures have their own particular disadvantages. Variability in the radiochemical purity of ^{131}I T₃, especially with respect to the degree of contamination with ^{131}I iodide, may cause considerable fluctuation in results from resin uptake or dialysis. This fluctuation may be minimized by comparison of the test sera with a simultaneously run control, or by use of ^{125}I T₃. Though isotope impurities are not of such significance in the R.B.C. uptake, here a correction for packed cell volume is essential. Finally, most methods are fairly laborious technically or time-consuming.

Recently the Thyopac-3 test (Radiochemical Centre, Amersham, 1969), a new in-vitro method, also based on

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protein-binding, has been marketed. Owing to the generosity of the Pharmaceutical Department of the Radiochemical Centre, we have been able to assess this test in a large number of patients and to compare it with an earlier technique (resin uptake, Clark, 1963). The Thyopac-3 test differs from the conventional uptake methods in that the radioactivity left in serum after incubation rather than that absorbed by the secondary binding material (R.B.C., resin, etc.) is measured. Therefore in thyrotoxicosis a low serum retention and not a high uptake is obtained, and in hypothyroidism the opposite, when compared with normal.

Methods

Thyopac-3 Test

Each test unit consisted of a glass screw-cap phial (69 by 17 mm.) containing measured quantities of buffer (with preservative), ^{125}I -labelled triiodothyronine, and absorbent granules. To each phial 0.1 ml. of serum was added, and phials were incubated in batches of 16 by rotating for 15 minutes at about 25–30 r.p.m. on a Matburn blood-cell suspension mixer at room temperature. Subsequently the phials were removed and sharply inverted twice to wash all granules off the cap and sides. After standing for a further minute, which allowed the granules to settle, 1 ml. of the supernatant fluid was pipetted from each phial into counting-tubes, and the radioactivity assayed in a well counter, to a total of 10,000 counts for each specimen. All specimens were tested in duplicate and a control serum was run in each batch. The mean radioactivity of the duplicate samples was then expressed as a ratio to that obtained from the control serum.

Resin uptake test

The resin uptake of ^{131}I L-triiodothyronine was performed according to a previously described method (Clark, 1963). The same control serum was used as for the Thyopac-3 test.

Sera

(1) Control serum consisted of pooled hospital laboratory discards, and was stored at -20°C . in 20-ml. aliquots. When required an aliquot was thawed and subsequently refrozen. Each was used until exhausted.

(2) *Test Sera.* (a) *Thyopac-3 Test.*—The sera from 411 persons were tested. Of these, 135 (76 men and 59 women aged 23 to 82 years) were patients from a general practice who had no clinical or serological evidence of thyroid disease and who were not on drugs known to affect thyroxine transport (normal group); 117 (27 men, 90 women) were patients referred to hospital with suspected thyroid disease, who by clinical and biochemical or radioisotope investigation were shown to be euthyroid (euthyroid group); 116 were patients with thyroid disease (79 thyrotoxic—11 men, 68 women; 37 hypothyroid—11 men, 26 women), confirmed by P.B.I. and ^{131}I thyroid uptake and by response to specific therapy (this group was unselected in that all patients with proved thyrotoxicosis or hypothyroidism seen during the period of evaluation of the Thyopac-3 test were included); the final group comprised 43 women who were in the second or third trimester of pregnancy (19) or were taking oestrogen containing oral contraceptives (24). (b) *Resin Uptake Test.*—Of the sera group mentioned above, 190 also had resin uptakes assessed (111 euthyroid, 58 thyrotoxic, 21 hypothyroid).

Results

The results obtained in the Thyopac-3 test in 411 patients are shown in Fig. 1. The normal range (95% confidence limits) of 0.91–1.21, mean 1.06, was derived from the normal group. Of the 117 in the euthyroid group, 5 (4.3%) had values outside the normal range, a proportion similar to that of the normal group, 5 (3.7%); 10 of the 79 thyrotoxic patients

FIG. 1.—Scatter diagram of results of Thyopac-3 test in normal people, in patients who were shown to be euthyroid, in those with thyroid disease (thyrotoxicosis and hypothyroidism), and in women who were pregnant or taking oral contraceptives.

(12.7%) and 4 of the 37 hypothyroid patients (10.8%) fell into the normal range. Of the pregnant/oral-contraceptive group, 34 were in the hypothyroid range (20.9% in normal range), as would be expected from previous experience. The mean and S.D. of the various groups are shown in the Table. In the

Results of the Thyopac-3 Test in the Groups Tested, Expressed as a Ratio to Control Pooled Serum

Group	No. in Each Group	Mean Ratio	S.D.
Normal { Total	135	1.06	0.08
Male	76	1.04	0.07
Female	59	1.09	0.07
Euthyroid	117	1.06	0.07
Thyrotoxic	79	0.80	0.09
Hypothyroid	37	1.28	0.07
Pregnant/oral contraceptives ..	43	1.30	0.10

normal group the mean ratio for males (1.04) differed significantly from that for females (1.09) ($P < 0.001$), in keeping with numerous previous observations. In addition, the mean of the normal group (1.06) differed significantly from unity ($P < 0.001$) presumably because the control serum did not accurately reflect the true norm.

Fig. 2 shows the relation between the resin uptake test and the Thyopac-3 test in 190 patients. Of 111 euthyroid patients, 7 (6.3%) were outside the normal range for the resin uptake test (0.82–1.28) and 5 (4.5%) outside the normal range for the Thyopac-3 test; 14 (24.1%) and 10 (17.2%) of the 58 thyrotoxic and 7 (33.3%) and 3 (14.3%) of the 21 hypothyroid patients lay within the normal ranges respectively for resin uptake and Thyopac-3 test; 12 of the euthyroid patients were outside the normal ranges of both tests, and six thyrotoxic patients and one hypothyroid patient were within. There was a negative relation between the two measures which was non-linear. An explanation for the former is given

FIG. 2.—Relation between results of Thyopac-3 and resin uptake tests in patients who were euthyroid or who had thyrotoxicosis or hypothyroidism, expressed as a ratio to the same control serum. The normal range for each test lies within the appropriate dotted lines.

in the Introduction. Non-linearity was apparently due to the fact that the resin uptake ratio showed a skewed distribution about the normal range, with the tendency for particularly high ratios in thyrotoxicosis, whereas the Thyopac-3 ratio was more evenly spread. The reason for this difference was not obvious and examination of the problem was deferred.

In a total of 176 duplicate estimations the standard deviation of the experiment was ± 0.022 as a ratio. Since all estimations were performed in duplicate the standard error of the mean of the duplicates was ± 0.016 as a ratio. In another 125 estimations comparing the control serum and another "standard" serum (supplied by the Radiochemical Centre) the latter gave a mean ratio of 0.94 ± 0.04 (S.D.) to the former, with a coefficient of variation of 4.41%.

Discussion

Though results from "uptake" tests based on thyroxine binding are comparative and not absolute, their value in diagnosis in thyroid disease is generally accepted (Clark, 1967). The Thyopac-3 test has proved to be an easy, quick, and accurate example of this type. There is minimal manip-

*The time of incubation, provided it is greater than 10 minutes, is immaterial, as supernatant radioactivity, with incubation at room temperature (21°C), remains constant after that period (own observations). This in itself can be a great advantage as clock-watching in the incubation phase is then unnecessary.

ulation (only two pipetting stages) and a short incubation period (15 minutes),* compared with times of 30 to 120 minutes for R.B.C. uptake and of 45 to 120 minutes for resin uptake. Incubation with coated charcoal is brief but centrifugation is necessary. The entire Thyopac-3 procedure for 16 samples from initial pipetting to final calculation of results following measurement of radioactivity can be completed in 60 minutes, and efficiency can be improved by increasing the number of batches done at any one time.

Diagnostic accuracy vies with that in other published methods, quoted previously, with about 85-90% of proved thyrotoxic and hypothyroid sera in the larger series of 411 patients giving abnormal results. In a direct comparison with a resin-uptake technique, in 190 sera tested the Thyopac-3 test was superior. The numbers of hypothyroid patients in particular, however, was rather small and resin uptake gave a less satisfactory performance than previously (Clark, 1963).

Duplicate estimations agreed closely, and it would appear that for normal practice a single estimation on any particular serum would suffice. In addition the relation between two particular sera when these were tested on many different occasions remained fairly constant (coefficient of variation 4.41%).

The small volume of serum required (0.1 ml. per assay if duplicate estimations are not required) could be a particular advantage, particularly as other methods employ at least 0.5 ml. of serum per single assay, and some considerably more.

Each Thyopac-3 kit contains a phial of "standard" serum to which unknown sera may be referred. In the present communication use was not made of this standard, as at the time of the investigation uniformity of standard was not obtainable and our own control serum was substituted. In future the availability of a "universal" standard serum will be of benefit in that direct comparison of results between different laboratories will be possible.

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