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[54] **HYPOTHYROID SERUM CONTROL**

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[58] **Field of Search** ..... 23/230 B; 424/1; 252/408

[56] **References Cited**

UNITED STATES PATENTS

3,516,794	6/1970	Murty et al.	23/230 B
3,743,482	7/1973	Eisentraut	23/230 B
3,775,615	11/1973	Eisentraut	23/230 B
3,776,698	12/1973	Eisentraut	23/230 B

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[57] **ABSTRACT**

Described is the preparation of a hypothyroid serum control using normal serum and serum containing elevated levels of thyroxine binding globulin. The control serum exhibits hypothyroid values in clinical tests that measure triiodothyronine uptake and thyroxine concentration.

11 Claims, No Drawings

HYPOTHYROID SERUM CONTROL

To diagnose thyroid disfunction and evaluate thyroid therapy, clinicians rely on tests which measure a patient's serum triiodothyronine (T_3) uptake level as well as tests which do quantitate the thyroxine (T_4) concentration. T_3 uptake levels are determined in serum using T_3 uptake tests (e.g., Triosorb); serum T_4 levels are guantitated using tests which measure total serum T_4 concentration (e.g. T_4 radioimmunoassay, Tetrasorb). Tests measuring total serum T_4 are dependent upon the actual thyroid hormone concentration present in the serum, whereas tests which measure T_3 uptake depend upon the thyroxine-binding protein (TBP) concentration in the serum as well as the concentration of thyroid hormone. Both tests are important in forming a clear understanding of the patient's thyroid state.

Circulating T_3 and T_4 are bound to several constituents of the blood of which the thyroxine binding globulin (TBG) fraction contains the major binding sites which are fixed in number. The binding of T_3 and T_4 on the TBG molecule is one of competitive protein binding, that is, unbound T_4 will replace T_3 and T_4 already on the molecule.

As there is only 1–2 mg of TBG/100 ml normal serum, the binding sites, equivalent to approximately 20 micrograms of T_4 per 100 ml of normal serum, are readily saturated by a small increase of T_4 concentration.

In hyperthyroidism, the binding sites on the TBG molecules are nearly saturated with T_3 and T_4; in hypothyroidism, the binding sites are highly unsaturated, resulting in an increased ability for T_3 uptake by the TBG molecule in the serum.

The Triosorb assay and other similar tests for T_3 uptake operate on the principle of competitive protein binding. Serum T_3 and T_4 are primarily bound to the binding sites on the TBG molecule. The number of unoccupied binding sites is determined by the addition of radioactive T_3 (T_3*) to serum in the presence of an adsorbing agent. When T_3* is added to serum, any excess not bound to the binding sites of the TBG molecule in the serum will be adsorbed onto the added agent. Resin sponges are the adsorbing agents for the Triosorb test. For example, in hyperthyroidism, most of the TBG binding sites are occupied by T_3 and T_4 and thus the added T_3* will not be taken up by the endogenous TBG molecules, but will be taken up by the test adsorbent. In hypothyroidism, the reverse is true. Thus, the amount of radioactive T_3 bound by the adsorbent directly reflects the thyroid state of the patient.

The tetrasorb assay for T_4 is also based upon the principle of competitive protein binding. To determine the T_4 concentration, T_4 is extracted from the serum releasing it from its binding protein, TBG. The serum extract is then added to a solution containing a limited quantity of exogenous TBG to which is bound radioactive T_4 (T_4*). A displacement reaction occurs in which the T_4 in the extract displaces the T_4* from the exogenous TBG. This displaced T_4* is then adsorbed onto the resin sponge used as the adsorbing agent. As more T_4 is added, more T_4* is displaced from the TBG. The amount of T_4* displaced from the TBG is therefore directly proportional to the amount of T_4 present in the serum extract. In hyperthyroidism there is more T_4 available in the serum extract to displace T_4* from exogenous TBG than in euthyroidism or hypothyroidism.

When normal levels of TBG are present in serum, these types of thyroid function tests reflect the actual state of the patient. When TBG and T_4 levels are elevated, as in pregnancy or following estrogen ingestion in the form of oral contraceptives, T_3 uptake tests indicate the patient to be hypothyroid. However, tests measuring total T_4 indicate an euthyroid or sometimes a hyperthyroid condition. Simply stated, pregnancy or estrogen ingestion results in an increase in the number of TBG molecules causing an increase in hormone binding sites with a concurrent rise in thyroid hormone levels. Thyroid function tests carried out on these sera at this time show an increased T_3 uptake, indicative of hypothyroid function and an increased T_4 level indicative of hyperthyroid function. It is the object of this invention to describe a control serum prepared from these types of sera and normal serum that will serve as a hypothyroid control serum for T_4 tests as well as T_3 uptake tests. By normal serum Applicants mean serum having normal T_3 and T_4 values and which may be human, beef, sheep, goat, or other animal serum. Only horse serum has been found to lack utility.

A serum is hyperthyroid by the tetrasorb and triosorb tests if the percentage of T_3 uptake is greater than 35, and the T_4 concentration is above 14.5 mcg per 100 ml of serum. A serum is euthyroid if the percentage of T_3 uptake is 25 to 35 and the T_4 concentration is 5.3 – 14.5 mcg per 100 ml of serum. Hypothyroid serum has a T_3 uptake of below 25 percent and less than 5 mcg of T_4 per 100 mls of serum.

Removing T_3 and T_4 from normal serum or serum containing elevated TBG levels, such as by the methods outlined below, results in a serum judged hypothyroid both by methods which measure T_4 concentration and T_3 uptake.

EXAMPLE 1

Ingredients	Quantity for 990 ml (420 vials)
1. Fresh, Normal Serum	2700 ml
2. Norit A, Neutral Decolorizing Carbon pharmaceutical grade. (Amend Drug Co., Irvington,N.J.)	480 g
3. Water, Purified	560 ml

Method

1. Add Item No. 2 to 2400 ml of Item No. 1 in a 4 liter Erlenmeyer flask (about 20% carbon weight to volume of serum).
2. Swirl gently at room temperature until the charcoal particles are dispersed in the liquid.
3. Place the mixture at 4°C. Stir very gently for about 24 hrs.
4. At the end of 24 hrs., centrifuge mixture at high speed in a refrigerated centrifuge (34,800 × g).
5. Decant the supernatant and centrifuge it again as in step 4.
6. After the second centrifugation, again decant the supernatant and filter it by vacuum through a millipore filter.
7. Pour the filtrate (1200 ml) into a suitable glass tray for lyophilization and lyophilize.
8. Transfer the lyophilized material to a 2 liter Erlenmeyer flask and add Item No. 3.
9. Let the mixture stand for 30 minutes, then aid the dissolving process by swirling gently.

10. Add 300 ml of Item No. 1 to the serum mixture obtained in step **9.**
11. Dispense 2.14 cc into a vial that will hold a 2 ml fill and lyophilize.

Vials are reconstituted with 2.0 ml deionized distilled water. This serum is used for a hypothyroid control in conjunction with all thyroid function tests, i.e., the resulting serum control should have a T_3 uptake level less than 25% by the Triosorb method and a T_4 concentration less than 5.3 mg/100 ml by the Tetrasorb method.

Modifications in Example 1 are of course possible. Centrifugation (steps 4 and 5) may be eliminated; the millipore filter (step 6) may, of course, be an Ertel apparatus; the serum may be human or animal; the requirement to dispense the mixtures (step 11) may, of course, be modified or done away with completely; the time of mixing (step 3) may be shortened or lengthened over a range of 3–30 hours; the percentage of carbon added may range from 5–20 percent, with a range of about 10 to about 20 percent being preferred.

This procedure removes over 99% of the T_3 and T_4 from the starting serum, effectively producing a T_3 and T_4-free serum, while not significantly affecting the total protein concentration, pH, or T_4 binding capacity of the serum.

The following example outlines the method used to remove T_3 and T_4 from serum containing elevated TBG levels. Modifications of this example similar to those outlined for Example 1 are also possible and encompassed by this invention.

EXAMPLE 2

Ingredients	Quantity for 900 ml (420 vials)
1. Serum containing a high thyroxine binding globulin concentration	1100 ml
2. Neutral Decolorizing Carbon, pharmaceutical grade	220 g
3. Fresh, normal serum	300 ml

Method

1. Add Item 2 to Item 1 in a 2 liter Erlenmeyer flask (about 20% carbon weight to volume of serum).
2. Swirl gently at room temperature for one minute so that charcoal particles are dispersed in liquid.

3. Place mixture at 4°C and stir very gently for about 24 hours.
4. At the end of 24 hours, centrifuge mixture at high speed in a refrigerated centrifuge (34,800 ×g).
5. Decant the supernatent and centrifuge it again as in Step 4.
6. After second centrifugation, again decant the supernatent and filter it by vacuum through a sintered glass filter.
7. Add 300 ml of Item 3 to 600 ml of serum obtained from Step 6. Swirl gently to mix.
8. Dispense 2.14 cc in a vial that will hold a 2 ml fill and lyophilize.

Vials are reconstituted with 2.0 ml deionized distilled water. This serum is used for a hypothyroid control serum in conjunction with all thyroid function tests.

The fill solution after reconstitution will contain about 7 percent protein. The resulting serum control should have a T_3 uptake level less than 25% by the Triosorb Method and a T_4 concentration less than 5.3μg% by the Tetrasorb method. The serum used in this example may be obtained, for example, from pregnant women or women on estrogen therapy.

A series of four separate experiments were carried out to show the effect on sera when treated as to Examples 1 and 2. The resultant sera were assayed by following the well recognized Triosorb and Tetrasorb protocols. The results of these experiments (Table I) show that serum treated according to the methods of this invention, may be used as a hypothyroid control serum for tests being carried out in clinical laboratories.

TABLE I

Experiment	Sera	% T_3 Uptake	T_4 mcg %
I	Pooled Normal Human Serum (NHS)	27.1	7.4
	Pooled Normal Human Serum after Example 1 treatment (HCS)*	15.1	0.8
II	Pooled Normal Human Serum (NHS)	31.9	3.6
	Pooled Normal Human Serum after Example 1 treatment (HCS)*	18.3	0.5
	2 HCS : 1 NHS **	21.2	3.3
III	Pooled Normal Beef Serum (NBS)	29.3	11.4
	Pooled Normal Beef Serum after Example 1 treatment (HBS)	17.8	0.4
	2 HBS : 1 NBS **	19.3	2.4
IV	Pooled Serum from Pregnant Women	17.6	13.0
	Pooled Serum from Pregnant Women after Example 2 Treatment	13.6	0.3

*This is the serum obtained after step 9 of Example 1
**This is the serum obtained after step 10 of Example 1

The results of experiment I show that the pooled normal serum was euthyroid by both tests. After treatment the serum was hypothyroid for both T_3 uptake and T_4 concentration.

The results for experiment II show that the hypothyroid values may be altered upwardly when before treatment sera and after treatment sera are intermixed, as in this experiment in a 2 to 1 ratio.

Experiment III was carried out with beef sera as representative of animal sera which have been shown to have utility. As the results show, pooled normal beef serum shows T_3 uptake and T_4 concentration in the euthyroid range. After treatment, this sera behaves similar to human sera and is in the hypothyroid range to T_3 uptake and T_4 concentration. As with human serum, the T_3 and T_4 values may be increased by mixing vari-

ous ratios of normal beef serum and the prepared hypothyroid beef serum.

Experiment IV shows the expected hypothyroid T_3 uptake and euthyroid T_4 concentration for pooled serum collected from pregnant women. After the treatment outlined in Example 2, however, the serum becomes hypothyroid for both T_3 uptake and T_4 concentration.

We claim:

1. A method for obtaining a hypothyroid serum control comprising the following steps:

A. adding neutral, decolorizing carbon to blood serum in an amount of about 5–20 percent based upon weight of carbon to volume of serum;

B. mixing the above mixture for about 24 hours at a temperature of about 4°C;

C. twice centrifuging the resulting slurry at 34,800 xg and at a temperature of about 4°C;

D. filtering the resultant supernatant;

E. lyophilizing the supernatant so obtained.

2. The method of claim **1** which includes adding untreated normal serum in an amount of about 10 to about 35 percent of the serum volume of step A to the filtered supernatant of step D.

3. The method of claim **1** wherein the serum is animal blood serum.

4. The method of claim **1** wherein the serum is human blood serum.

5. The method of claim **1** wherein the serum is beef blood serum.

6. The method of claim **4** wherein the serum is T_4-euthyroid, hypothyroid, or hyperthyroid and T_3-euthyroid or hypothyroid.

7. The method of claim **6** wherein the serum is T_3-hypothyroid.

8. The method of claim **6** wherein the serum is T_4-euthyroid and T_3-euthyroid.

9. A control serum judged hypothyroid by methods which measure T_4 concentration and T_3 uptake comprising blood serum which has been treated by the method according to claim **1.**

10. In clinical chemistry procedures for measuring a person's serum triiodothyronine uptake level and thyroxine concentration, the improvement of which comprises the use of a single control serum standard which is a hypothyroid reference control serum for both triiodothyronine uptake level and thyroxine concentration tests.

11. The improvement of claim **10** in which the hypothyroid reference control serum preparation comprises the following steps:

A. adding neutral, decolorizing carbon to blood serum in an amount of about 5–20 percent based upon weight of carbon to volume of serum;

B. mixing the above mixture at a temperature of about 4°C;

C. twice centrifuging the resulting slurry at 34,800 g and at a temperature of about 4°C;

D. filtering the resultant supernatant;

E. lyophilizing the supernatant so obtained.

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