June 24, 1969 W. DI GIULIO 3,451,777

METHOD AND APPARATUS FOR DETERMINING THE THYROID HORMONE CONTENT OF BLOOD

Filed Aug. 20, 1965

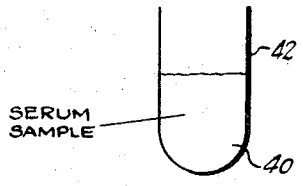


FIG. 1

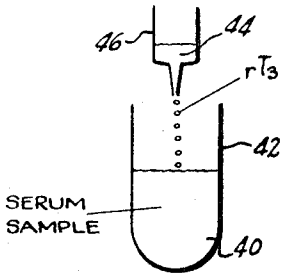


FIG. 2

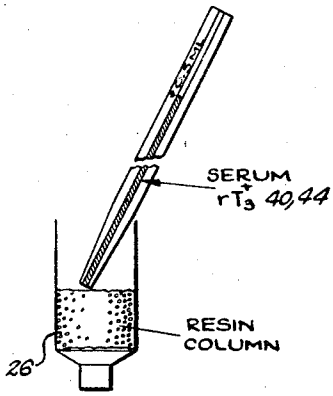


FIG. 3

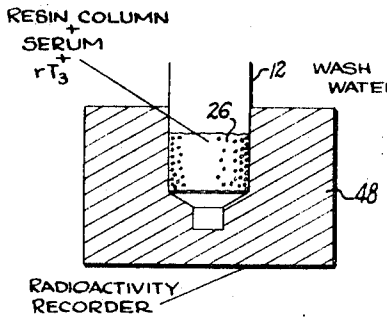


FIG. 4

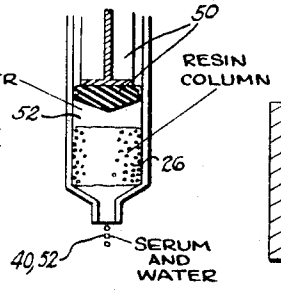


FIG. 5

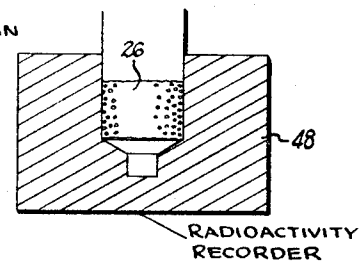


FIG. 6

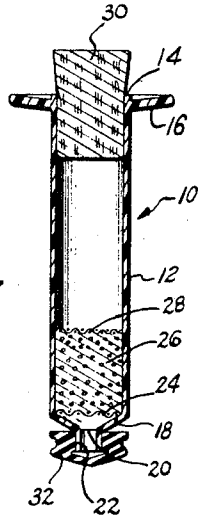


FIG. 7

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METHOD AND APPARATUS FOR DETERMINING THE THYROID HORMONE CONTENT OF BLOOD

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ABSTRACT OF THE DISCLOSURE

Method and apparatus for determining the thyroid hormone content of blood utilizing a granular resin having an affinity for a radioactive T_3 hormone. A predetermined amount of a radioactive T_3 hormone is mixed with a selected volume of a serum, and a measured quantity of the mixture is introduced into a column of the granular resin so that the mixture is completely contained within the column. An initial count of one minute of the radioactivity of the column containing the mixture is made in a conventional well counter. After a lapse of two minutes, wash water is expressed through the column by a plunger, and a final count of one minute of the column is again made in the conventional well counter. The T_3 resin uptake of the blood sample is determined by a comparison of the two readings.

This invention relates generally to the field of blood testing, and more particularly to an improved method and apparatus for determining the thyroid hormone content of blood.

Thyroid hormones, such as triiodothyronine, or T_3 as it is commonly known, are produced in the thyroid gland and are carried in the blood to the body cells where the hormones ultimately produce their well known metabolic effects. The thyroid hormones regulate the activity of most human cells in carrying out the particular functions of these cells. Consequently, when the thyroid gland puts out an excess of these hormones, the body cells are overactive, the body burns up excess food and the patient has the condition known as hyperthyroidism. In hypothyroidism, the reverse situation caused by a deficiency of thyroid hormones takes place.

It is well known that the thyroid hormones do not exist freely in large amounts in the plasma but are bound to specific protein fractions of that tissue. In this bound form, the hormones are transported throughout the body. The thyroid hormones are specifically bound to two distinct plasma proteins, which are normally present in such low concentrations as to defy practical methods of quantitation. Nevertheless, the binding strength or capability of the protein fractions to bind the thyroid hormones and the quantity of binding proteins available has been shown to be generally constant within narrow limits in various blood samples from most humans.

It is also well known that certain resins have this ability to bind the thyroid hormones. In other words, these resins have an affinity for the thyroid hormones, so that in the presence of these well known resins, a fraction of the thyroid hormones becomes bound to the resins even in the presence of serum proteins. The magnitude of this fraction is influenced primarily by the quantity of thyroid hormone in the blood when the binding capacity of thyroxine binding proteins is within normal limits. Consequently, it has been the practice to test indirectly for the thyroid hormone content of blood by contacting the blood with a radioactive thyroid hormone, and a quantity of a resin having an affinity for the thyroid hormone. In the case of a hyperthyroid, in which the thyroid gland of the patient was overactive, the protein

fraction in the serum approaches saturation with thyroid hormone. Consequently, the resin attracts and binds a large proportion of the radioactive thyroid hormone. The extent to which the resin takes up radioactive hormone from the blood sample is an indirect measure of the thyroid hormone content of the blood sample. This measurement is sometimes simply stated as "T_3 resin uptake," so in a hyperthyroid situation there is a high T_3 resin uptake. In a hypothyroid situation, the amount of thyroid hormone present in the patient's serum is reduced so that the serum proteins are "starved" for hormones. As a result, more of the added radioactive hormone will be bound to plasma proteins and less to the resin having an affinity for the hormone. Consequently, the test, in such a situation, will show a lesser take-up of hormone by the resin, i.e., a low T_3 resin uptake.

In carrying out the above test, the usual procedure is to obtain serum from the patient, place this serum in a test tube or glass vial, add a predetermined amount of resin to the vial, and then add radioactive T_3 to the serum-resin mixture. In one method the T_3 is added directly to the resin. The radioactivity of this mixture is recorded and then the mixture is incubated for a period of time between thirty minutes and two hours. In all current methods some form of agitation or mixing is required during this period. After the incubation period, the resin must be separated from the serum, following which the amount of radioactive material bound to either the serum alone or the resin alone is determined by making another radioactivity recording. This separation is generally accomplished by allowing the resin to settle (by centrifugation or by gravity) and either (a) withdrawing a known fraction of the overlying serum or (b) washing the settled resin by adding several volumes of water decanting the water-serum mixture after each addition of water.

Another known procedure involves embedding finely divided resin having an affinity for the thyroid hormone in a sponge. The serum and the radioactive T_3 are then deposited in the sponge, following which the sponge must be periodically compressed with a plastic rod in order to assure some measure of contact of the resin with the radioactive T_3 and the T_3 in the serum. Separation of serum and resin is accomplished by washing the sponge. These procedures are objectionable because (1) the agitation required to thoroughly mix the resin with the serum is inconvenient, (2) if there is a variance in the amount of resin added the results are inconclusive and (3) the separation of the serum from the resin following mixing is difficult.

It is an object of this invention, therefore, to provide an improved method and apparatus for obtaining an indirect measure of the thyroid hormone content of blood which is faster and easier to perform than the procedures described above, avoids all requirements for agitation of a resin-serum-radioactive hormone mixture, which positively avoids errors due to incomplete separation of the serum and the resin following competing of the serum and the resin for the radioactive thyroid hormone added to the mixture, and which significantly reduces the sensitivity of this test to small variations in the quantity of resin employed.

A further object of this invention is to provide an improved method and apparatus for obtaining an indirect measure of the thyroid hormone content of blood in which an excess of resin in the form of a fixed column is utilized, so that the amount of resin necessary to surround the available serum and the added radioactive T_3 is more than sufficient and so that complete and uniform contact of the resin with the serum and radioactive T_3

aixture is assured by depositing the serum T_3 mixture in he column of resin.

Still a further object of this invention is to provide ap-aratus for obtaining an indirect measure of the thyroid iormone content of blood which is readily manufactured, asily shipped, readily utilized by unskilled technicians n carrying out the method of this invention, and useful n carrying out other similar tests requiring intimate esin-liquid contact.

Further objects, features and advantages of this in-'ention will become apparent from a consideration of the ollowing description, the appended claims, and the ac-:ompanying drawing in which:

FIGURES 1–6, inclusvie, are diagrammatic illustra-ions showing the successive steps employed in the prac-ice of the method of this invention; and

FIGURE 7 is a vertical sectional view of the apparatus of this invention.

With reference to the drawing, the apparatus of this nvention, indicated generally at **10** in FIG. 7, is first briefly described herein to facilitate a later description of he method of this invention. The apparatus **10** consists of an upright tubular member **12** having an open top nd **14**, illustrated as being surrounded by a flange **16**, nd a downwardly tapering bottom wall **18** which is formed with an opening **20** defined by a downwardly ex-ending tubular extension **22** of the body **12**. A perforated netal disk **24**, of a diameter corresponding to the inner liameter of the tubular member **12**, and preferably formed of a metal screen material, is supported on the ottom wall **18** at the outer periphery thereof. A column **26** of a grauluar resin having an affinity for the T_3 hor-none is supported on the disk **24** and is confined by the ontainer **12**. A second perforated metal disk **28** is sup-orted on the upper end of the column **26** at a position etween the upper and lower ends of the tubular mem-er **12**. A conventional cork or stopper **30** is illustrated n a position closing the upper end **14** of the tubular nember **12**. A rubber stopper or cover member **32** is shown mounted on the tubular member extension **22** so as to close the lower end of the container **12**.

The particular resin employed to form the column **26** orms no part of the present invention since resins for his purpose are well known. In general the resin em-loyed is an ion exchange resin, specific examples of which are Amberlite IRA 400 Cl, 20–50 mesh chloride form, manufactured by Rohm-Haas Company, Phila-delphia, Pa. and Dowex I manufactured by Dow Chemi-al Company, Midland, Mich., U.S.A. In a preferred embodiment of this invention, one gram of the resin is placed on the screen **24** to form the column **26** after the resin has been dried at room temperature for twenty four hours to facilitate dispensing and weighing. Before the upper disk **28** is inserted, the cap **32** is applied and a liquid buffer material is added to the gram of resin on the disk **24**. The main purpose of the buffer is to keep the resin particles wet. The preferred buffer is a mixture of 24.2 gm. hydroxyaminomethane and 10.0 gm. maleic anhydride in one liter of water, with the pH of the mix-ture adjusted to 7.4 by the addition of hydrochloric acid or sodium hydroxide. After a period of several hours, the resin in the column has swollen to its final wet size and the disk **28** is inserted and placed on top of the column. The cork **30** may then be inserted and the apparatus **10** stored at a temperature of about 4° C. until its use is required.

In the practice of the method of this invention, utiliz-ing the apparatus **10**, a sample **40** (FIG. 1) of the pa-tient's serum to be tested is placed in a vial or test tube **42**. Radioactive thyroid hormone **44** (FIG. 2), herein-after referred to as rT_3, from a container **46** is then added to the serum sample **40**, as shown in FIG. 2. A predeter-mined amount of radioactive thyroid hormone is mixed in the test tube **42** according to the volume of the serum sample **40** contained therein as shown in FIG. 2. In a preferred embodiment of the invention one drop (ap-proximately 0.05 cc.) of a solution which contains be-tween 0.1 to 0.25 microcurie of rT_3 is added for each 0.5 ml. of serum in the serum sample **40**.

The cork **30** and the cap **32** are then removed from the apparatus **10** to allow the buffer in the container **12** to drain out through the container extension **22**. Some of the buffer remains in the spaces between the resin particles in the column **26**. Subsequently, a predetermined volume of the mixture of serum **40** and rT_3 related to to size of the column **26**, and in this case exactly 0.5 ml. of the mixture is deposited on the top end of the column **26**, as shown in FIG. 3. As this mixture enters the col-umn **26**, it displaces an equal amount of buffer which was retained in and around the spaces adjacent to the resin particles in the column **26**, and this displaced buffer flows out of the container **12** through the extension **22**.

As shown in FIG. 4, the container **12** is next placed in conventional apparatus **48** capable of counting and re-cording the radio activity in the resin column **26** with the serum **40** and rT_3 **44** therein. In a preferred embodiment of the invention, an initial count of one minute is taken.

After a lapse of a predetermined time period, two min-utes in a preferred embodiment of the invention, a plunger assembly **50** is inserted in the open upper end **14** of the container **12**, and the plunger **50** is utilized to take in and express wash water **52** through the column **26**. In a pre-ferred embodiment of the invention, one or two washes with the water **52** is accomplished with the plunger as-sembly **50**, so as to express the serum sample **40** out the bottom end of the container **12** as shown in FIG. 5. The container **12** with the resin column **26** therein is then replaced in the radioactivity recorder **48** (FIG. 5) and a count corresponding to the initial count illustrated in FIG. 4 is repeated. In a preferred embodiment of the invention, this subsequent count is also for one minute.

The T_3 resin uptake of the blood sample **40** is deter-mined by comparing the above two radioactivity record-ings. In other words, the higher the second recording (FIG. 6) relative to the initial recording (FIG. 4) the higher will be the T_3 resin uptake, and presumably the higher the amount of thyroid hormone in the initial blood sample **40** (assuming the binding capability of thyroxine binding proteins is normal). Expressing the second re-cording as a percentage of the first recording as the up-take of T_3 by the resin column **26**, normal serum, that is serum from a patient who is neither hyperthyroid or hypothyroid, will have a T_3 resin uptake percentage in the neighborhood of fifty percent. In practice the precise range of normal must be determined. This range is ap-proximately six percent above or below the value for T_3 resin uptake which is obtained by testing serum collected from a large number of normal persons and pooled to-gether. A T_3 resin uptake higher by more than six percent than the value obtained using pooled serum indicates a hyperthyroid condition in the blood sample **40**. A T_3 resin uptake percentage which is lower than the value obtained using pooled serum by more than six percent indicates a hypothyroid condition in the blood sample **40**.

From the foregoing description, it is seen that the method and apparatus of this invention for determining the thyroid hormone content of a blood sample is capa-ble of providing results of improved accuracy and repro-ducibility. In all cases, the amount of resin utilized to form the column **26** is substantially more than that re-quired to make complete contact with the serum and rT_3 mixture **40**, **44** (FIG. 3) which is added to the column **26**. That is, the column of resin is sufficiently large to contain the entire sample. The extent of the contact be-tween serum and resin is determined by the spacial ar-rangement of the resin particles and their dimensions, and not by agitation of the mixture. The excess of resin relative to the serum makes the test insensitive to small fluctuations in the quantity of resin used per test, since the excess resin does not significantly participate in the

competition for T_3. The fact that the resin is in the form of a body or column, which is positively fixed in place by the disks **24** and **28** and the wall of the tubular container **12**, to which the serum is added facilitates handling of the apparatus **10**, adding of the serum to the column **26**, and final separation of resin and sample. The perforated disks **24** and **28** function to retain the resin in container **12** while allowing serum **40** and water **52** to pass through the resin. Variations in volumes of samples of serum **40** employed are readily detected in variations in magnitudes in the initial radioactivity recordings from apparatus **48** if multiple determinations are performed using the serum-T_3 mixture in FIG. 2. Such variations, even if undetected, cause lesser variations in test results than would be the case when the test is performed without an excess of resin.

It is to be understood that while the apparatus **10** of this invention is described herein in connection with the T_3 resin uptake test, this apparatus can be used in connection with other blood and similar tests such as in testing for iron deficiency anemia. Consequently, the apparatus **10** is to be understood as having general utility in situations in which a column **26** of active particles, such as the resin, is required to make complete contact with a liquid sample.

It will be understood that the method and apparatus for obtaining a measure of the thyroid hormone content of serum which are herein disclosed and described are presented for purposes of explanation and illustration and are not intended to indicate limits of the invention, the scope of which is defined by the following claims.

What is claimed is:

1. A method for determining an index of the thyroid hormone content of a serum sample comprising the steps of:

(a) adding a quantity of radioactive T_3 hormone to said sample so as to form a mixture of said sample and said radioactive T_3 hormone;

(b) introducing said mixture completely into a fixed column consisting entirely of a resin having an affinity for the T_3 hormone so that a portion of said quantity of radioactive T_3 will become bound to said resin;

(c) measuring the radioactivity of said fixed column with said mixture therein;

(d) removing all of said mixture from said fixed column except the portion of said radioactive T_3 bound to said resin by expressing a wash fluid through said fixed column; and

(e) thereafter measuring the radioactivity of said fixed column.

2. A method for determining an index of the thyroid hormone content of a serum sample of predetermined volume utilizing a resin having an affinity for the T_3 hormone comprising the steps of:

(a) adding a predetermined quantity of radioactive T_3 hormone to said sample so as to form a mixture of said sample and said radioactive T_3 hormone;

(b) arranging said resin in the form of a column in an amount substantially exceeding the amount required to make complete contact with said mixture to be tested;

(c) depositing said mixture to be tested on the upper end of said column so that said mixture can flow completely into said column;

(d) measuring the radioactivity of said column with the mixture therein;

(e) removing said mixture from said column a predetermined time interval after making the deposit; and

(f) measuring the radioactivity of said column to determine the amount of the radioactive T_3 in said sample which was bound to the resin in said column.

3. A method for determining an index of the thyroid hormone content of a serum sample of predetermined volume utilizing a granular resin having an affinity for a radioactive thyroid hormone comprising the steps of:

(a) arranging said granular resin in the form of a fixed column in a tube between transversely positioned screens;

(b) introducing into said tube a liquid buffer in an amount in excess of that needed to wet the resin particles in said column;

(c) adding a predetermined quantity of the radioactive thyroid hormone to said sample so as to form a mixture of said sample and said radioactive thyroid hormone;

(d) removing from the tube the amount in excess of the liquid buffer needed to wet the resin particles in said column;

(e) depositing a measured portion of said mixture on the upper end of said column in an amount insufficient to contact all of the resin particles forming said column and so as to displace a corresponding volume of said liquid buffer from said column;

(f) measuring the radioactivity of said column with the mixture therein;

(g) removing said mixture from said column a measured time interval thereafter by expressing a wash liquid through said tube and column; and

(h) measuring the radioactivity of said column to determine the amount of radioactive thyroid hormone in the measured portion of said sample which was bound to the resin in said column.

4. The method according to claim **3**, wherein the step of removing said mixture from said column comprises introducing a wash liquid into said tube to immerse said column, and expressing said wash liquid by depressing a plunger in said tube.

5. A method for determining an index of the thyroid hormone content of a serum sample comprising the steps of:

(a) adding a quantity of radioactive T_3 hormone to said sample so as to form a mixture of said sample and said radioactive T_3 hormone;

(b) introducing said mixture completely into a fixed column consisting of a resin having an affinity for the T_3 hormone so that a portion of said quantity of radioactive T_3 will become bound to said resin, the resin forming said column being in excess of that required to receive all of the introduced mixture;

(c) measuring the radioactivity of said fixed column with the mixture therein;

(d) removing said mixture from said column a predetermined time interval after introducing the mixture thereinto by expressing a wash fluid through said column; and

(e) measuring the radioactivity of the washed column to determine the amount of the radioactive T_3 in said sample which was bound to the resin in said column.

6. Apparatus for obtaining an index of the thyroid hormone content of a serum sample comprising an upright container having openings at the upper and lower ends thereof, vertically spaced screens in said container, and a column of a granular resin having an affinity for said hormone confined in said container between said screens.

7. Apparatus for obtaining an index of the thyroid hormone content of a serum sample comprising an upright transparent tube having a bottom wall formed with an opening and an open top end, a screen in said tube supported on said bottom wall, a vertically extending column consisting entirely of a resin having an affinity for said hormone supported on said screen, and removable closure means on said tube for closing the open top end thereof and the opening in said bottom wall.

8. Apparatus for obtaining an index of the thyroid hormone content of a serum sample comprising an upright transparent tube having open upper and lower ends, a first transverse screen supported in said tube adjacent to said lower end, a column of granular resin having an affinity for said hormone supported on said screen, a second transverse screen positioned on the upper end of

said column, removable closure means on said tube for closing said upper and lower ends, and a plunger insertable into said tube when the closure means are removed for expressing washing liquid through said granular resin and out said lower end.

9. Apparatus for obtaining an index of the thyroid hormone content of a serum sample comprising an upright container having openings at the upper and lower ends thereof, vertically spaced screens in said container, a column of granular resin having an affinity for said hormone confined in said container between said screens, and a plunger positioned in said container above said resin and said screens for expressing a wash fluid through the confined resin.

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