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[54] **METHOD FOR DETERMINING THYROXINE IN BLOOD SERUM AND REAGENT THEREFOR**

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[56] **References Cited**

UNITED STATES PATENTS

3,414,383 12/1968 Murphy .....23/230 B

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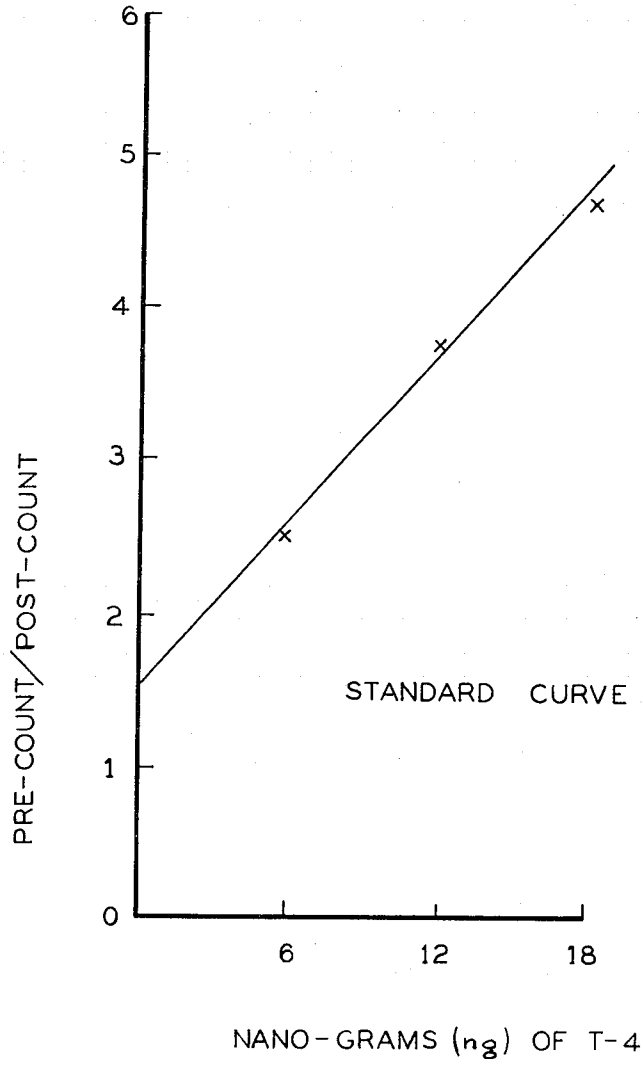
[57] **ABSTRACT**

An improved method for determining the thyroxine content of blood serum with the aid of a radioactive thyroxine tracer employs a reagent consisting of blood serum from which most of the naturally occurring thyroxine has been removed, for example by passing the serum through an ion-exchange column. Also, by comparing the radioactivity of an unknown sample with reference standards containing the same amount of alcohol from the same source, the usual and time-consuming alcohol evaporation step is eliminated.

9 Claims, 1 Drawing Figure



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METHOD FOR DETERMINING THYROXINE IN BLOOD SERUM AND REAGENT THEREFOR

BACKGROUND OF THE INVENTION

The serum protein, thyroxine-binding globulin ("TBG") has a relatively high and specific affinity for binding the thyroid hormone substance thyroxine (hereinafter referred to as "T–4"). It is also known that if radioactive T–4, that is T–4 containing radioactive iodine such as iodine-125, is added to a solution containing barbital buffered TBG, essentially all of the T–4 will be bound to the TBG. If stable T–4 is then added to the TBG solution, the radioactive T–4 will be displaced from the TBG in proportion to the amount of stable T–4 that is added. If another T–4 binding agent, such as an ion-exchange resin, is now added to this system, it will bind the radioactive T–4 that has been displaced from the TBG.

Various techniques based on the above principles have been devised. A particularly convenient method employing ion-exchange resin membranes to adsorb the displaced radioactive T–4 is disclosed in the copending application of Ella M. Bettinger and James L. Brown, Ser. No. 821,097, filed May 1, 1969.

The degree of radioactive T–4 displacement and hence the amount of stable thyroxine added to the TBG can be determined by removing the ion-exchange resin from the system and comparing the radioactivity now emitted by the TBG solution with its original radioactivity. When increasing amounts of stable T–4 are added to solutions containing the same amounts of radioactive T–4 and TBG and treated as above, the radioactivity of the TBG solution decreases with each successive increase in added stable T–4. By plotting the radioactivity of the treated sample against the amount of stable T–4, a graph is obtained like that shown in FIG. 1. If unknown amounts of T–4 are added to the TBG solution and treated in an identical manner, the amount of T–4 thus added can be read from the graph.

For this determination, diluted blood serum is customarily used as the source of TBG. It has not been the practice to subject it to any further treatment.

In order to determine T–4 in blood serum, the serum T–4 must first be separated from the binding proteins. This is accomplished by denaturing the proteins with alcohol. The denatured proteins release most (approximately 80 percent) of their bound T–4 which is removed in the alcoholic supernatant after centrifugation.

In methods known heretofore, this alcoholic extract is evaporated to avoid possible errors introduced by alcohol in the test mixture. Generally, 30 to 120 minutes are required to complete this step, depending upon the number of samples tested.

SUMMARY OF THE INVENTION

Among the several objects of the invention may be noted the provision of an improved method for determining the thyroxine content of blood serum; the provision of such a method which requires substantially less time than methods used heretofore; the provision of a special thyroxine-binding globulin reagent which improves the accuracy of the method of the invention; and the provision of a kit which facilitates the practice of the method of the invention. Other objects and features will be in part apparent and in part pointed out hereinafter.

The present invention is thus directed to the method of determining the thyroxine content of blood serum using radioactive thyroxine as a tracer compound which comprises the steps of adding to a known quantity of a reagent consisting essentially of a solution containing thyroxine-binding globulin and radioactive thyroxine, an alcoholic extract of a sample of blood serum whose thyroxine content is to be determined and an ion-exchange resin, maintaining the globulin solution in contact with the resin for a predetermined length of time, separating the anion exchange resin from the globulin solution, and comparing the radioactivity of the globulin solution with that of globulin solutions to which have been added known amounts of stable thyroxine dissolved in the same volume of alcohol from the same source as that present in said alcoholic serum extract. The invention also includes a special reagent for use in carrying out the above-described method and a kit for facilitating the practice of the method.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is a graph depicting an illustrative standard curve obtained by plotting the radioactivity of treated thyroxine-binding globulin samples against amounts of stable thyroxine.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Using the method of the present invention, the time required to determine thyroxine in blood serum is substantially shortened since evaporation of the alcoholic extract is unnecessary. Moreover, using the special TBG reagent disclosed herein, the accuracy of the method is substantially improved because the slope of the line shown in FIG. 1 is steeper than that obtained with untreated dilute TBG reagent under the same conditions.

The following example illustrates the practice of the invention:

For example, about 10 ml. of blood whose thyroxine is to be determined is withdrawn and allowed to coagulate. The serum is removed and 1 ml. is added dropwise to 2 ml. of 95 percent ethanol in a centrifuge tube and the contents are well mixed to denature the serum proteins. This alcoholic mixture is then centrifuged at 2,500 rpm for 4–5 minutes and 0.3 ml. of the supernatant liquid is withdrawn and transferred to a suitable test vial which contains 4 ml. of the special TBG solution whose preparation is described hereinafter.

In the same manner, several control samples are prepared. In place of the ethanolic serum extract, 0.3 ml. of an ethanolic solution containing a known amount (e.g., 0, and 12 nanograms) of stable thyroxine are added.

A strip of ion-exchange resin membrane is then added to each test vial. Since thyroxine is amphoteric, the ion-exchange resin may be either anion-selective or cation-selective. Many such resins are available in the form of membranes. For example, a commercially available anion-selective resin suitable for the purposes of this invention is designated "AR–111" (manufactured by Ionics Incorporated, Watertown, Massachusetts).

After the resin strips are added, the vials are capped and rotated for exactly 1 hour at room temperature. It

is convenient to use one of the commercial rotators especially designed for this purpose. Since the resin uptake of radioactive thyroxine is a function of rotation time, it is essential that the rotation time be the same for both the unknown and control samples. At the end of the 1 hour rotation time, the resin strip is removed with a forceps and discarded. The radioactivity of each vial is then counted and recorded as the "post-count." A minimum of 10,000 counts per minute will insure an error of less than 2 percent. The "pre-count" is also advantageously determined by counting an unused vial containing the TBG-radioactive T–4 solution. The pre-count/post-count ratio is calculated for each of the samples. The values for the standard samples are then plotted against the amount of added T–4, a straight line is drawn through the points, and the thyroxine content of the unknown serum samples is read from the resulting graph (see FIG. 1). Since the alcohol extracts approximately 80 percent of the T–4 from the serum, the value read from the graph should be divided by the extraction efficiency (approximately 0.8) to give the actual thyroxine content of the serum. This corrected value then represents the amount of T–4 (nanograms) in each 0.1 ml. of serum which is numerically equivalent to the number of μgm. of T–4 per 100 ml. (μgm. %) of patient serum.

The special TBG solution is conveniently prepared by passing the blood serum through a column containing a suitable anion-exchange resin which is capable of adsorbing the thyroxine but not the serum protein.

A suitable procedure for preparing the special TBG solution is as follows. To 137 ml. of serum is added 3280 ml. of a pH 8.6 barbital buffer solution containing 0.64 percent sodium chloride, 0.23 percent barbital sodium, and 0.6 percent barbital. A small amount of radioactive thyroxine (approximately 0.24 millicuries) is also added to serve as a tracer for determining the efficiency of the extraction.

An extraction column for this purpose is prepared by adding a sufficient amount of resin to a suitable glass column to provide a bed volume of 1,200 ml. and a flow rate of approximately 25 ml. per minute. For this purpose, a strongly basic quaternary ammonium type resin is suitable. For example, a commercially available resin of this kind designated "IRA–400" (manufactured by the Rohm and Haas Company) is satisfactory, but other anion-selective or cation-selective resins may be used instead.

The resin is added to the column as a water slurry and a layer of water is always maintained above the resin. The column is connected to a reservoir containing the serum solution to be extracted, and flow of the serum through the column is begun. Periodically a 1 ml. sample of the eluate is removed and its radioactivity counted to measure the efficiency of the extraction.

The radioactivity of the eluate should be 10 percent or less that of the stock solution. The extracted serum solution is then preferably passed through a 0.22 micron filter. More (approximately 0.30 millicuries) radioactive thyroxine is then added to the eluate and the latter is diluted with six times its volume of the aforementioned buffer solution. The resulting solution should have a pH of 8.6.

The usefulness and accuracy of the method is increased if the analyst is provided with a kit containing all the special reagents and other supplies required. For example, a useful kit consists of a number of 4 ml. test vials made of glass and provided with screw caps. The vials should include 4 cc. of the above TBG solution and should be matched for radioactivity. The standard samples should contain known amounts of stable thyroxine (e.g., 0 and 12 nanograms) in 0.3 ml. of alcohol. A container of alcohol from the same source used to prepare the control samples should also be supplied. The kit should also include the required ion-exchange resin, the latter preferably being in the form of membrane strips. The other necessary equipment such as syringes, radiation counter and rotator are standard laboratory equipment.

In view of the above, it will be seen that the several objects of the invention are achieved and other advantageous results attained.

As many changes could be made in the above methods and products without departing from the scope of the invention, it is intended that all matter contained in the above description shall be interpreted as illustrative and not in a limiting sense.

What is claimed is:

1. The method for determining the thyroxine content of blood serum using radioactive thyroxine as a tracer compound which comprises the steps of

adding to a known quantity of a reagent consisting essentially of a solution containing thyroxine-binding globulin and radioactive thyroxine,

a. an alcoholic extract of a sample of blood serum whose thyroxine content is to be determined, and

b. an ion-exchange resin,

maintaining the said globulin solution in contact with the said resin for a predetermined length of time;

separating the ion-exchange resin from the globulin solution; and

comparing the radioactivity of the globulin solution with that of globulin solutions to which have been added known amounts of stable thyroxine dissolved in the same volume of alcohol from the same source as that present in said alcoholic serum extract.

2. The method according to claim 1 wherein said reagent comprises a buffered solution of radioactive thyroxine and blood serum from which most of the naturally occurring thyroxine has been extracted.

3. The method according to claim 1 wherein the radioactive thyroxine is iodine-125.

4. The method according to claim 1 wherein the globulin solution is maintained in contact with the resin by rotating a sealed vessel containing said solution and resin.

5. The method according to claim 1 wherein said ion-exchange resin is in the form of a membrane strip.

6. The method for preparing a reagent for use in determining the thyroxine content of blood serum which comprises passing diluted blood serum through a column containing an ion-exchange resin to extract most of the naturally occurring thyroxine from the serum, and adding radioactive thyroxine to the resulting eluate.

7. A reagent for use in determining the thyroxine content of blood serum which comprises a buffered solution of radioactive thyroxine and blood serum from

which most of the naturally occurring thyroxine has been extracted.

8. A reagent according to claim 7 wherein the radioactive thyroxine contains iodine-125.

9. A kit for determining the thyroxine content of blood serum comprising a buffered solution of blood serum containing not more than about 20 percent of the normal amount of thyroxine and a sufficient amount of thyroxine-I125 to act as a tracer, two control samples containing a known amount of thyroxine dissolved in a predetermined volume of alcohol, a quantity of alcohol from the same source used to prepare the control sample, and a plurality of resin membrane strips whose dimensions permit them to fit loosely in the test containers.

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