United States Patent [19]

Fader et al.

[11] **3,716,632**

[45] **Feb. 13, 1973**

[54] TEST COMPOSITION AND METHOD FOR THE IN VITRO DETERMINATION OF THYROID FUNCTION

[75] Inventors: **Marshall Lloyd Fader; James Edward Christner,** both of Elkhart, Ind.

[73] Assignee: **Miles Laboratories, Inc.,** Elkhart, Ill.

[22] Filed: **April 27, 1970**

[21] Appl. No.: **32,396**

[52] **U.S. Cl.** .....**424/1,** 23/230 B, 252/301.1 R

[51] **Int. Cl.** **A61k 27/04**

[58] **Field of Search** .....424/1, 2, 7; 252/301.1; 23/230 R, 230 B

[56] **References Cited**

UNITED STATES PATENTS

3,485,587	12/1969	Keston	23/230 B
3,507,618	4/1970	Murty et al.	424/1 X
3,516,794	6/1970	Murty et al.	424/1 X

Primary Examiner—Benjamin R. Padgett

Attorney—Joseph C. Schwalbach, Michael A. Kondzella, Louis E. Davidson and Harry T. Stephenson

[57] **ABSTRACT**

The thyroid hormone content of blood serum can be readily determined by using an in vitro radioactive thyroid function test system containing a "protein error" pH indicator such as bromcresol purple. Such a "protein error" indicator provides color and detects the presence of the test sample, inhibits the formation of free iodide and decreases the adsorption of radioactive tagged thyroid substance to the walls of the container enclosing same.

16 Claims, No Drawings

TEST COMPOSITION AND METHOD FOR THE IN VITRO DETERMINATION OF THYROID FUNCTION

BACKGROUND OF THE INVENTION

Numerous tests have recently been developed to determine thyroid function in vitro without exposure of the patient to ionizing radiation. For example, specific plasma proteins known as thyroxine-binding proteins or TBP are the primary binding sites for the hormones produced by the thyroid gland. Since the TBP have a strong affinity for thyroid hormone, they will bind any free thyroid hormone available until they become saturated. In this test, such hormone is made available to the TBP in a sample of the patient's serum and a secondary binding site consisting of a resin powder or cross-linked dextran (Sephadex) is provided to take up any of the added radioactive triiodothyronine or thyroxine (hereinafter referred to as T_3 or T_4, respectively) not bound by the TBP. Thus, the test provides an estimation of the saturation level of the TBP in a given serum sample which is an indirect but reliable indication of thyroid function. A large resin uptake indicates hyperthyroidism whereas a small resin uptake indicates hypothyroidism.

Two such tests for thyroid function are described in U.S. Pat. Nos. 3,206,602 and 3,451,777. Since no color change is involved in either test, the operator can become confused if the procedural steps are interrupted. In addition, the decomposition of radioactive iodinated T_3 to the free iodide form, combined with considerable adsorption of the aqueous buffered radioactive T_3 solution to glass or plastic containers, produces a significant error in the determination of the thyroid hormone content of the serum sample.

SUMMARY OF THE INVENTION

The main object of the present invention is to provide a thyroid function test solution containing a pH indicator which possesses the three fold advantage of (1) providing a color change when the serum sample is added thereto (2) inhibiting the formation of free iodide from radioactive iodinated T_3 and (3) decreasing the adsorption of radioactive iodinated T_3 to the walls of the vessel containing the same.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Although thyroid function tests may be performed in many different ways, one common method involves adding an appropriate amount of a buffered aqueous solution of radioactive triiodothyronine or thyroxine and serum to a column of ion exchange resin such as Amberlite IRA 400 made by Rohm and Haas Co. of Philadelphia, Pa. or a cross linked dextran such as Sephadex G-25 made by Pharmacia Corp. of Uppsala, Sweden. The column is then drained and the radioactivity measured in a gamma ray counting instrument for 1 minute. The column is thereafter eluted with distilled water and the radioactivity remaining on the column is again measured for 1 minute. The percent retention is then calculated by comparing the two readings. Retentions of 41 percent to 61 percent are considered normal with cross-linked dextran (Sephadex) columns and 25 percent to 35 percent with resin columns.

It has now been found that by adding a "protein error" pH indicator to an aqueous radioactive T_3 solution prior to combining with the serum sample or by adding same to the combined T_3 solution and serum sample, one can achieve a highly improved test system.

The pH indicator employed in the present invention is of the protein error type which changes color in the presence of albumin without a significant actual change in pH. When such an indicator is added in a concentration of about 5. to 70 micrograms per milliliter of aqueous I^{125}-T_3 reagent buffered at a pH of 5 the reagent changes from its initial color to a second color when the serum is added. This second color acts as a marker which will appear at varying heights on the resin or dextran column depending on the last operation step completed in the test procedure, since the color will move with the solvent front.

A preferred indicator is bromcresol purple which changes color from yellow to purple within the pH range of 5.2 to 6.8. When mixed with aqueous radioactive T_3 solution, the yellow-orange color will turn to a grey-green color when serum is added at a ratio of 0.05 milliliter of serum to 0.45 milliliter of reagent mixture. Thus, the green color indicates the presence of the serum. Other satisfactory indicators which can be employed depending on the pH of the buffer system are (1) chlorphenol red, which changes from red to violet at a pH of 6.5 (2) tetrabromphenol blue, which changes from yellow to blue at a pH of 3.0 (3)bromphenol blue, which changes from yellow to purple at a pH of 3.0 (4) bromcresol green, which changes color from yellow to blue at a pH of 3.0 and (5) bromthymol blue, which changes from green to yellow at a pH of 6.5. In practice, the colors of the color change are not important as long as a difference can be easily seen.

Preferably, the improved T_3 test reagent with pH indicator of this invention will contain the following:

Ingredient	Amount
Anhydrous citric acid	43.2 grams
Dibasic sodium phosphate	125.0 grams
Formaldehyde (38% aqueous solution)	8.1 milliliters
I^{125} triiodothyronine	4.0 micrograms
Triiodothyronine unlabelled	26.0 micrograms
Bromcresol purple	0.18 grams
Distilled water q.s. to	3.0 liters

In addition to the color change which occurs in the use of a pH indicator of the type disclosed, another advantage resides in the unforeseen property of the indicator to inhibit the formation of free iodide from the I^{125}-T_3 employed in the T_3 reagent. This is important, since the gamma counters cannot distinguish between I^{125}-T_3 and free I^{125}, and all of the dose I^{125} is counted initially during the test procedure. Washing with water removes any free I^{125} from the column, since only the hormonal materials remain thereon. Thus, if the percent of free iodide is above 5 percent of the total dose counts, the T_3 ratio is subject to a significant error of from 5 percent to 10 percent.

In a series of tests, a buffered aqueous solution of I^{125}—T_3 containing 60 micrograms of bromcresol purple per milliliter prepared as shown in the foregoing example was stored under various conditions in a polyethylene

container and the percent of free iodide formed was compared with a similar solution containing no pH indicator. The initial solutions employed contained about 2 percent free iodide. After 90 days at room temperature, the radioactive T_3 solution containing the indicator showed 2.25 percent free iodide compared to 5.2 percent free iodide in the control sample containing no indicator. After 60 days at 40° C., the T_3 test solution with pH indicator contained 4.25 percent free iodide, whereas the control sample had a free iodide content of 9.0 percent.

A still further advantage of the use of a pH indicator in T_3 test solution is to inhibit the adsorption of radioactive T_3 to the walls of a plastic or glass container. Propylene glycol and albumin have been used in the past to reduce such adsorption but these additives interfere with the thyroid function test. In actual tests, aqueous radioactive T_3 solutions containing one of several indicators at a concentration of 60 micrograms per milliliter of solution were rotated on a wheel in a polyethylene container for 14 days and the percent activity remaining was compared with a control sample containing no indicator. The results obtained showed that over 90 percent of the radioactivity (after correction for physical decay) remained in those solutions containing bromthymol blue, chlorphenol red, bromcresol purple and tetrabromphenol blue, whereas with solutions containing phenol red or bromcresol green, 80 percent of the radioactivity remained, while those containing bromphenol blue retained 70 percent of the radioactivity in solution. Under the same test conditions, the control sample not containing one of the mentioned indicators retained only 32% of the radioactivity in solution. Similar results are obtained with glass containers.

Decreased adsorption of like magnitude has been observed when plastic or glass containers are prewashed prior to filling with radioactive -T_3 reagent devoid of indicator. The term "prewashed" means contacting (such as by rinsing, soaking or flushing) the interior surfaces of said containers with an aqueous, buffered solution of one of the pH indicators hereinbefore disclosed. Bromcresol purple or bromthymol blue are the preferred indicators. These are employed at a concentration of from 50 to 70 micrograms per milliliter of water buffered to a pH of about 5.

It is to be understood that the T_3 employed in the test compositions of this invention can be made radioactive with either I^{125} or I^{131}, although I^{125} is preferred, since it has a half-life of 56 days compared to I^{131} which has a half-life of 8 days.

What is claimed is:

1. In a test composition for determining thyroid function comprising a radioactive thyroid hormone and a buffer for establishing the pH of the composition in a predetermined range, the improvement which comprises adding to the composition a color responsive amount of a protein error type pH indicator which changes color in response to protein at the pH of the said buffer.

2. A test composition as in claim 1 wherein the buffer is used to maintain the pH of the composition at about 5.

3. A test composition as in claim 1 wherein the indicator is selected from the group consisting of bromcresol purple, chlorphenol red, tetrabromphenol blue, bromphenol blue, bromcresol green and bromthymol blue.

4. A test composition as in claim 1 wherein the indicator is bromcresol purple.

5. A test composition as in claim 1 wherein the indicator is bromthymol blue.

6. A test composition as in claim 1 wherein the indicator is present in a concentration of about 60 micrograms per milliliter of composition.

7. A test composition as in claim 1 wherein the thyroid substance is triiodothyronine.

8. In a method for determining thyroid function wherein blood serum is added to a composition comprising a radioactive tagged thyroid hormone and a buffer for establishing the pH of the composition in a predetermined range, the improvement which comprises the addition of a color responsive amount of a protein error type pH indicator which changes color in response to protein at the pH of the buffer prior to the addition of the serum.

9. A method as in claim 8 wherein the indicator is selected from the group consisting of bromcresol purple, chlorphenol red, tetrabromphenol blue, bromphenol blue, bromcresol green and bromthymol blue.

10. A method as in claim 8 wherein the indicator is bromcresol purple.

11. A method as in claim 8 wherein the indicator is present in a concentration of about 60 micrograms per milliliter of composition.

12. A method as in claim 8 wherein the thyroid substance is triiodothyronine.

13. A method of inhibiting the adsorption of radioactive triiodothyronine from an aqueous solution thereof on the walls of a polyethylene container which comprises adding to said aqueous solution an adsorption inhibiting amount of a protein error type pH indicator.

14. A method as in claim 13 wherein the indicator is selected from the group consisting of bromcresol purple, chlorphenol red, tetrabromphenol blue, bromphenol blue, bromcresol green and bromthymol blue.

15. A method as in claim 13 wherein the indicator is bromthymol blue.

16. A method as in claim 13 wherein the container is prewashed with an aqueous solution of bromcresol purple at a concentration of from 50 to 70 micrograms per milliliter of water.

* * * * *