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CHARLES L. KROLL, Sc.D., director
regulatory operations

August 16, 1976

29-00139-05G

Nuclear Regulatory Commission
Washington, D. C. 20555

Attention: Mr. Douglas Collins

Gentlemen:

Reference is made to our specific license, 29-00139-05G, filed pursuant to 10 CFR § 32.71 for the manufacture and distribution of by-product material for use under general license of 10 CFR § 31.11 and to Mr. Douglas Collins' comments of January 7, 1976 concerning the "Precaution" section of the package inserts for the following products.

1. Thyrostat 4/FTl
2. Thyrostat FTl
3. Thyrostat 3
4. Digoxin Immutope Kit
5. Gastrin Immutope Kit

The "Precautions" section of the inserts for these products have been revised as indicated in the attached specimens in conformance with our commitment of January 12, 1976.

We trust the revised package inserts are satisfactory.

Sincerely,

C. L. Kroll

GSR/lc
Enclosure

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J3-414A

THYROSTAT®-4/FTI DIAGNOSTIC TEST KIT

Revised February 1976

For Quantitative Measurement of Total Serum Thyroxine or Free Thyroxine Index for the Evaluation of Thyroid Function

For *In Vitro* Diagnostic Use
For Professional Use Only

PRINCIPLES OF THE TEST

Total Serum Thyroxine

The circulating thyroid hormones produced by the thyroid gland are bound to specific plasma proteins known collectively as thyroxine-binding proteins (TBP), and are in equilibrium with a small fraction of the free thyroid hormones circulating in the plasma. The first step in the assay procedure involves liberation of the bound thyroxine, and extraction of the major portion of the liberated and free thyroxine with an alcoholic solvent. Subsequent to the extraction step, the procedure follows the basic principle of saturation analysis, and requires a specific thyroxine-binding protein, a radiolabeled derivative of thyroxine, and a purified sample of thyroxine to serve as a reference standard. With the saturation analysis technique, there is competition between labeled and unlabeled thyroxine for a fixed number of protein binding sites. As the concentration of unlabeled thyroxine (obtained from the extraction step) increases, less of the radiolabeled thyroxine will be retained by the binding proteins. The quantity of thyroxine which is not bound by the specific binding protein is measured by introducing an adsorbent material that will only bind free thyroxine. The relative amounts of free and bound thyroxine are determined by isolating the adsorbent and measuring the radioactivity associated with it. The absolute quantity of thyroxine (T_4) in a serum sample is determined from a standard curve prepared with known amounts of a thyroxine standard preparation. In the Thyrostat-4 test, the thyroxine is extracted from serum with ethanol and the alcoholic extract is mixed with ^{125}I-labeled thyroxine that is bound to thyroxine-binding proteins. An organic adsorbent is used to separate bound thyroxine from free thyroxine.

When the thyroid gland produces an excess of thyroid hormones, as in hyperthyroidism, the concentration of T_4 in the serum will be elevated, and a greater proportion of the radiolabeled thyroxine will be bound to the added adsorbent. Conversely, when the thyroid hormone production is decreased, as in hypothyroidism, the concentration of T_4 in the serum will be decreased, and a smaller proportion of the radiolabeled thyroxine will be bound to the added adsorbent.

Free Thyroxine Index

In the absence of abnormalities in serum TBP concentration, the measurement of total serum thyroxine provides an accurate means of assessing thyroid status. In conditions where TBP concentration is altered, it is also necessary to obtain an estimate of the unoccupied binding sites of the TBP, in order to obtain an accurate indication of thyroid status. The Thyrostat-FTI procedure provides a means for simultaneous measurement of total serum thyroxine and estimation of unoccupied binding sites of the TBP. The result obtained by this procedure provides an indirect measure of free thyroid hormone, which correlates closely with thyroid status, regardless of serum TBP concentration.

The first step in the assay procedure involves liberation of the bound thyroxine, and extraction of the major portion of the liberated and free thyroxine with an alcoholic solvent. Subsequent to the extraction step, the procedure follows the basic principle of saturation analysis, and requires specific thyroxine-binding proteins, a radiolabeled derivative of thyroxine, and a normal control serum. With the saturation analysis technique, there is competition between labeled and unlabeled thyroxine for a fixed number of protein binding sites. As the concentration of unlabeled thyroxine (obtained from the extraction step) increases, less of the radiolabeled thyroxine will be retained by the binding proteins. The quantity of thyroxine which is not bound by the specific binding proteins is measured by introducing an adsorbent material that will only bind free thyroxine. The relative amounts of free and bound thyroxine are determined by isolating the adsorbent and measuring the radioactivity associated with it. The estimation of unoccupied binding sites of the TBP is achieved by adding a small quantity of unextracted patient serum to the incubation medium prior to addition of the adsorbent material. The amount of radiolabeled thyroxine taken up by the adsorbent material will be influenced by the number of unoccupied TBP binding sites present in the patient serum sample. As the number of unoccupied binding sites increases, the amount of radioactivity taken up by the adsorbent material will decrease. The net effect of addition of unextracted patient serum is adjustment of assay results to compensate for alterations in TBP concentration. Serum thyroxine measurements adjusted for TBP concentration provide an indirect measure of serum-free thyroxine, which is highly correlated with thyroid status. An "index" of free thyroid hormone is obtained by comparing adsorbent uptake of radiolabeled thyroxine in a patient sample, with adsorbent uptake of radiolabeled thyroxine for a normal control serum sample assayed in an identical manner. When the thyroid gland produces an excess of thyroid hormones, as in hyperthyroidism, the increased level of free thyroxine will be reflected by an increased uptake of radiolabeled thyroxine by the adsorbent material, when compared with the uptake for a normal control serum. Conversely, when thyroid hormone production is decreased, as in hypothyroidism, the decreased level of free thyroxine will be reflected by a decreased adsorbent uptake of radiolabeled thyroxine, compared with the uptake for a normal control serum.

In the Thyrostat-FTI test, the thyroxine is extracted from serum with ethanol, and the alcoholic extract is mixed with ^{125}I-labeled thyroxine bound to thyroxine-binding proteins. An organic adsorbent is used to separate bound thyroxine from free thyroxine. The normal control serum is of animal origin.

NOMENCLATURE

The Thyrostat-4 *in vitro* diagnostic test for the measurement of total serum thyroxine is based on the classic Murphy-Pattee "competitive protein binding analysis" procedure, recently redesignated "radiotransin assay." It has been suggested by an *ad hoc* committee of the American Thyroid Association[1] that this method of measuring thyroxine concentration be identified as "Thyroxine (displacement)," abbreviated "T_4(D)," and expressed as micrograms per 100 milliliters of serum (μg./100 ml.).

When there are pronounced alterations in the binding capacity of the transport proteins, the T_4(D) test, used in conjunction with the commonly used T_3 test (Resin Triiodothyronine uptake-RT_3U) provides an indirect measure of the concentration of free T_4, which is highly correlated with the thyroid state.[2] This indirect measure of free T_4 is commonly referred to as the "free thyroxine index," with the American Thyroid Association suggested designation being "Thyroxine-resin T_3 index," abbreviated T_4-RT_3. The T_4-RT_3 is the mathematical product of the results of the T_4(D) and RT_3U tests.

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Revised February 1976

THYROSTAT®-FTI DIAGNOSTIC TEST KIT

For Quantitative Measurement of Free Thyroxine Index for the Evaluation of Thyroid Function

For *In Vitro* Diagnostic Use
For Professional Use Only

PRINCIPLES OF THE TEST

In the absence of abnormalities in serum thyroxine-binding proteins (TBP) concentration, the measurement of total serum thyroxine provides an accurate means of assessing thyroid status. In conditions where TBP concentration is altered, it is also necessary to obtain an estimate of the unoccupied binding sites of the TBP, in order to obtain an accurate indication of thyroid status. The Thyrostat-FTI procedure provides a means for simultaneous measurement of total serum thyroxine and estimation of unoccupied binding sites of the TBP. The result obtained by this procedure provides an indirect measure of free thyroid hormone, which correlates closely with thyroid status, regardless of serum TBP concentration.

The first step in the assay procedure involves liberation of the bound thyroxine, and extraction of the major portion of the liberated and free thyroxine with an alcoholic solvent. Subsequent to the extraction step, the procedure follows the basic principle of saturation analysis, and requires specific thyroxine-binding proteins, a radiolabeled derivative of thyroxine, and a normal control serum. With the saturation analysis technique, there is competition between labeled and unlabeled thyroxine for a fixed number of protein binding sites. As the concentration of unlabeled thyroxine (obtained from the extraction step) increases, less of the radiolabeled thyroxine will be retained by the binding proteins. The quantity of thyroxine which is not bound by the specific binding proteins is measured by introducing an adsorbent material that will only bind free thyroxine. The relative amounts of free and bound thyroxine are determined by isolating the adsorbent and measuring the radioactivity associated with it. The estimation of unoccupied binding sites of the TBP is achieved by adding a small quantity of unextracted patient serum to the incubation medium prior to addition of the adsorbent material. The amount of radiolabeled thyroxine taken up by the adsorbent material will be influenced by the number of unoccupied TBP binding sites present in the patient serum sample. As the number of unoccupied binding sites increases, the amount of radioactivity taken up by the adsorbent material will decrease. The net effect of addition of unextracted patient serum is adjustment of assay results to compensate for alterations in TBP concentration. Serum thyroxine measurements adjusted for TBP concentration provide an indirect measure of serum-free thyroxine, which is highly correlated with thyroid status. An "index" of free thyroid hormone is obtained by comparing adsorbent uptake of radiolabeled thyroxine in a patient sample, with adsorbent uptake of radiolabeled thyroxine for a normal control serum sample assayed in an identical manner. When the thyroid gland produces an excess of thyroid hormones, as in hyperthyroidism, the increased level of free thyroxine will be reflected by an increased uptake of radiolabeled thyroxine by the adsorbent material, when compared with the uptake for a normal control serum. Conversely, when thyroid hormone production is decreased, as in hypothyroidism, the decreased level of free thyroxine will be reflected by a decreased adsorbent uptake of radiolabeled thyroxine, compared with the uptake for a normal control serum.

In the Thyrostat-FTI test, the thyroxine is extracted from serum with ethanol, and the alcoholic extract is mixed with ^{125}I-labeled thyroxine bound to thyroxine-binding proteins. An organic adsorbent is used to separate bound thyroxine from free thyroxine. The normal control serum is of animal origin.

NOMENCLATURE

It has been suggested by an *ad hoc* committee of the American Thyroid Association[1] that the measurement of total serum thyroxine by the classic Murphy-Pattee "radiotransin assay" be identified as "Thyroxine (displacement)," abbreviated "T_4(D)," and expressed as micrograms per 100 milliliters of serum (μg./100 ml.).

When there are pronounced alterations in the binding capacity of the transport proteins, the T_4(D) test, used in conjunction with the commonly used T_3 test (Resin Triiodothyronine uptake-RT_3U) provides an indirect measure of the concentration of free T_4, which is highly correlated with the thyroid state.[2] This indirect measure of free T_4 is commonly referred to as the "free thyroxine index," with the American Thyroid Association suggested designation being "Thyroxine-resin T_3 index," abbreviated T_4-RT_3. The T_4-RT_3 is the mathematical product of the results of the T_4(D) and RT_3U tests.

Nomenclature has not been suggested for tests that provide a direct measurement of "free thyroxine index," as opposed to the calculation procedure described above. For purposes of simplicity, the direct measurement of "free thyroxine index," as embodied in the Thyrostat-FTI procedure will be abbreviated FTI(M).

RATIONALE FOR USE

The measurement of total serum thyroxine (T_4) by the T_4(D) procedure represents a significant advance in the *in vitro*

J3-415A

J3-358A

THYROSTAT®-3 DIAGNOSTIC TEST KIT

Revised February 1976

For Quantitative Measurement of Serum Liothyronine (T_3) Uptake for the Evaluation of Thyroid Function

For *IN VITRO* Diagnostic Use
For Professional Use Only

PRINCIPLES OF THE TEST

The currently accepted principles underlying the T_3 (synonymous with liothyronine and triiodothyronine) uptake test are as follows:

The circulating thyroid hormones produced by the thyroid gland are bound to specific plasma proteins known collectively as thyroxine-binding proteins (TBP), and are in equilibrium with a small fraction of the free thyroid hormones circulating in the plasma. A change in the number of unoccupied TBP binding sites will alter the free thyroid hormonal level in the blood, which can be indirectly measured through the use of the T_3 uptake test.

In the T_3 uptake test, a supply of labeled exogenous thyroid hormone (in the Liothyronine I 125 Buffer Solution) is added to the patient's serum together with a secondary binding site (the Thyrostat-3 Adsorbent Tablet). A portion of the liothyronine ^{125}I will become bound to the binding sites of the TBP that are not occupied by the thyroxine whereas some, not bound to the TBP, will become bound to the adsorbent.

When the thyroid gland produces an excess of thyroid hormones, as in hyperthyroidism, the number of unoccupied TBP binding sites is reduced and a greater proportion of the added hormone will become bound to the adsorbent. Conversely, when thyroid hormone production is decreased, as in hypothyroidism, the number of unoccupied TBP binding sites is increased and a greater proportion of the added liothyronine ^{125}I will become bound to the TBP resulting in a decreased uptake by the adsorbent. (In normal pregnancy, although the free thyroid hormone level is normal, there is an increased production of TBP, so that more binding sites are available resulting in the binding of a greater proportion of the added liothyronine ^{125}I to these sites as in hypothyroidism.)

Therefore, the use of the T_3 uptake test provides an estimate of the unoccupied binding sites of the TBP in a given serum sample. This, in turn, gives an indirect estimate of the amount of endogenous circulating thyroid hormone, and therefore an indirect but reliable indication of thyroid function.

In summary, a large T_3 uptake indicates hyperthyroidism, while a small T_3 uptake indicates hypothyroidism (or normal pregnancy).

RATIONALE FOR USE

The T_3 uptake test represents a significant advance in the search for a simple and reliable test of thyroid function. The test is an *in vitro* procedure which avoids any exposure of the patient to ionizing radiation. Equally important is the fact that the test is diagnostically significant in the presence of unrelated nonthyroidal factors which are known to complicate interpretation of other thyroid function tests. Although other thyroid function tests may be affected for considerable periods of time by the prior administration of most iodine-containing preparations, the T_3 uptake test is not so affected at the normal dose level at which these drugs are used. Anxiety, hypertension, congestive heart failure, or administration of mercurial agents also have no effect on the test.

The technique readily falls within the scope of any hospital or office laboratory with ordinary isotope facilities and is simple, rapid, and inexpensive enough to be used as a general screening test. Moreover, the test is consistently reliable when repeated at frequent intervals.

Note: While the T_3 uptake test is a very useful aid in the evaluation of thyroid function, it should not be used as the sole basis for such an evaluation. In any patient, the clinical state is probably the best indication of thyroid status, and any laboratory test must be interpreted with caution when test results do not agree with clinical evidence.

The Thyrostat-3 test offers further advantages in the performance of the T_3 uptake test. Unlike many of the T_3 uptake procedures employing anion exchange resins, Thyrostat-3 test results are not significantly affected by variations in time or temperature during contact with the Thyrostat-3 Adsorbent Tablet (test results are essentially unchanged at normally encountered room temperatures ranging between 20° and 25° C.).

The use of ^{125}I rather than ^{131}I considerably lengthens the shelf-life of the liothyronine employed in the test because of the longer half-life of ^{125}I and the fact that it emits no beta rays to affect the stability of the liothyronine. Moreover, with ^{125}I labeled material, radiation exposure to the technician is lowered. Radioactivity is well within good counting range of modern equipment, and *in vitro* counting is quite efficient.

The half-life of ^{125}I is 60 days. The isotope decays in a complex fashion with emission of x-rays and gamma rays whose radiation energies are 27.5 kev. and 35.4 kev, respectively. There is no beta emission.

REAGENTS

The Thyrostat-3 *in vitro* diagnostic test for quantitative measurement of serum liothyronine (T_3) uptake for the evaluation of thyroid function is available in 25-, 100-, and 500-test kits.

The Thyrostat-3 25-test kit provides 25 plastic test tubes, 1 vial of Adsorbent Tablets (28), 1 bottle of Liothyronine I 125 Buffer Solution (50 ml., containing 0.03 μCi or less per ml.), 1 Control Pak containing 1 vial (1.0 ml.) Normal Control

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Gastrin IMMUTOPE® KIT

For Quantitative Measurement of Serum Gastrin Levels by Radioimmunoassay

For *In Vitro* Diagnostic Use

For Professional Use Only

DETERMINATION OF SERUM GASTRIN LEVELS BY RADIOIMMUNOASSAY

Measurement of body constituents by the technique of radioimmunoassay offers a bioanalytical tool that combines the extreme sensitivity of radioisotope methodology with the extreme specificity of immunological techniques. The procedure requires a specific antibody, a radiolabeled antigen, a pure sample of the antigen to serve as a reference standard, and a means of separation of free antigen from antibody-bound antigen. The procedure follows the basic principle of saturation analysis, where there is competition between labeled and unlabeled antigen for a fixed number of antibody binding sites. As the concentration of unlabeled antigen (the substance actually being measured) increases, less of the added radiolabeled antigen will be bound to the antibody. When equilibrium has been reached in the antigen-antibody reaction, the free and bound components of the mixture are separated, and the relative amounts of each are determined by measuring the radioactivity of the separated components. The absolute quantity of unlabeled antigen in the sample being analyzed is determined by comparing the assay results to a standard curve prepared with known amounts of the unlabeled antigen.

In the Gastrin IMMUTOPE Kit, antibody to synthetic human gastrin I serves as the specific antibody, synthetic human gastrin I labeled with iodine-125 serves as the labeled antigen, and synthetic human gastrin I is used as a reference standard. An anion exchange resin is used to separate free gastrin from antibody-bound gastrin, and serum gastrin levels are expressed as picograms (pg., 10^{-12} g.) of gastrin per milliliter of serum (pg./ml.).

RATIONALE FOR USE

The measurement of serum gastrin levels by radioimmunoassay has been of value in studying physiological processes involving the gastrointestinal hormones,[1-5] and as an aid in detecting tumors of the Zollinger-Ellison variety.[2,6-8] The measurement of serum gastrin levels by radioimmunoassay has several advantages over the standard bioassay procedures. The sensitivity, simplicity, specificity, and rapidity with which the radioimmunoassay can be performed, remove the obstacles to routine clinical determination of serum gastrin that were associated with the more complex bioassay procedure.

The use of ^{125}I rather than ^{131}I considerably lengthens the shelf-life of the radioiodinated Gastrin employed in the test, and reduces radiation exposure to laboratory personnel. The half-life of ^{125}I is 60 days. The isotope decays in a complex fashion with emission of x-rays and gamma rays whose radiation energies are 27.5 kev and 35.4 kev, respectively. These energies are well within the detection capability of modern counting equipment. There is no beta emission.

GASTRIN CHEMICAL AND BIOLOGICAL PROPERTIES

Gastrin is a linear polypeptide with seventeen amino acid residues (heptadecapeptide), that is produced by the mucosal lining of the gastric antrum.[9] Gastrin release is stimulated by feeding, alkaline pH, cholinergic stimulation and mechanical distention of the gastric antrum. Human gastrin occurs in two almost identical chemical forms, which possess identical biological activities. Human gastrin I is a heptadecapeptide with a molecular weight of 2,096; human gastrin II is a heptadecapeptide with a molecular weight of 2,176, due to the presence of a sulfate ester on the tyrosyl residue at position 12. Both terminal groups of the gastrin molecule are blocked: the N-terminal by the formation of the pyroglutamyl condensation, and the C-terminal by the presence of an amide. The excess of dicarboxylic acids in gastrin confers a strong negative charge to the molecule, which is reflected in its electrophoretic and chromatographic behavior.[10] Gastrin is adsorbed by anion exchange resins.

Yalow and Berson[11] have studied the nature of immunoreactive gastrin in human plasma and extracts from gastrointestinal tissues, and have observed the presence of two immunoreactive components of plasma gastrin. One component has the characteristics of heptadecapeptide gastrin, while the other component (BG, big gastrin) has a less acidic charge than heptadecapeptide gastrin and based on Sephadex gel column chromatography has an estimated molecular weight of about 7000. The BG component usually represents the major fraction of plasma gastrin, and appears to have the same biologic and immunologic potencies as heptadecapeptide gastrin.

The primary biologic response to gastrin is the production of hydrochloric acid by the parietal cells of the stomach. Release of gastrin from the antral mucosa into the circulation, following a variety of stimuli, results in a potent stimulus to gastric secretion of hydrochloric acid. Gastrin release is inhibited when the intragastric pH is reduced to 3.0 and eliminated at pH 1.5 or less,[2] completing a cycle of feed back controls.

Gastrin has effects on all major gastrointestinal activities including secretion, motility, and absorption. These physiological and pharmacological actions are discussed in detail in papers by Sanders and Schimmel,[9] and Grossman.[10]

CLINICAL APPLICATIONS

By use of the radioimmunoassay technique, fasting gastrin levels have been shown to be markedly elevated in patients

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Digoxin IMMUTOPE® Kit

For Quantitative Measurement of Serum or Plasma Digoxin Levels by Radioimmunoassay

For *IN VITRO* Diagnostic Use
For Professional Use Only

DETERMINATION OF SERUM OR PLASMA DIGOXIN LEVELS BY RADIOIMMUNOASSAY

Measurement of body constituents or administered compounds by the technique of radioimmunoassay offers a bioanalytical tool that combines the extreme sensitivity of radioisotope methodology with the extreme specificity of immunological techniques. The procedure requires a specific antibody, a radiolabeled antigen, a pure sample of the antigen to serve as a reference standard, and a means of separation of free antigen from antibody-bound antigen. The procedure follows the basic principle of saturation analysis, where there is competition between labeled and unlabeled antigen for a fixed number of antibody binding sites. As the concentration of unlabeled antigen (the substance actually being measured) increases, less of the added radiolabeled antigen will be bound to the antibody. When equilibrium has been reached in the antigen-antibody reaction, the free and bound components of the mixture are separated, and the relative amounts of each are determined by measuring the radioactivity of the separated components. The absolute quantity of unlabeled antigen in the sample being analyzed is determined by comparing the assay results to a standard curve prepared with known amounts of the unlabeled antigen.

In the Digoxin IMMUTOPE Kit, antibody to digoxin (raised in rabbits by administration of digoxin coupled to human serum albumin) serves as the specific antibody, purified digoxigenin labeled with ^{125}I serves as the labeled antigen, and purified digoxin is used as a reference standard. Powdered charcoal is used to separate free digoxin from antibody-bound digoxin, and digoxin levels are expressed as nanograms (ng., 10^{-9}g.) of digoxin per milliliter of serum or plasma (ng./ml.).

RATIONALE FOR USE

The measurement of serum or plasma levels of digoxin by radioimmunoassay has proved to be a valuable adjunct in the clinical diagnosis of digoxin toxicity. Since excessive accumulation of digoxin is a major factor in the development of toxicity, and there is some constancy in myocardium-to-serum digoxin ratios,[1] determination of serum digoxin concentration can be of help in the diagnosis of digoxin intoxication.[2]

Measurement of serum or plasma digoxin by radioimmunoassay has the advantages of speed and simplicity over the originally used double-isotope derivative method[3] and has the advantages of simplicity and specificity over bioassay methods.[4] The more recently developed ^{86}Rb uptake method has yielded results that are in good agreement with results obtained by radioimmunoassay, but requires a day to obtain results versus one hour required for radioimmunoassay.[5]

The Digoxin IMMUTOPE Kit utilizes ^{125}I labeled digoxigenin in lieu of ^{3}H labeled digoxin that is employed in many of the procedures described in the literature. The use of ^{125}I rather than ^{3}H avoids the problems associated with sample preparation and availability of liquid scintillation counting equipment. In addition, the use of internal counting standards to correct for quenching associated with the presence of variable quantities of bile pigments or hemoglobin[6] is avoided with the ^{125}I label. The half-life of ^{125}I is 60.2 days. The isotope decays in a complex fashion with emission of x-rays and gamma rays whose radiation energies are 27.4 kev and 35.4 kev, respectively. These energies are well within the detection capability of modern solid crystal gamma scintillation detectors. There is no beta emission.

DIGOXIN CHEMICAL AND BIOLOGICAL PROPERTIES

Chemical Properties: Digoxin is a pure glycoside obtained from the leaves of *Digitalis lanata*. Like all cardiac glycosides, digoxin consists of a steroidal portion or aglycone, and a glycosidic portion, consisting of three digitoxose sugar residues. Digoxin is formed upon partial hydrolysis of the naturally occurring Lanatoside C found in *Digitalis lanata*. It differs from digitoxin by the presence of an extra hydroxyl group at the C-12 position. Because of this structural difference, digoxin shows increased polarity and decreased lipid solubility, resulting in a marked difference in the pharmacokinetics of the two compounds.

The pharmacologic activity of cardiac glycosides is contained exclusively in the steroidal (aglycone or genin) portion of the molecule. The sugars possess no intrinsic activity, but they enhance the pharmacologic activity of the aglycone several times, presumably by increasing solubility or enhancing the ability of the drug to penetrate cell membranes. The pharmacologically active aglycone portion of the digoxin molecule, devoid of the sugar residues, is referred to as digoxigenin, and is the radiolabeled component of the Digoxin IMMUTOPE Kit.

Biological Properties: Digoxin is well absorbed from the gastrointestinal tract, with approximately 80 percent of an oral dose being eventually absorbed. Following oral administration, peak serum levels are found at one to two hours. Absorption is not diminished by food or fasting, although the shape of the curve defined by serum levels has a somewhat lower and more extended peak.[7]

Doherty[8] and co-workers administered tritium labeled digoxin to human subjects and found a more or less constant relationship between tissue and serum levels. Concentrations of digoxin were always highest in the heart, followed by liver and kidney. The ratio between heart and serum concentration was rather constant at 30:1.[8] The relationship between tissue and serum concentration and the fairly uniform serum concentration over several hours during the post-absorptive phase, provides the basis for the clinical use of serum digoxin determinations.[9]

There is some controversy as to the nature of the binding (specific vs. nonspecific), but it is generally accepted that digoxin forms a complex with the plasma membrane-bound enzyme Na^{+}, K^{+} ATP-ase. It has been shown that all cardiac glycosides inhibit ATP-ase activity in cardiac and other tissues, and that concentrations causing inhibition are in a range known to cause a positive inotropic effect.[7] As with other cardiac glycosides, digoxin acts primarily on the heart to (1) increase the force of systolic contraction; (2) slow conduction and lengthen the refractory period through the AV node and bundle of His; and (3) alter cardiac vagal activity.

Digoxin is excreted largely unchanged in the urine. Loss in the stool accounts for about 15 percent of a single dose, with virtually all of this being derived from bile. Of clinical importance is the direct relationship between glomerular filtration rate and the clearance of digoxin. Patients with renal impairment have a significantly prolonged serum half-life and tend to accumulate the drug. Anephric subjects exhibit a very long serum half-life and have markedly increased excretion via the stool, apparently representing a secondary adaptive mechanism for limiting the progressive accumulation of digoxin in such patients.[7]

CLINICAL APPLICATIONS

The clinical use of digitalis and its component glycosides is accompanied by a distressingly high prevalence of toxic manifestations, the most serious of which are arrhythmias and disturbance of conduction. Many factors contribute to the development of digitalis toxicity, with perhaps the most important factor being an accumulation of excessive amounts of digitalis in the body and in the myocardium in particular.[10] Since there is a degree of correlation between serum and tissue levels of digoxin, the measurement of serum digoxin levels provides useful information in the diagnosis of digoxin toxicity. In view of the multiple factors governing individual response to cardiac glycosides, however, it must be stressed that serum digoxin measurements should be viewed as just one of many important factors to be weighed in a complex clinical setting.[11]

The measurement of serum digoxin levels, in conjunction with careful investigation of adherence to prescribed medication schedules, has also been of value in detecting patients who fail to comply with the prescribed dosage regimen.[12,13]

J3-313C