

Screening and bioassay of thyroid hormones



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The purpose of this section is double: to describe the methods for determination of the hormones, which became separate by the thyroid gland and to describe the investigation of the thyroid function in the experimental models. To information over hypothalamisch pituitäre thyroidsystem and, you see its investigation the sections on hormones of the preceding Pituitary (N. 7) and hypothalamischen hormones (N. 9). The thyroid gland separates two kinds of hormones: the thyroid hormones i. e. Lthyroxin (T) and Triiodothyronine (T₃) the metabolic functions have and into neural development to be referred and calcitropic the hormone, Calcitonin. The system of functions for the metabolic regulation, which is helpful by the thyroid hormones, is to the complex system for regulation calcium and phosphate balance full → completely differently, led is by (thyro) Calcitonin, ready hormones of the Parathyreoid glands and the Calciferolhormone (in former times the vitamin D) produced by the liver and the kidneys and activated in the skin. The biological main effects of T₃ and T₄ are on growth and development (e. g., development of Tadpoles), its calorogenic effect (increase of the fundamental metabolic rate), the cardiovascular function (sensitivity of the heart increases to the Benzcatechinaminen) and metabolic functions (Lipid, coal hydrate and Kollagenmetabolismus). The primary back discussion effect is inhibition of the thyroid-suggesting isolation of the hormone (TSH). These effects can be used, in order thyroid hormone correspondences and - stoffwechselprodukte to examine. The thyroid hormones adjust iodine lifting and - application in the thyroid, and its activity can be restrained by Antithyroiddrogen. Historical biological drug tests are based on Morphogenese and neural development in the amphibians (Biedl 1916; Pit tri verse and Tata 1959; Copp et al. 1962; Turner and

Premachandra 1962). Thyroid hormones cause premature metamorphosis into that amphibiously for Tadpoles. Since → observation first by Gudernatsch (1913a, 1913b) this phenomenon by the numerous workers with the purpose of the adjustment of this answer for the sample thyroidal of the substances (Bomskov 1937) was studied. Within a short period the treatment with thyroid hormones causes the transformation of the Tadpole into a small frog with growth of the members, the lungs and other terrestrial equipments, and suggests the synthesis of the enzymes Morphogenese and transformation mediating. The Axolotl (*Ambystoma mexicanum* or *tigrinum*) was used like a Testgegenstand, in order to study the metamorphosis, which is caused by thyroid hormones. This animal loses the Kiemen and forms the lungs and changes at the same time the form of its end piece (Huxley and Hogben 1922; Zavadovsky and Zavadovsky 1926; Haffner 1927). Another basic rule activity of T3 and T4 is metabolic activation and increased energy expenditure. Kreitmair (1928) standardised thyroid preparations using the weight loss of the guinea pigs after 1 week of the festiveness ment as parameter. A guinea pig unit was at least reduced de →), which is as the dose ned, those the body weight of the guinea pigs with an initial weight 250-300g within 7 days by 10%. Another function role by Calcitonin is helpful. Hypocalcemic the hormones Calcitonin by Copp one discovered (Copp et al. 1962; Copp 1964). Calcitonin develops from parafollicular the C-cells of the thyroid. Its function antagonist is Parathyroid hormone. The biological drug testing of the Calcitoninvorbereitungen is accomplished, by determining its ability to lower the plasma calcium in the rat. Sample of serum (thyro) Calcitonin has one signi →) inclination diagnostic role for thyroid cancer ulcer. As with other hormones, research methods of the <https://assignbuster.com/screening-and-bioassay-of-thyroid-hormones/>

biological drug tests of the thyroid hormone activity up to direct measure of the thyroid hormones (Thyroxin and Triiodothyronine) and their Stoffwechselprodukte, up to investigations over enzymatic steps in the thyroid hormone synthesis and - inactivating, up to identi → the cation of the thyroid hormone receivers than members of the superfamily of the nuclear receivers and up to signaling are get ahead, that by operation difficulty of the thyroid hormones to their receivers are caused..

Thyroid Hormone Receptors

Obligatory proteins of the Kerntriiodothyronine were puri → OD and characterized by Torresanai and Anselmet (1978). Ichikawa and DeGroot (1987a, 1987b) described puri → the cation and the marking Rattelleberder nuclear thyroid hormone receiver and thyroid hormone receivers in a human Hepatomazellform. Apriletti et al. (1988) reported spacious puri → cation of the nuclear thyroid hormone receiver rat liver and sequence speci → C of the operation difficulty of the receiver to DNA. Ichikawa et al. (1988) and Ichikawa and Hashizume (1991) published methods of aqueous two-phases (Dextran and PL glycol) study of the nuclear thyroid hormone receivers distributing. Glucocorticoids, other Steroidhormone, thyroid hormones and vitamin-derived hormones (inclusively retinoids) all have their effects by the regulation of the hormone-accomodating goal genes within the cell core. William and Franklyn (1994) repeated the physiology of the Steroidschilddrüse hormone nuclear receiver Superfamily. A receiver-connected protein of the nuclear hormone, which restrains transactivation by the thyroid hormone and retinoic the sour receivers, became of Burris et al. described (1995). Two different genes code two different thyroid hormone

receivers, thyroid hormone receiver α and thyroid hormone receiver β and these two thyroid hormone receivers frequently on different levels in the different fabrics are CO-expressed. Chiellini et al. (1998) \rightarrow high affinity subtype selective Agonist sketched ligand for the thyroid hormone to one receiver β . The expression of thyroid hormone receptor ISO form in the rat growth disk cartilage in vivo became of Ballock et al. described (1999). Yuan et al. (1998) Coaktivator protein of the thyroid hormone (CASE) described a component of a receiver-connected complex, which affects directly the nuclear receivers on a ligand-dependent way. The sequence thyroid hormone answer of the element and the reinforcement retinoid of the x-receivers for thyroid hormone reactivity became of Wu et al. investigated.

Thyroidectomy

PURPOSE AND RATIONALE

Experiments for pharmacological evaluation of thyroid hormones and analogs were performed in thyroidectomized rats. Bomskov (1937) described the method of thyroidectomy in various animal species, such as tadpoles, frogs, birds, goats, dogs, cats, rabbits, guinea pigs, rats and mice, based on the clinical experience with thyroid resection in humans.

PROCEDURE

The thyroid in rats consists of three lobes (left, median, and right). The rat is anesthetized, e. g., with pentobarbital, and placed on a surgical table. The fur of the neck is removed with electric clippers and the area disinfected. A median skin incision of 2.0 cm length is made. On both sides large salivary glands and maxillary lymph nodes are found. They are pushed aside, making

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visible the musculus hyoideus covering the trachea. This muscle is split in the midline. The isthmus of the thyroid connecting both lobes is located below the thyroid cartilage. The lobes and the isthmus are separated with blunt forceps from the trachea and the blood vessels ligated. Alternatively, the thyroid can be removed by electrocauterization. In most cases, the parathyroid glands are severed by the operation, and postoperative substitution with calcium lactate 1% in drinking water is advised.

In Vivo Tests for Thyroid Hormones

Oxygen Consumption

PURPOSE AND RATIONALE

Basal metabolic rate, oxygen consumption and CO₂ production are increased by thyroid hormones. This has been used for diagnostic procedures in humans as well as for evaluation of thyroid hormones and their derivatives in animals (indirect calorimetry). The historical method based on survival time of mice placed individually into tightly closed glass jars (Smith et al. 1947; Basil et al. 1950; Gemmill 1953) was modified, measuring time until occurrence of convulsions. The method was based on the increase in oxygen consumption associated with the markedly increased metabolic rate at high doses of thyroid hormones.

PROCEDURE

This is a description of the now obsolete assay: mice are placed individually into 200-ml wide-necked bottles. The bottom of the bottles is covered with filter paper to soak up the urine. The bottles are tilted to an angle of 60° and rotated five times per minute in a special apparatus. The time until

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asphyctic seizures occur is noted. Immediately after observation of seizures, the mouse is released for recovery. Due to the de→ned muscle work, the time to seizures is shortened in controls to 20-30 min.

EVALUATION

Average time to seizures was calculated and dose- response curves were established.

MODIFICATIONS OF THE METHOD

Similar studies were reported by: Bomskov 1937; Lilienthal et al. 1949; MacLagan and Sheahan 1950; Reineke and Turner 1950; Anderson 1954; Heming 1964; Turner 1969

Several apparatuses have been designed to measure oxygen consumption in animals, e. g., by Holtkamp et al. (1955).

Stock (1975) described an automatic, closed-circuit oxygen consumption apparatus for small animals. A Perspex animal chamber is surrounded by a water jacket except for one end, which has a removable cover plate. This cover, as well as allowing access to the chamber interior, also holds the connections for the oxygen delivery line and the pressure line. For experiments involving injections, infusions, and blood sampling, catheters are passed through, and sealed into rubber bungs which are then forced into holes in the cover plate. A rubber gasket forms an airtight seal between the cover and the chamber. Within the chamber, the animal is supported on a wire grid over a layer of self-indicating soda lime and silica gel. A major determinant of sensitivity in this system is the dead space of the chamber.

Chambers with internal dimensions of 20 - 10 - 10 cm are suitable for animals such as mice and rats up to about 250 g body weight. Fixed volumes of oxygen are introduced into the chamber by an automatic syringe dispenser (Fisons Scientific) which draws pure oxygen from a spirometer through a drying tube filled with silica gel. When chamber pressure exceeds atmospheric by about 3 mmH₂O, the microdifferential pressure switch (KDG Instruments) inactivates the dispenser. The dispenser is reactivated when the pressure differential drops below this threshold value. The volume of oxygen dispensed is adjusted to the smallest volume that, with a single action of the syringe, will return chamber pressure to above the threshold value. The particular dispenser used in this system has the advantages of being (1) gas tight and (2) when activated will complete its pump cycle even if the chamber pressure exceeds the threshold value in midcycle. A discrete fixed volume of oxygen is delivered each time it is activated. To obtain the rate of oxygen consumption it is merely necessary to record the pump rate.

Inhibition of Iodine Release

PURPOSE AND RATIONALE

The thyroid gland has a high avidity for iodine, uptake of which may be measured by isotope-labeled iodine (

¹³¹I), in a dose-related and time-dependent manner. The release of ¹³¹I from the thyroid in rats is inhibited by treatment with thyroxine (Wolff 1951), and the degree of inhibition is related to the dose administered (Perry 1951). This phenomenon was used to compare activity of thyroid hormone derivatives with the standard thyroxine.

For analytical and diagnostic purposes, direct quantitation of thyroid hormones is now achieved by methods such as radioimmunoassay and HPLC chromatography, and by measuring feedback inhibition of thyroid hormones directly via the decrease in serum TSH.

PROCEDURE

Male Sprague-Dawley rats weighing 180-240g are fed a commercial laboratory chow without or with addition of 0.03% propylthiouracil (reference compound for thyroid peroxidase inhibition). Food is withheld 8 h before the injection of 25 μ C131I or 125 I intraperitoneally. Radioactivity over the thyroid region of the neck is determined 40 h later (if necessary under sedation). This reading is taken as time zero and all further counts made at 24-h intervals may be expressed as a percentage of time-zero counts after correction for physical decay of the 131I isotope. After the reading at time zero, the diet is changed to a feed containing 0.03% propylthiouracil, and several doses of the test preparation or the standard are injected subcutaneously at 24-h intervals up to a total of four doses. The daily loss of 131I is inversely proportional to the dose of thyroid hormone.

EVALUATION

Percentage of time-zero counts after 96 h of I remaining in the thyroid after the last of four doses is plotted against logarithm of dose. From these dose-response curves, potency ratios are calculated.

The method has been used by several authors: Reineke and Turner 1950; Anderson 1954; Turner and Premachandra 1962

CRITICAL ASSESSMENT OF THE METHOD

The assay described here was used for quantitative estimates and has now been replaced by analytical determination of thyroid hormone contents. For human drug formulations, bioequivalence studies are required when generic formulations are assessed. This approach of measuring the uptake and release of labeled iodine may be modified for short-term uptake of ^{131}I or ^{125}I as a parameter of thyroid peroxidase inhibition by antithyroid drugs, and other drugs affecting thyroid function.

Anti-Goitrogenic Activity

PURPOSE AND RATIONALE

Thyroid weight and size are controlled by the action of thyroid-stimulating hormone (TSH) on thyroid tissue. In rats, increased secretion of TSH induces thyroid enlargement and weight increase within a few days (addressed as goiter formation). In normal animals the secretion of TSH by the pituitary is regulated by feedback of thyroid hormones. The administration of goitrogenic compounds which block thyroid hormone synthesis and/or secretion reduces the concentrations of circulating thyroid hormones (T) and their pituitary effect (negative feedback inhibition of TSH secretion), releasing TSH from its feedback inhibition. The TSH rise induces hyperplasia of the thyroid follicles as indicated by the dose-related increase of thyroid weight. Hyperplasia is prevented by injection of thyroxine, triiodothyronine or thyroid hormone analogs.

PROCEDURE

Male Sprague-Dawley rats weighing 150-180 g are used in groups of eight to ten animals. During the treatment period, 0.1% propylthiouracil (PTU) is added to the food or to the drinking water, in order to achieve a stable baseline of thyroid weight. Over a period of 2 weeks, the rats are treated (preferably by gavage) with various doses of the test compound or the thyroxine standard (10-40 µg/kg). PTU controls are treated with the suspension medium or saline injections only. At autopsy on day 14, the thyroid glands are dissected out and weighed rapidly to avoid evaporation loss. Thyroids may also be lyophilized first to weigh dry matter. The two- to three-fold increase of thyroid weight by PTU is reversed dose-dependently to normal values by thyroid active substances.

EVALUATION

Dose-response curves are plotted and potency ratios with confidence limits may be calculated.

MODIFICATIONS OF THE METHOD

Similar studies were reported by: Reineke et al. 1945; Pitt-Rivers and Tata 1959; Turner and Premachandra 1962; Wiberg et al. 1964; Ortiz-Caro et al. 1983; Pisarev et al. 1994

The effect of PTU-induced baseline suppression is monitored and ascertained by measuring serum TSH, T4 and T. The dose-related inhibition of the TSH rise by thyroid substances is used as the parameter to assess goiter prevention.

Tensile Strength of Connective Tissue in Rats, Modified for Thyroid Hormones

These studies are an example of evaluating the biological effect of high doses of thyroid hormones on tissues other than those involved in the increase of metabolic rate. Thyroid hormone secretion affects almost all tissues in the body, and high doses may exert unwanted effects on connective tissue.

Antithyroid drugs general views Antithyroiddrogen obstruct synthesis, release and/or the auxiliary activity of the thyroid hormone and lower the basic conversion. They are used in the treatment of the thyroid disturbances (Hyperthyreose). The reconciliation of the isolation T4/T3 reduces thyroidal inhibition of the pituitären gland, zunehmenTSH isolation and causes then the goitrogene answer. This answer was used to determine over Antithyroiddrogen and for Siebungverfahren at most was used. It is however nonspeci → C and can by some different mechanisms, including enzyme induction of glucuronyltransferases be caused. The goitrogene answer is from the considerable interest in the toxicology, because it can be produced by some means during the early drug evaluation, which the bio-synthesis and/or inactivating of the thyroid hormones change in an unexpected way. Inhibition of the iodine elevation in the rats PURPOSE AND BASIC PRINCIPLE

Propylthiouracil (PTU) and a broad spectrum of the drugs can restrain thyroid hormone synthesis. Some these drugs are used, in order to treat thyrotoxicosis. As consequence of the Schilddrüseperoxydasehemmung the iodine lifting is reduced through and contents in the thyroid. This

phenomenon is mix dependent and can appear to increase thyroid weight in the rats (McGinty and Bywater 1945) at the untereren doses as those. The historical parameter of iodine contents was replaced, by measuring a lifting and the release of ^{131}I .

PROCEDURE

^{131}I . Groups of the male Wistar rats age 26-28 days and weigh 40-45 g, set within metabolism frameworks. They are drawn in normal diet, and potassium iodide is added the drinking water. In modes \rightarrow a cation of the method (for toxicology studies), can be added the test means or the reference standard (some concentrations) of the diet over a length of time by 10 days, and the quantity of the means taken by each rat then computed expressed by the total food consumption in 10 days and in the milligram daily paper per kilogram body weight. After 10 days of the treatment, the rats are sacri \rightarrow ced and the thyroids dismembered freely from the adjacent fabric and from the cap. The thyroid is weighed and determined iodine contents. In the daily doses of between 0. 1 and 10. 0 mg/kg, Thiouracil reduces iodine contents of the thyroid in a mix-dependent way. Higher doses De \rightarrow nitely are necessary, in order to increase thyroid weight.

EVALUATION

responding to the dose curves of the test means and reference standard are plotted, and force conditions with fraud \rightarrow dence delimitations can be computed. CHANGES of the METHOD Walker and Levy (1989) used transplantable tablets of Propylthiouracil, in order to cause thyroid malfunction in the rats. Lift marked iodine in place of of iodine contents one measures. Release of marked iodine knows through protirelin (TRH) injection <https://assignbuster.com/screening-and-bioassay-of-thyroid-hormones/>

to determine over thyroid function or become lively as quantitative biological drug testing for the effect of the hypothalamischen hormone TRH.

Antithyroidal of effects in the animal samples the Sauerstoffverbrauch in iodine-treated mice was used as biological drug testing, modes → OD for Antithyroidtätigkeit. PURPOSE AND BASIC PRINCIPLE historical biological drug testing are based on Sauerstoffverbrauch, which is increased acutely potassium iodidetreated the mice, with the result of a decrease of the asphyxiation time (thyroid activation). This effect is the dose-dependent, which are fought through antithyroidal means, and which is to time to the cramps because of the reduced metabolic rate extended. The methods is based on increased Sauerstoffverbrauch after thyroid hormones (section. N. 5. 1. 1) are applied. CHANGES of the METHOD thyroid weight was an early parameter for querying the Antithyroidtätigkeit. Rabbit treated with goitrogenen means or with Kohl (Chesney et al. 1928 exclusively drawn in; Navy et al. 1929) a tenfold increase of the thyroid weight to shown, histological announce as hyperplasia without kollodiale arrangement. These phenomena were waived by iodine treatment (Bomskov 1937).

Kropfanordnung as side effect of non steroidal anti- →, in ammatory the drugs became of Mueller et al. studied (1985). Calcitonin general views calcitropic the hormones (thyro) Calcitonin was discovered in the C-cells of the thyroid gland of Copp (Copp et al. 1962; Copp 1964, 1994). This hypocalcemic hypophosphatemic basic rule of the thyroid gland (Austin and heath 1981) became thyrocalcitonin of deer et al. (1964), Munson and deer (1966), Raisz et al. (1967) and MacIntyre (1992) characterized. Its calcitropic effects on bone and kidney function are opposite those of the Parathyroid of hormone. Calcitonin develops from parafollicular the C-cells of the thyroid.

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Calcitoninabsonderung can be evaluated using the located gedurchströmten pig thyroid (Pento 1985) in vitro. Radioimmunoprobe for Calcitonin are present (Tashjian and Voelkel 1979), and sort speci → C methods for Calcitoninermittlung must be regarded. Samples for Calcitoninempfänger were described (Nissenson et al. 1985). Overviews on effects of the exogenous Calcitonin were given by Deftos (1989); Braga (1994); Embankment oh et al. (1999). The biology and the clinical meaning of the Calcitoninengenpeptide were repeated (Reginster 1993; Silverman 2003; Zaidi et al. 1990). Decrease of the serum calcium at the rats

PURPOSE AND BASIC PRINCIPLE the biological drug testing of the Calcitoninvorbereitungen using their ability to lower the plasma calcium accomplished in the rat. Also with the pharmacopeias, existing using the international reference preparation for the Calcitonin (pigs) of gefriertrocknetem puri → OD pork Calcitonin was accepted, and during the international reference preparation consisting this procedure for the Calcitonin (salmon) of gefriertrocknetem puri → OD synthetic Lachscalctonin. These samples for Calcitoninquantitative regulation however were replaced now by a physicochemical method for pharmaceutical quality control. Either intravenous or subkutane administration can be selected. International standards for Lachscalctonin, Aal Calcitonin and the Asu 1-7 correspondence of Aal Calcitonin are expenditure work CCIT (Zanelli et al. 1990). A second international standard for pig and human Calcitonins was manufactured by an international cooperative working group, those on drug testing biological in vivo rat hypocalcemia (Zanelli et al. 1993) are based. If the groups of at least female Wistar rats → of the VE, weighing 100-120g, PROCEED are used. Three doses standard preparation (1, 3 and 9 MU per rat) and three doses test

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preparation are intravenously injected. Then 1 h is taken back after injection, blood under bright anaesthesia. Plasma calcium is determined through →, ame Photometrie or by Atomabsorptionsphotometrie. EVALUATION responding to the dose curves of decreases at the plasma calcium manufactured and force conditions with fraud will become →) dence delimitations computed.

CHANGES of the METHOD

similar studies were reported past: Kumar et al. 1965; Munson et al. 1968; Rittel et al. 1976; Schwartz et al. 1981; Michelangeli et al. 1983; Findlay et al. 1985; Dollar and Maxl 1990; Deming et al. 1994 Yates et al. (1990) determined the acute hypocalcemic answers to individual subkutanen injections of the Calcitoninvorbereitungen into intact young Swiss mice man of the ICR, which weighed 12-20 G. Calcitonin of the Stingray and the SH gold →) became of Sasayama et al. marked (1992, 1993). Kapurniotu and Taylor (1995) led hypocalcemic in-vitroproben in the mice by analysis of the serum calcium 1 h after subkutaner injection of lactambridged correspondences of the human Calcitonin through. Effect of Calcitonin on Osteoclasts in vitro PURPOSE AND BASIC PRINCIPLE Calcitonin proceeds mainly in accordance with inhibition osteoclastic of the bone admission (Friedman and Raisz 1965; Aliapoulios et al. 1966). Zaidi et al. (1990, 1994) the development reported and on the validation of three microbioassays for the Calcitonin, which was based on calcitonin caused inhibition of the activity of the located osteoclasts. PROCEDURES of thigh legs and Schienbeine are removed from the newborn Wistar rats. The bones are released to fötalem calf rum, benzyle penicillin (100 µU/ml) and Streptomycin by the adhering

soft fabrics and means 199 HEPES moderate by the cut over their Epiphyses in supplemental with heat-inactivated (100 µg/ml). Osteoclasts are mechanically divided, by exciting the bones of each rat with a Skalpellblatt into a 1 ml-means curetting and the abolition with a pipette. Larger fragments will let agree for 10 s, before the Supernatant on suitable substrate fall one leaves (bone disks, PlastikPETRISCHALEN or glass cover glasses). Motilität created system the morphologic appearance of the stained osteoclasts is used like an index, in order to determine the condition cell plasma tables of the activity. Osteoclasts are agreed upon on coverglass in the micro titer wells and become for minute of 20 at 37°C. expenditure-bred. The cover glasses are removed into different wells, each contained 100 µl means, put washed with means 199 and. After a further Ausbrütung for minute of 30 (37°C), those series dilutions (tenfold) salmon or human Calcitonin or test preparations or suitable dilutions of the plasma samples are added. The cells are →), nally for 2 h, which are expenditure-bred →), which are stained in the 10% Glutaraldehyd xed and with Toluidinblau. The condition of the Motilität of everyone, which is osteoclast on each cover glass, is counted, by observing the characteristic deformation, which these cells go through, when Motilität is restrained; a freely mobile cell marked by a smooth outline by increased staining intensity over everything or partially its periphery, while a immotile cell usually an irregular slat-outline without call →, Edränder shows. The number immotile cells is counted and expressed on each cover glass, how a percentage of the total number cells counted. Cytoplasmatic spread system Osteoclasts are agreed upon in the fabric cultural plates (35 millimeters) and expenditure-bred at 37°C, so that minute permits 20 sediment formation and accessories. The cells are washed with <https://assignbuster.com/screening-and-bioassay-of-thyroid-hormones/>

means 199 and to 2 ml the same means into everyone well are then put. The plates are put converted into the Ausbrütungsraum of phase contrasting microscope. Pictures osteoclasts are noted on a time lapse video equipment. A pursuit of their will outline by a digitization system brought into a computer, programmed, in order to measure the range within each pursuit. Those outline of osteoclast everyone before or after the additive of Calcitonin or from carrier to the cultures are noted. For each variable outline by six osteoclasts after a 60-Minute-Ausbrütung in the area and again in the 40 pursued, which is following minimal the additive of the hormone. The central surface taken off by six osteoclasts, after Ausbrütung is expressed as percentage of the central surface osteoclasts before the additive of the hormone or the carrier. Bone photograph system of copies of the human kortikalen thigh bone are received from the donors (patients, who died without proof of the bone illness). The adhering soft fabric is removed and the bone crust cut longitudinal in disks (0, 1 millimeters strongly). The disks are then cut into pieces (approximately 3 millimeter of 2). It through ultrasonication (minute of 15, in the sterile distilled water), drained cleaned stored by immersion 80% in aqueous ethanol for 2 h, and, in order to dry at room temperature. Osteoclasts located 199 in means will fallen on 12-16 bone disks, which were put well in 18 Millimetermultiweltellers. After Ausbrütung (37°C, minute of 15), the disks are removed, and washed easily supplemented in the minimum substantial means with 10% FCS and antibiotics, as described above. They are put to well-being contained → VE into different wells, each to six disks in 900 µl means. After further Ausbrütung (37°C, the 10% humidity → OD CO, minute of 10), is contained added µl 100 of the means the test concentration of the hormone or the

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Testlösung. Human PTH (1-34) (0, 1 U/ml) one uses, in order to determine function effects of the contamination of osteoblasts. The Calcitoninentsprechungen is examined with different concentrations (tenfold dilutions). Finally bone disks are expenditure-bred over night (37°C, the 10% humidity →) OD CO₂ 18 h). The cells are →), which is examined by transferred light microscopy in the Glutaraldehyd xed, with Toluidin blue stained and. Osteoclasts and in-full of seeds cells are counted. The disks are bleached then by immersion in the sodium hypochlorite solution for 30 minimum and drain 80% in aqueous ethanol. Finally are they squirt covered with the gold, randomized and in an electronic microscope of scanning examined. The numbers osteoclastic weakening, each de →), which is by a continuous edge ned, are counted. The range of the bone surface resorbed is computed, by one outline the concavity into a digitization tablet pursued, connected with a microcomputer. Surfaces of the admission can be expressed as per cent age of the means of the tax answer. EVALUATION data of each sample using the classical methods for analysis of the parallel line samples are analyzed. Estimations of the relative forces are computed of the parallel machine log book addressing on the dose lines of the test preparations and the reference preparation. Osteoclasts are divided and absent-minded mechanically by the long bones of the newborn rat at the low densities on disks devitalized of the cattle cartilage bone. The result areas of the bone weakening are quanti →) OD with mikrometric precision, by them electron microscopy as well as computer-assisted image analysis scanning. These →) ndings are used, in order to develop a formal biological drug testing for Calcitonin. Reacts to receiver operation difficulty and camp accumulation in located cells

PURPOSE AND BASIC PRINCIPLE the human cancer of the breast cell form

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T47D to Calcitonin and his correspondences by receiver operation difficulty and accumulation of the camp. This can as biological sample (Findlay et al. 1980, 1983, 1985 to be used; Grey et al. 1992; Küster and Hilton 1992; Curtains et al. 1993). PROCEED the human cancer of the breast cell form T47D originally by polarizing Urals Erguss of in →) one manufactured, ductal cancer of the breast (Horwitz et al. 1978) ltrating are. Washed for obligatory experiments cell-monomolecular films with 0. 02% EDTA before treatment with 0. 125% Trypsin in 0. 02% EDTA for minute of 2 at 37°C, introduction of the complete means before centrifugation with 200 g and Resuspension in the complete means. Iodination of Calcitonin is accomplished with 125 using the Chloramin t method. For obligatory experiments T47D, which cells in the isotonischen buffer shifted, the Lachscalctonin 125I-labeled are added, which is mixed with the different concentrations of the unlabelled Calcitonin, or correspondences and at 20°C for 1 H. Nonspeci expenditure-bred →) C operation difficulty as the operation difficulty of the 125I marked Lachscalctonin is determined in presence of the surplus (2 µg/ml) unlabelled Lachscalctonin. Suggestion of Adenylate Cyclase into the intact T47D cells by Calcitoninentsprechungen is determined, by measuring production of the camp [3H] in the cells, prelabeled is also, adenine [3H]. Zellulare Atp laughter become by Ausbrütung with [3 H] adenine 2, 8 - (0, 5-2 µCi/ml) for 2 h at 37°C in 12 wohlen cultural plates in RPMI mark 16