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CATECHOL ESTROGEN RECEPTOR ASSAY OF 7,12-DIMETHYLBENZANTHRACENE (DMBA) INDUCED RAT MAMMARY TUMORS.

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Catechol estrogens are the major metabolites of estrogen in human and animals. Recently, 2-hydroxyestradiol showed appreciable inhibition of tumor growth in DMBA induced tumor. Thus, we investigated the binding of catechol estrogens and estradiol to cytosol receptor of DMBA induced rat mammary tumors.
Famale Donryu rats were given DMBA by gastric intubation. In mammary tumors of 14 rats, we carried out radio-receptor assay of catechol estrogens and determined their receptor concentrations and the affinity of their receptors using a Scatchard plot.
The receptor concentration of catechol estrogens was twice higher than that of estradiol in rat mammary tumor cytosol. On the other hand, affinity constants (Ka) of estradiol, catechol estradiol, and catechol estrone in rat mammary tumor cytosol were 2.81×10^8, 1.71×10^8, and 1.11×10^8 (M^{-1}), respectively. It is suggested that the binding activity of catechol estrogens is exhibited to the cytosol receptor of DMBA induced rat tumors.

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A SENSITIVE DETERMINATION OF ACTH IN PLASMA USING DIRECT RADIOIMMUNOASSAY AND ITS CLINICAL APPLICATION

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A sensitive determination of ACTH in plasma was established using direct radio-immunoassay(RIA)method and investigated its clinical application. RIA procedure was performed by delayed assay method at 4°C for 48 hours and B/F separation was achieved by 2nd antibody method. The antiserum(final dilution 1:22500)supplied by Dr.Orth, produced a linear standard curve between 0.5 to 20 pg/tube. The dilution curves of several patients's plasma were parallel with the standard curve. Recoveries were 100+9.8% . The C.V. were about 4-10%. The concentrations of ACTH in normal subjects were less than 125 pg/ml(n=96). Farthermore, plasma levels were increased in patients with Cushing's disease and markedly elevated in patients with Addison's and Nelson's disease , Whereas those in patients with Cushing's syndrom and Isolated ACTH deficiency were undetectable. Plasma levels, in CRF infusion test in normal subjects, were 1.5-2 times increased transiently after 15 min and in insulin infusion test in normal subjects, were 5-10 times elevated after 45 min. In conculusion, the present method has the advantages of high sensitivity(0.5pg), small sample volume(50ul), responsibility of trerance tests and correlation with disease.

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FUNDAMENTAL AND CLINICAL EVALUATION OF PTH-MM RADIOIMMUNOASSAY.

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PTH radioimmunoassay(RIA) using antisera which recognize primarily the middle-molecule(MM) of parathyroid hormone was studied for clinical evaluation of various parathyroid status. PTH-MM RIA system used was a INC PTH-MM RIA kit. The following fundamental data were obtained. The minimal detectable dose was 10 pmol/L.;recovery of PTH-MM added to the serum was 97.4%; inter-assay variance was less than 9.8%(C.V.) and intra-assay variance was 2.7-8.9%. Serum levels of PTH-MM were determined in 40 normal subjects (N), 6 patients with primary hyperparathyroidism(PH), 45 patients with secondary hyperparathyroidism(SH) and 3 patients with hypoparathyroidism(HO). The mean PTH-MM level in N was 22.6±8.4 pmol/L, in PH was 507±524 pmol/L, in SH was 4179±4533 pmol/L, and in HO was 32.7±1.7 pmol/L. In 6 patients with hyperparathyroidism, serum PTH-MM level was significantly decreased from 2517±2652 pmol/L to 48.3±40.8 pmol/L after parathyroidectomy for abnormal gland(s). Good correlation was observed between serum PHT-MM levels and PTH-C levels in 92 subjects;such as r=+0.98, y=175.3x-145.3. However, there is no patients was found discrepancy between PTH-MM and PTH-C level in this series. The data suggested that the PTH-MM RIA was useful for clinical experiments.

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OSTEOCALCIN IN BLOOD.

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Osteocalcin, bone gamma caboxyglutamic acid containing protein (BGP), M.W.5,800, is currently studied in the metabolism of bone. The synthesis of osteocalcin is localized in the osteoblast. In the resorption of bone, osteocalcin is released into the blood. The assay of osteocalcin in blood was studied concerning about the daynamic analysis in the metabolism of bony disorders.

The assay of osetocalcin was performed using Osteocalcin Kit (INC, U.S.A.).The concentration of osteocalcin was increased with the aging; up to 14 years old in males, and upto 12 years old in females. After this, the concentration in the blood was decreased in both sexes. This convex curve was very similar to the height velocity curves and also corresponded to the concentraiton of 1,25 hydroxy-Vitamin D_3. The osteocalcin in the blood will be depndent of the growth in the pediatric group.Diurnal changes was found in the healthy adults, maximal and minimal concetration was observed in the night and daytimes, respectively. In the patients with renal failure, osetocalcin was increased; in the group of patients received maximal dosage of steroids, it was decresed. In the patients with osteonecrosis, osteocalcin was increased, but when treated it was normalized.

The assay of osteocalcin will be informative to study of the metabolism of bone.