299 QUANTITATION OF CIS-PT DNA ADDUCTS IN PHAGE PM2 DNA.

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Monoclonal antibody (mAb) 62-5 was derived from a mouse immunized with cis-Pt modified calf thymus single strand DNA. From the results of enzyme-linked immunosorbent assay (ELISA), this antibody recognized either denature or native cis-Pt modified DNA, but not unmodified DNA or free cis-Pt. The binding of mAb 62-5 to cis-Pt-dsDNA was competitively inhibited by cis-Pt modified DNA, polydG, poly(dGdC) or polydG.polydC.

To measure small amount of cis-Pt DNA adducts in PM2 phage, PM2 DNA treated with various doses of cis-Pt were used as standardized samples and measured by means of atomic emission spectrophotometry. The number of cis-Pt PM2 DNA adducts against mAb 62-5 per DNA molecule was determined by ELISA with standardized DNA samples in the same microtiter plate. The results showed that cis-Pt PM2 DNA adducts against mAb 62-5 is proportional to the inactivation efficiency of cis-Pt PM2 DNA after transfected into host cells.

⊙300 STUDY OF SERUM STEROIDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

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The most common form of congenital adrenal hyperplasia (CAH) is due to 21-hydroxylase deficiency, which results in deficient biosynthesis of cortisol and accumulation of its precursors.

A high performance liquid chromatography (HPLC) system for the analysis of serum steroids was developed. Serum samples were extracted by Extrelut Column 1 (Merck) and eluted with ethylacetate. The eluate was evaporated in a centrifugal evaporator at 40°C and the residue was redissolved by mobile phase (methanol/water=52/48, v/v). Determination of serum steroids was accomplished by reversed phase (C-18) HPLC with UV (250±4nm) detection. The retention times of the steroid standards (cortisone, cortisol, dexamethasone, corticosterone, 11-deoxycortisol, androstenedione, deoxycorticosterone, testosterone, 17-hydroxyprogesterone) were between 4 and 19 min. The within-run precision was 2.7-7.8% (n=10) with good linearity (r=0.999). The serum concentrations of cortisone and cortisol in normal adult were estimated to be 37.5±29.3 ng/ml and 36.5±14.5 ng/ml, respectively, which were much higher than that found in the patient with CAH. Whereas, the patient with CAH had a serum level of 173.8 ng/ml of 17-hydroxyprogesterone, which is undetectable in the serum of normal subjects.

This method is simple and fast in simultaneous analysis of several steroids which are clinically important. The method may offer aid in diagnosis for cases with positive CAH neonatal screening test and may also be applied in monitoring therapy.