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EVALUATION OF A SPECIFIC AND DIRECT RADIOIMMUNOASSAY TECHNIQUE FOR PROINSULIN MEASUREMENT IN HUMAN SERUM.

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This work describes a highly specific human proinsulin RIA developed by using biosynthetic proinsulin as immunogen, standard and tracer. Proinsulin was radioiodinated by the iodogen method, presenting great stability after QAE-Sephadex A-25 purification. The antiserum was raised in guinea pig and does not cross-react with insulin and C-peptide ($<0.01\%$), recognizing the junction between the insulin B-chain and C-peptide. The technique employed was the PEG assisted second antibody RIA in a non-equilibrium system.

The assay sensitivity of the order of 0.007 pmol/ml proved to be suitable for the mesurement of low circulating proinsulin levels by the use of concentration techniques. The recovery of extracted with Sep-Pak C18 cartridges and concentrated proinsulin was 115% and the recovery of unextracted one was between 94 to 126%. Precision was 7.3-17.1% (CV, intra-assay) and 9.2-13.9% (CV, inter-assay).

The RIA was applied in the measurement from low (normals, 0.018 ± 0.004 pmol/ml, mean $\pm$ SD) to high levels (patients with insulinoma, 0.227 ± 0.097 pmol/ml; mean $\pm$ SD) confirming its validity.

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DIRECT LABELLING OF ANTI-CEA MoAb WITH Tc99m: EVALUATION THE DEGREE OF FRAGMENTATION AND IN VIVO STABILITY.

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The mercaptoethanol reduction based techniques used for labelling of antibodies may yield some degree of fragmentation.
During our experient anti CEA MoAb (IOR-1) and human IgG (Sandoglobulin) were reduced with ME at two different molar ratio (1000; 3000:1). After reduction (30 min) the solution were purified on Sephadex G-50 column, and fraction collected were frozen (-70°C) or lyophilized. The number of free SH groups by Ellman's reagent were determined before and after of reduction and lyophilization. Structural integrity of reduced MoAbs were determined by non-reduced PAGE and BIO-DAD HPLC system with a UV detector. Chromatography study were performed using a BIO-Sil TSK-250 column for mol. weight sizing. Samples (20 µl) were injected and eluted at flow rate of 0.5-1 ml/min with 0.02 M phospate buffer, pH:6.8. Serum stability and cysteine challange assay were done followed by biodistribution study in nude mice bearing CEA positive human colorectal adenocarcinoma xenografts.
HPLC analysis of reduced and radiolabelled anti CEA showed one major peak (> 90 %) with a retention time identical with unreduced MoAb. Results of PAGE study showed slight fragmentation (3-10 %) only. Labelling efficiency (at 50 µg $SnCl_2x2H_20$) was more than 95 %. Serum stability was 94 % at 4 hrs, 89,1 % at 24 hrs. Dissociation in cysteine challange assay (25 mM) was less than 10 % also. Biodistribution showed high activity in blood pool, liver, Kidney and tumour (8.3 %ID/g). The number of endogenous SH generated was between 1.2 - 3,4 SH per MoAb mol. (< 10% of SH available).
In summary, this model experiments indicates that standard ME reduction do not produce structural perturbations of MoAb only slight fragmentation occuring.

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QUALITY CONTROL OF Tc-99m-LABELLED MONOCLONAL ANTIBODIES

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The search of rapid and sensitive analytical methods for Q.C. of radiolabelled monoclonal antibodies are of special importance. In this work the labelling efficiency, composition/activity distribution and serum stability of Tc-99m labelled anti-CEA (IOR-1), MAK-47 and hIgG were studied by using TLC, HPLC, radio-HPLC and non reduced PAGE methods.
HPLC analysis of MoAbs was performed using BIO-RAD HPLC system. Chromatographic separation was performed on BIO-Sil SEC column (600 x 7.5mm) for molecular weight sizing. Samples (20 µl) were injected and eluted with 0.02 M NaH_2P0_4/0.05 M Na_2S0_4 buffer (pH 6.8) at a flow rate of 0.8 ml/min. Protein absorbance was monitored at 280 nm, gamma radiation was measured simultaneously. In PAGE study samples (cold and labelled) were applied to 7.5 % gel, electrode solution contained 0.2 M phosphate buffer of pH 7.2. Electrophoresis was carried out at 4 mA per gel for 24 hrs. Each sample were tested simultaneously on two gel, one was stained and the other was frozen and sliced to 2 mm sections and counted to determine the distribution of radioactivity.
The labelling efficiency (%) and stability determined by TLC was as follows:

	20 min	3 hrs	6hrs
IOR-CEA	98.1±0.2	96.0±0.4	95.0±0.4
MAK-47	97.9±0.3	98.1±0.2	97.1±0.3
hIgG	98.2±0.5	98.0±0.2	96.0±0.3

These results were confirmed by HPLC and PAGE as well. The HPLC study showed low dimer fraction (< 3%) and high monomer IgG (> 90%) content. Slight fragmentation (< 5 %) was detected by PAGE also.
In summary, the methods used during our experiments provide usefull information about the composition/activity distribution and stability of Tc-99m labelled monoclonal antibodies.

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" Beta-endorphines, cortisol and testosterone radioinmunoassay to evaluate stress in patients with non-insulin-dependent diabetes mellitus (Type II DM) ".

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Non-insulin-dependent diabetes mellitus is the most common form of diabetes, accounting for 85-90 % of the diabetic population. The aim of the present research was the simultaneous evaluation of stress hormones and degenerative oxidant injury blood signs in type II-DM patients (DM: n=12, mean age 61.5 ± 7.7 years) and in a control group (C: n=10, mean age 63.1 ± 5.2 years). All patients with DM were in treatment with sulphonylureas.

Radioinmunoassay of plasma cortisol, beta-endorphin and testosterone was performed. In addition, routine haematological tests, dyslipemia, plasmatic glucose level, plasmatic fructosamine and stress oxicative parameters: erythrocyte susceptibility to oxidant injury, plasmatic thio-barbithuric-acid reactive substances (TBARS) and plasmatic antioxidant capacity (PAC) were evaluated.

Results : cortisol (ug/dl) :D=17.9 ± 1.8 vs C=13.3 ± 0.6 ($p<0.05$); beta-endorphines (pm/l): D=9.27 ± 1.16 vs C=5.09 ± 0.84 ($p<0.02$); testosterone (ug/dl): D=3.74 ± 0.40 vs C=4.52 ± 0.40 (N.S.); glucose level (mg/dl): D=149.0 ± 12.6 vs C=87.9 ± 11.8 ($p<0.025$); fructosamine (mg/dl): D=649.2 ± 46.9 vs C=303.9 ± 39.3 ($p<0.001$); red blood cell susceptibility (nmTBARS/ml erythrocyte): D=7.72 ± 0.30 vs C=6.59 ± 0.20 ($p<0.01$); plasmatic TBARS (nm/ml) D=21.4 ± 3.2 vs C=12.0 ± 2.1 ($p<0.05$) and PAC (%): D=6.96 ± 5.38 vs C=20.87 ± 2.37 ($p<0.05$).

Results obtained showed statistically significant differences of specific Type II DM indicators, stress hormones and oxidative parameters, between DM patients and a control group. In Type II DM there is an increased effect of stress and an accelerated oxidative aging. These results suggest the necessity to continue studying possible relationships between both inespecific processes.