chromatography in procedure which took two working days. The enzyme was obtained with a yield of 30.8%. The overall purification was about 5882 fold. The effect of temperature and optimum pH of the enzyme activity was also examined. And we observed the pH curve has more than one maximum value (mainly at pH 8.0 and pH 9.5). This type of curve may be seen for diprotic systems and indicate that the active site of the enzyme may contain several ionizable groups.

P04-95
Variations in salivary function in a rodent model of pre-diabetes

L. Rodrigues[1], R. Mouta[1], C. Barra[1], A. R. Costa[2], F. C. e Silva[2], O. Lopes[2], M. Lima[3], C. Antunes[4] and E. Lamy[2]
[1]University of Évora, Reguengos de Monsaraz, Portugal, [2]ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Évora, Portugal, [3]Departamento de Biologia, Universidade de Évora, Évora, Portugal, [4]CNC - Centro de Neurociências, Universidade de Coimbra; ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterranicas, Universidade de Évora, Évora, Portugal

Diabetes is a widespread disease representing an enormous part of the total health costs. An early diagnostic could be of extremely importance both for the understanding and prevention of this pathology. Saliva is a fluid with increasing interest as a source of biomarkers for disease diagnostic and saliva protein composition changes have already been reported for diabetic individuals. However, the studies were performed after the onset of the disease and it is unknown if salivary changes are present in the early stages of development of the disease or a characteristic of overt diabetes. Wistar rats have been selected for their glucose intolerance (GIR). GIR females were compared with Wistar females with normal glucose tolerance (control) for changes in saliva protein composition and salivary gland histology. Fasting glycemias were observed to be normal (<95 mg/dl) in GIR animals, indicating an absence of a diabetic state. However they presented an abnormal increase in glycemia after a glucose bolus. For salivary parameters a marked increase in total protein concentration and alpha-amylase activity occurred in GIR animals, comparatively to controls. After separation of salivary proteins by SDS PAGE differences between the experimental groups for some protein bands, with apparent molecular masses ranging from 20 to 55 kDa were observed. Different expression of alpha-amylase at salivary gland duct level is also apparent for pre-diabetic animals. Although preliminary, these results suggest changes in saliva occurring before the onset of diabetes, reinforcing the interest of further investigation of saliva composition for the diagnostic of pre-diabetic condition, ultimately allowing an early intervention and eventually the prevention of disease development.

P04-96
Designing targeted drug delivery systems by targeting different cell surface receptors for thyroid cancer treatment

F. G. Martínez and **C. G. Montero**
Department of Citology and Histology Normal and Pathological, Faculty of Medicine, University of Seville, Seville, Spain

Introduction: Nanotechnology has lead to an improvement in the diagnostics techniques and treatment of several diseases, using nanoparticles which transport the therapeutic agent specifically to the pathological area. Among many possible applications, tumors have been the most often investigated. Thyroid cancer is the most frequent endocrine neoplasm, and although differentiated cancers have in general a very good outcome, undifferentiated tumors, as in the case of anaplastic thyroid cancer, are more aggressive and do not respond to treatment. Our long term goal is to develop a targeted drug delivery system for thyroid cancer treatment, and for this purpose, we have first focused our work in the evaluation in cell cultures of polymer nanocarriers directed to potential targets on the surface of thyroid cells by fluorescence microscopy.

Experimental Methods: Model polymer nanocarriers (NCs) were prepared by coating green fluorescent polystyrene spheres (100 nm) by surface adsorption with different antibodies against the thyrotropin receptor (TSHR), and the epidermal growth factor receptor (EGFR), which is known to be overexpressed in a number of cancers. Binding and internalization of anti-TSHR or anti-EGFR NCs were tested by fluorescence microscopy in human thyroid follicular control cells (Nthy-ori 3-1), cells derived from a follicular carcinoma (FTC-133), and an anaplastic thyroid cancer cell line (8505C).

Results and discussion: All tested antibodies directed to TSHR and EGFR recognized thyroid cells as a monomolecular antibody. However, when coupled to nano-sized carries systems, only anti-EGFR nanocarriers bound efficiently to thyroid cells, although with different binding patterns, depending on the cell line. Furthermore, anti-EGFR NCs were internalized by thyroid cells in culture, mainly in the case of anaplastic thyroid cancer cell line. Our results provide an avenue to explore anti-EGFR NCs as transporters to specifically deliver therapeutic agents to thyroid cancer cells.

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P04-97
Localization of pyruvate kinase M2 (PKM2) expression in non-small cell lung cancer

A. Piotrowska[1], C. Kobierzycki[1], B. Werynska[2], M. Majchrzak[2], B. Pula[1], P. Dziegiel[1] and D. Rakus[3]
[1]Department of Histology and Embryology, Wroclaw Medical University, Wroclaw, Poland, [2]Lower Silesia Centre of Pulmonary Diseases in Wroclaw, Wroclaw, Poland, [3]Department of Animal Molecular Physiology, Wroclaw University, Wroclaw, Poland

Lung cancer is the most commonly diagnosed cancer in the world and the first cause of death due to neoplastic disease. Despite advances in diagnosis and treatment, prognosis still remeins unfavourable.

Pyruvate kinase isozyme type M2 (PKM2), the enzyme catalyzing the rate-limiting final step of glycolysis, is consistently altered during tumorigenesis and is highly upregulated in tumors. Moreover, PKM2 promotes the Warburg effect. The aim of our study was to investigate the expression of PKM2 in non-small cell lung cancer (NSCLC) and in cancer associated fibroblasts (CAFs), which are known to support tumor growth and may play a key role in the acquisition of drug resistance in tumor cells.

The studies were conducted on 154 archival paraffin blocks of NSCLC from patients treated in the Lower Silesia Centre of Pulmonary Diseases in Wroclaw. Immunohistochemical reactions with PKM2 antibody were performed on paraffin sections using DAKO AutostainerLink 48. Expression of PKM2 was evaluated using the semi-quantitative immunoreactive (IRS) scale of Remmele and Stegner.

PKM2 was expressed significantly higher in NSCLC as compared to tissues of adjacent non-malignant lung tissues (NMLT). In NSCLC, PKM2 was expessed in cancer cells, as well as CAFs and tumor associated macrophages (TAMs). PKM2 expression in