GENETIC VARIABILITY OF B AMYLASE IN KERNELS OF SOME Triticum aestivum L. CULTIVARS XIV-054

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Separation by disk-electrophoresis in Tris-B-alanine buffer pH 8,2 of total B-amylase of 85 wheat cultivars has shown the presence of 12 zymogram types comprising 2-4 fractions each; according to their mobility a total of five fractions can be distinguished. The electrophoresis was performed in polyacrylamide gel obtained with: 5 g cyanogum 41, 0.32 ml TEMED, 50 mg amonium persulphate, all brought to 100 ml buffer. The glass tubes were 7,5 cm long and 0.6-0.7 cm in diameter. The electrophoresis was performed at 250 V, the current ranging between 2.5-3.5 mA/tube at room temperature, for 75-90 min, while the dye Amido black 10B reached the bottom of the tube.

Experimental production of therapeutic plasma proteins by chromatography XIV-055

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The demand for plasma proteins, e.g. coagulation proteins, IgG-preparations albumin and others is continuously increasing. A few chromatographic pilots have been constructed and installed in the recent years to produce proteins.

An integrated system producing Factor VIII and IX concentrates, specific and intravenous IgG preparations and albumin has been installed by the National Institut of Haematology and Blood Transfusion in Budapest. The equipment and the basic method, was developed by Curling and Berglof for albumin and IgG chromatography. The Factor VIII and IX production is carried out according to Wickerhauser and Brummelhuis.
The semi-automatic chromatographic system prepares 50 l of human plasma in one batch. Each batch can provide the products mentioned above. The system is very flexible, thus new steps to win other proteins, e.g. Antithrombin III by affinity chromatography can easily be added. Experiences of over one year's operation are discussed. Cost analysis based on productivity, consumption of energy, chemicals and manpowr are also given by the authors.

POLYMORPHISM OF RED CELL GLYOXALASE I IN SERBIA, YUGOSLAVIA XIV-056

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Electrophoretic analysis of human hemolysates showed that glyoxalase I (GLO I, S-D-lactoylgluthatione methylglyoxal lyase (isomerising), EC 4.4.1.5) is a polymorphic enzyme. The aim of this study was to obtain data on incidence of GLO phenotypes and allelic frequencies in Serbia, Yugoslavia and compare it with the results of some other mediterranian population studies. Red cell glyoxalase I phenotypes were determined in 258 unrelated adults from the population of Serbia. Phenotypes of erythrocyte GLO I were distinguished by the horizontal starch gel electrophoresis. After electrophoresis, the gels were sliced and stained for GLO activity by the method of Kompf et al. (Hum. Genet. 27: 141-143, 1975). In the population of Serbia only common phenotypes were observed with the following frequencies: 0.147 for GLO 1 0.473 for GLO 2-1 and 0.379 for GLO 2. The GLO^1 gene frequency was estimated to be 0.384.

INDIVIDUAL PROTEINS IN DIAGNOSIS AND TREATMENT OF RENAL DISEASES XIV-057

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Proteinuria is basic laboratory parameter in diagnosis of renal diseases. The author uses two methods for the determination of the individual proteins-bidimensional immunoelectrophoresis and own modification of immunodiffusion for identification ofalbumin, transferin, alpha-2macroglobulin and immunoglobulins (G,A,M). The possibilities of the two methods are compared. The selectivity is determined by orthostatic proteinuria, nephrites, pyelonephrites, purpura of Schönlain-Henoch and diabetishe nephropathy in children. There are different kinds of selectivity by all studied diseases. The dynamics of selectivity is a basic parameter for the evolution of diseases and the effects of the treatment by renal diseases in children.

PHYSICAL BASIS OF HAEMOGLOBIN BEHAVIOUR ON AGAR ELECTROPHORESIS. XIV-058

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On agar gel electrophoresis at pH 6.2, foetal haemoglobin (HbF) migrates at the velocity of electroendosmosis. Some other haemoglobins migrate more slowly, apparently because of interaction with an agar component. It has been shown [1] that such haemoglobins differ from HbF at amino acid residues in the β-β cleft, by way of substitutions which yield a higher net positive charge in this region. The binding site includes that for 2,3-diphosphoglycerate but is more extensive.

The responsible agar component is readily extracted from the dry powder in water, and precipitates with cetyl pyridinium chloride. The residual cold-insoluble material yields a gel showing less electroendosmosis than before and no separation of HbA and HbF. Both electroendosmosis and separating capacity are fully restored by reconstituting the original gel composition.

Gel chromatography studies show that the responsible agar component (Factor X) is heterogeneous and very large, also that haemoglobin binding is readily reversible (at least in the presence of citrate). Consequently, apparent K_{AV} varies with gel porosity and also with relative concentration of X. The only barrier to determining the equilibrium constant is that the molar concentration of X is unknown. Factor X preparations contain sulphate but only a minority of the total sulphate content of the agar.

[1] W.P. Winter & J. Yodh, Science 221 (1983) 175-178.

COPPER REMOVAL IN HEMOCYANINS:DIFFERENCES BETWEEN MOLLUSCAN AND CRUSTACEAN HEMOCYANINS. XIV-059

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The kinetics of the copper removal by cyanide has been studied in Carcinus m. (Crustacea) and Octopus v. (Mollusca) hemocyanin (Hc). In both Hcs the two copper ions at the active site are removed sequentially. Carcinus Hc reacts with the ligand only when $[CN^-] \geq 1$ mM. The reaction is rate-controlled by the equilibrium constant of Hc with CN^-. No site-site interactions are evident in absence of Ca (II) and Mg (II) ions. Octopus Hc gives a ligand exchange reaction between O_2 and CN^- at $[CN^-] < 1$ mM. At higher concentrations the copper is lost and the rate limiting reaction is the removal of the metal. The rate constant decreases with the time both in absence and in presence of Ca (II) and Mg (II), indicating strong negative interactions between the active sites. These results indicate that in Carcinus Hc the active sites can behave indipendently from each other. In contrast, in Octopus Hc the removal of one copper from an active site decreases the accessibility of the neighbouring ones vs. CN^-. This fact explains the uncompleted removal of copper even when $[CN^-]$ is > 50 mM.