# TWO-DIMENSIONAL GEL ANALYSIS OF PROTEIN CHANGES IN MATURING ERYTHROID BURSTS.

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Cultures of erythroid bursts have been used primarily for studies of hemoglobin synthesis, particularly in relation to the switch between fetal and adult hemoglobin. In this study we have compared the synthesis and accumulation of hemoglobin with the accumulation of other non-hemoglobin proteins in the erythroid bursts using two-dimensional polyacrylamide gel electrophoresis (2D PAGE). Peripheral blood from normal individuals was used for erythroid cultures. Cultures were established in methylcellulose. BFUE were harvested from cultures on even numbered days starting with the day of culture until day 14 when the bursts are mature. 2D PAGE was performed as previously described (Clin. Chem. 28:925, 1982). Gels were stained with an ultrasensitive silver stain. The proteins observed in the 2D gel pattern appear to be primarily cytosol proteins as none of the major membrane proteins are present nor the proteins previously observed using 2D PAGE. Hb accumulation was observed as early as day 4 of culture and increased throughout the period of culture. Carbonic anhydrease could also be identified and was seen as early as day 6, however, only appeared as a major spot after day 10. A spot observed in lymphocytes (the cells cultured) was observed to increase during the period of culture and was also observed to be a major red cell lysate protein. Ten other proteins changed either qualitatively or quantitatively over the period of culture. Several of these which decreased in culture relative to the starting pattern also do not appear to be red cell lysate proteins. We conclude that protein changes associated with erythroid burst maturation can be detected by 2D PAGE.

Memo:

## POLYPEPTIDE DIFFERENCES BETWEEN LYMPHOCYTES AND LYMPHOBLASTS DETECTED BY TWO-DIMENSIONAL ELECTROPHORESIS.

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Two-dimensional electrophoresis (2-DE), because of its resolving power, allows a systematic search for protein differences between related cell populations. Prior studies of abnormally proliferating cells, using 2-DE, have focused on established cell lines or autoradiographic analysis of polypeptide patterns. In this study we have investigated the use of 2-DE coupled with ultrasensitive silver staining to identify polypeptide differences between lymphocytes and lymphoblasts, obtained directly from peripheral blood of normal and leukemic individuals. Specifically, we have searched for the presence of 325 major polypeptide spots common to T and non-T peripheral blood lymphocytes in 2-DE patterns of lymphoblasts obtained from six children with common acute lymphoblastic leukemia. We have also searched for major lymphoblast spots that were missing from lymphocyte 2-D gels. Ten of the 325 lymphocyte spots studied could not be detected in lymphoblast patterns. In addition, 15 major spots observed in lymphoblast 2-D gels were missing from lymphocyte patterns. One of the 15 spots was observed in platelet and the other in neutrophil 2-D gels. None of ten additional neutrophil or 22 platelet restricted spots were observed in lymphoblast 2-D gels. These observations suggest that 2-DE could provide a useful approach for the detection of changes in cellular proteins associated with various leukemias, and for the identification of key polypeptides for monoclonal antibody production.

Memo:

# GENETIC ANALYSIS OF OVARY SPECIFIC PROTEINS IN DROSOPHILA MELANOGASTER

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Stage specific proteins of Drosophila melanogaster during the process of embryogenesis were investigated by high resolution, two-dimensional polyacrylamide gel electrophoresis. Previous work had shown that named OVA-1A protein is present only in the ovary and not in embryos and adult somatic tissues (head or thorax), that is, this protein is ovary specific.

By screening analysis of many mutant stocks in Drosophila melanogaster, we identified genetic variants of OVA-1A (named OVA-1B and OVA-1C) which alter the mobility in SDS-polyacrylamide gel electrophoresis. The genetic factor(s) responsible for these changes of molecular weight mapped to locus 21 on the X chromosome (between w and m). Possible biological function of the OVA-1A protein during oogenesis will be discussed.

**Memo:**

## ELECTROPHORETIC ANALYSIS OF PANCREATIC PROTEASES AND ZYMOGEN ACTIVATING FACTORS IN MICE

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Mouse pancreatic proteases were analyzed by one and two dimentional electrophoresis. Two kinds of trypsins (Try-I group and Try-II) and a kind of chymotrypsin (Chy-I) were determined by specific and non-specific protease staining. Try-I group and Try-II were derived from different trypsinogens (Try G-I group and Try G-II). Chy-I was derived from a single chymotrypsinogen (Chy G). Try G-II was activated by not only intestinal extract but also bovine trypsin. However Try G-I group was only activated by intestinal extract.

Intestinal activating factors were analyzed by two dimentional electrophoresis. Mouse enterokinase which can activate bovine trypsinogen had a slow mobility. There were at least two kinds of activating factors in addition to the enterokinase in mouse intestine. One factor could not activate bovine trypsinogen, but could activate Try G-I group and Try G-II. The other factor could not activate both bovine trypsinogen and Try G-I group but could activate Try G-II. Though it has not been clear what intestinal activating factors can activate Chy G, there are factors which can convert chymotrypsinogen into chymotrypsin directly. These data suggest that intestinal activating factors play an important role in activating mechanisms of mouse pancreatic zymogens.

Memo:

# IDENTIFICATION OF FETAL POLYPEPTIDES IN AMNIOTIC FLUID USING TWO-DIMENSIONAL ELECTROPHORESIS.

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We have established the two-dimensional pattern for polypeptides present at levels of >0.001 g/L in mid-gestational amniotic fluid (week 15-18) and in matched samples of maternal plasma using a modification of the method of O'Farrell [O'Farrell (1975) J. Biol. Chem., 250, 4007-4021]. The purpose of this study was to describe a population of non-maternal polypeptides in amniotic fluid, to determine how their amount and type changed during gestation, and to investigate their origins by analyzing for their presence in fetal body fluids such as plasma and urine. We have identified 23 proteins that were common constituents of both amniotic fluid and plasma and 6 maternal plasma proteins that were not present at detectable levels in mid-gestational amniotic fluid. We found that amniotic fluid contained 7 soluble polypeptide groups, ranging in molecular mass from 25,000->200,000 daltons, for which no counterparts could be detected in maternal plasma. Two of these "amniotic fluid-specific" polypeptides were identified as fibronectin and alpha fetoprotein and one additional group may be of possible uterine origin. We have compared the polypeptide pattern of mid-gestational amniotic fluid with that obtained at term (week 38-42) and have begun to quantitate the relative amounts of each polypeptide present in the two sample populations using computer assisted densitometric analysis [Vo et al., (1982) Clinical Chemistry, 28, 867-875].

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Memo:

# CHARACTERIZATION OF SERUM PROTEINS INDUCED BY PARTIAL HEPATECTOMY

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The changes of the serum proteins after partial hepatectomy have been examined by means of polyacrylamide gradient gel and two-dimensional electrophoresis, and three proteins (named Spot A, B, and C) which rapidly increased in amount after hepatectomy have been detected (1). In the present study, we investigated the characteristics and the time-dependent changes of those proteins.

The molecular weights of Spot A and B were estimated to be about 800,000 and that of Spot C was about 900,000. The pIs of Spot A and B were 4.9 and 5.1, respectively. Spot C showed a wide pI range (pI 5.5-6.5). Spot A and B were PAS-positive proteins.

Time-dependent changes of those proteins were examined. Male rats (Wistar strain, 150 g) were partially hepatectomized under ether narcosis according to the method of Higgins and Anderson (2), and the quantities of those proteins at 1, 2, 3, 4, 7, and 10 days after the hepatectomy were measured. Those proteins rapidly decreased from the serum at 4-7 days after the hepatectomy. This decrease was parallel with the regeneration of the liver cells. Therefore, we suggest those proteins are closely related to the regeneration of the liver cells.

1. Kadofuku, T., Iijima, T., Sato, T., Tei, I., and Makino, Y., The Physico-Chemical Biology 26, 295 (1982)
2. Higgins, G.M,et al., Arch. Pathol. 12, 186 (1931)

Memo: