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Gene locus for α_1 -antitrypsin (PI) assigned to human chromosome 14

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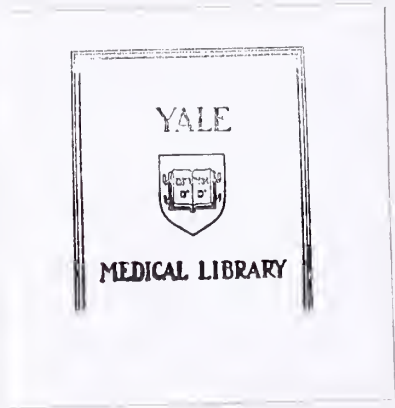



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GENE LOCUS FOR α_1 -ANTITRYPSIN (PI)
ASSIGNED TO HUMAN CHROMOSOME 14

STEPHEN J. PEARSON

1983





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GENE LOCUS FOR
 α_1 -ANTITRYPSIN (PI) ASSIGNED
TO HUMAN CHROMOSOME 14

By

Stephen J. Pearson

A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Medicine

1983

ABSTRACT

In order to study the involvement of human chromosomes in the expression of human liver-specific functions, somatic cell hybrids, were produced between a rat hepatocarcinoma cell line and normal human fetal liver cells. The rat hepatoma line was HGPRT deficient, and hybrids were selected in medium containing HAT and ouabain. The presence of human liver-specific proteins α_1 -antitrypsin, albumin, α -fetoprotein, transferrin and ceruloplasmin was analyzed by immunoelectrophoretic techniques applied to concentrated serum-free hybrid culture supernatants. A subset of hybrids secreted an antigen that was immunologically identical to human α_1 -antitrypsin (AAT or PI). Neither parental line supernatant, fetal calf serum, nor normal rat serum reacted with this antiserum. It is concluded that interaction of the rat hepatoma genome with that of the human fetal liver cells has activated the human PI locus. In 19 primary HAT-selected and 5 azaguanine back-selected hybrids, human PI production segregated concordantly with human chromosome 14. All other human chromosomes were excluded by discordant clones. Assignment of the PI gene to chromosome 14 is consistent with data of others localizing the GM immunoglobulin heavy chain gene cluster to this chromosome since family studies have established linkage between PI and GM.

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INTRODUCTION

1. Somatic Cell Hybridization. The development of techniques for the production and analysis of somatic cell hybrids has provided an important tool for the investigation of cellular functions. During the nineteenth century many investigators had observed multinucleated eucaryotic cells in vivo and fusion was suggested as a possible mechanism. These cells were described in tumors (Virchow, 1858), tissue from tuberculous patients (Rokitansky, 1855) and bone marrow (Robin, 1849). With the introduction of tissue culture techniques, reports appeared describing the spontaneous fusion of animal cells in culture (Lambert, 1912; Lewis, 1927). These and numerous other studies described spontaneous fusion between cells of similar origin.

Barski and colleagues (1960) were the first to report the isolation and description of a somatic cell hybrid between two different cultured mouse lines. The isolation was fortuitous in that the spontaneous hybrid had selective growth advantage and was thus easily separated from the parental cells. Because spontaneous fusion is a relatively rare event and it is extremely difficult to separate isolated fused cells from a sea of parental cells, techniques were needed to facilitate both of these processes.

A report by Warthin (1931) describing numerous multinucleated cells in tonsil tissue from patients with measles suggested the possibility of using virus to induce fusion of cultured cells. The use of measles (Enders, 1954), mumps (Henle et al 1954) and parainfluenza viruses (Marston, 1958) were reported. Of prime importance was the description by Okada (1958,1962) of the use of inactivated hemagglutinating virus of Japan (HVJ) for rapidly and reproducibly fusing suspensions of Ehrlich ascites tumor cells. This has become the standard agent for viral fusion. Harris and Watkins (1965) then showed that Sendai virus, another name for HVJ, induced fusion between

cells of different species and that the fused cells were viable. The mechanism of fusion with inactivated virus is unclear but probably results from nonenzymatic adsorption of virus particles to the cell surface via the viral envelope. This then fuses with an adjacent cell surface and forms an intercellular bridge (Harris, 1970). The cytoplasm then begins to merge. Other investigators have utilized the chemical polyethylene glycol, PEG, to promote fusion (Pontocorvo, 1975). It presumably acts in a similar fashion by causing adjacent cell surfaces to adhere and their membranes to fuse followed by cytoplasmic merging.

A number of studies showed that spontaneously hybridized cells and the same cells fused with virus do not differ in growth potential, chromosomal composition or morphology (Engle et al, 1969 and Littlefield, 1966). The use of these agents was therefore felt to increase the frequency of otherwise rare events and to make possible hybridization of cells that do not fuse spontaneously.

Once cytoplasmic fusion has occurred there is apparently no intracellular mechanism to recognize "self", and the cytoplasmic contents function in harmony. Johnson and Harris (1969), using conventional DNA radioactive labeling techniques, showed that once fused the multinucleated cells, or heterokaryons, synthesize protein and mRNA and that all nuclei contributed to the synthesis.

After formation of multinucleated heterokaryons, nuclear fusion must occur if daughter cells, with the genetic information of each parent, are to be produced. Harris et al (1966), Okada (1962), Yamanaka and Okada (1966) and Rao and Johnson (1970) have used DNA labeling to show that when cells of similar origin are fused a synchronization of the nuclei's cell cycle was rapidly achieved. Synchrony was also observed in heterokaryons of different species (Harris, 1966), although not as frequently. For extended viability

and reproduction of the heterokaryons nuclear fusion must occur. Since the nuclei have become synchronized they enter mitosis simultaneously, and nuclear fusion occurs as a single spindle network aligns the chromosomes from each parent. Cell division then results in two mononucleate daughter cells (synkaryons), each with the genetic complement of the parent cells. In heterokaryons where synchronization has not occurred, where there are more than two nuclei, or where unusual forms of mitosis occur, daughter cells with variable chromosomal and nuclear composition have been noted. These cells have been shown to have decreased viability and reproductive potential (Harris, 1966).

Once the parental cells have been induced to fuse, a selection procedure must be used that separates hybrid cells from the unfused parental populations. A doubly selective system was described by Littlefield (1964, 1966) which utilized enzyme deficient cell lines. By incorporating toxic substances, harmful to one or the other cell line, he was able to select hybrid cells from mixtures of parental and hybrid cells. He first isolated mutant clones of a mouse fibroblast cell line that was deficient for thymidine kinase (TK). A second strain of mouse cells, resistant to 8-azaguanine, a purine analog, was obtained by selection. This strain was shown to lack activity of the enzyme hypoxanthine guanine phosphoribosyl-transferase (HGPRT).

With two enzyme deficient cell lines, one in the purine biosynthetic pathway and the other in the pyrimidine pathway, Littlefield could then subject the parental cells to fusion procedures and select hybrid cells by cultivation in hypoxanthine, aminopterin and thymidine containing media. This medium, called HAT, was doubly selective. TK deficient cells could not obtain thymidine monophosphate since de novo synthesis of tetrahydrofolate is inhibited by aminopterin. Because of their enzyme deficiency these cells

cannot utilize thymidine in the medium, and thus pyrimidine production was inhibited and the cells could not reproduce. Aminopterin also inhibits endogenous production of inosine monophosphate. Since HGPRT deficient cells cannot utilize exogenous hypoxanthine for conversion to inosine or guanosine monophosphate, this shuts down nucleic acid biosynthesis in these cells.

Since hybrid cells contain the chromosomal complements of both parents, the enzyme deficiency of one parental line was compensated by the other. Littlefield showed that suspected hybrids selected in this manner were indeed hybrids by chromosomal analysis and by retreatment with 8-azaguanine and BrdU, a pyrimidine analog. Both chemicals caused killing of hybrid cells, indicating that the cells contained both enzymes.

The model described above has been utilized by most investigators for the selection of hybrid cells after fusion. However, two distinct cell lines adapted to culture and treated to select mutants are necessary. The use of normal human cells in fusion experiments is facilitated by the relative resistance of rodent and rodent hybrid cells to ouabain (Baker et al, 1974). This glycoside compound inhibits the plasma membrane Na^+/K^+ ATPase and is cytotoxic to human cells at certain concentrations. Therefore, hybridization experiments utilizing rodent and human parental cell lines generally employ ouabain to select against unfused human cells and HAT medium against an HGPRT or TK deficient rodent parental line.

Once fused and selected, hybrid cell lines are propagated with standard nonselective tissue culture techniques. In culture, hybrids between cells of the same species are generally stable in terms of their chromosomal composition with only slow, unpredictable loss of chromosomes over extended periods in culture (Barski et al, 1960; Engel et al, 1969; Littlefield, 1966). Morphological changes of chromosomes in hybrids of the same species were noted (Engel et al, 1969).

The situation in hybrids of two distinct species is different in that there is a preferential loss of one parent's chromosomes. This phenomenon was first described by Weiss and Green (1967) who studied the fusion products of human diploid fibroblasts and TK deficient mouse fibroblasts. The chromosomal complement of the hybrids was not the expected sum of the parental lines. They noted that while the mouse genome appeared to be intact, a variable number of human chromosomes were found in each cell. Since the mouse line was TK deficient, the activity of this enzyme in any hybrid was assumed to be of human origin. By treating different hybrid lines with BrdU they selected against those with TK activity and found that surviving cells also lacked a human chromosome identified as E-17 or 18. They therefore postulated that the gene for thymidine kinase was carried on that chromosome. This assignment was later confirmed by Migeon and Miller (1968).

Using similar techniques with human leukocytes and HPRT deficient mouse L cells, Nabholz et al (1969) confirmed the X-linkage of 8-azaguanine resistance in man. They proposed that the loss of human chromosomes occurred early, during initial hybrid cell divisions, and lead to a variety of segregant clones. These clones remained stable enough for phenotypic and karyotypic analysis. Thus, somatic cell hybridization was established as an accepted tool for genetic analysis.

The mechanism for preferential loss of human chromosomes from human/rodent hybrids is obscure. Early studies with interspecific hybrids suggested that the loss was random (Weiss and Green, 1967; Matsuya and Green, 1969; Nabholz et al, 1969). More recent work has shown that it is apparently a non-random process (Croce et al, 1973; Norum and Migeon, 1974; Rushton, 1976; Ruddle and Creagan, 1975; Reardon, 1981). The basis for non-random segregation has not been explained but may relate to culture

conditions and their selective pressures. For example, in human/rodent hybrids selected with HAT medium all hybrids contain the human X, since it carries the gene for HGPRT, and without which the cell would not divide. In a similar fashion, particular combinations of human chromosomes may confer a selective advantage under certain culture conditions and would therefore be found more frequently than expected by random. Other studies have shown that hybrids formed with differing human parental lines retain different non-random complements of human chromosomes (Allderdice, 1973). These studies point out the need for careful chromosomal analysis and caution against defining gene location or linkage on the basis of concordant isoenzyme markers exclusively.

A variety of parental cells have been used to produce inter- and intraspecific hybrids. In fusion of two differentiated diploid lines the resultant hybrids are slow-growing. If at least one parent is a permanent or transformed cell line, the hybrids are generally rapidly growing and retain the immortality of the transformed parent.

In somatic cell hybrids derived from cultured cell lines that express differentiated functions, those functions may be continually expressed or extinguished. Further, differentiated traits not present in the parental lines may become activated. Many studies have utilized cells from different tissues in order to study the genetic interactions of cells from histologically distinct sources.

Early reports by Silagi (1967) and Davidson and colleagues (1966,1968) demonstrated that certain parental functions were not expressed in hybrids. These studies looked at the production of melanin in hybrids between a Syrian hamster melanoma and unpigmented mouse fibroblasts. Over one hundred hybrid lines were analyzed and all were amelanotic. It was hypothesized that the fibroblasts contributed a regulatory substance that blocked

expression of genes for melanin production. This process has been called extinction and is the basis for a large body of work that has been reviewed by Davidson (1974) and Davis and Adelberg (1973). The latter paper contains a discussion of possible mechanisms of extinction and cautionary statements for the interpretation of regulatory data from somatic cell hybridization experiments.

Activation of human phenotypes not expressed by the human parental line has also been reported. Darlington et al (1974) produced hybrids between diploid human leukocytes, which did not secrete human albumin, and mouse hepatoma cells that secreted mouse albumin. These hybrids produced both human and mouse albumin, as determined by immunodiffusion. Whether the human phenotypic activation resulted from the loss of a repressing substance or interaction of an activating substance from the mouse parental line could not be determined.

Later, Rankin and Darlington (1979) described hybrids of mouse hepatoma and human amniocytes. The human cells in culture produced no detectable extracellular proteins. However, with hybridization human albumin, transferrin, ceruloplasmin, and α -1-antitrypsin were produced.

Activation of mouse liver enzymes in rat hepatoma/mouse lymphoid hybrids has also been reported (Brown and Weiss, 1975; Malawista and Weiss, 1974). This appears to be a generalized phenomenon of interspecific hybridizations with the expression of differentiated functions dependent, in part, on the developmental status of the parental lines.

The study of regulatory mechanisms has been facilitated by the use of differentiated cells, especially hepatocytes or hepatoma cell lines. Hepatoma cell lines have been adapted to tissue culture and demonstrated to express differentiated liver specific functions in culture (Kaighn and Prince, 1971; Darlington et al, 1974; Szpirer and Szpirer, 1975; Szpirer and

Szpirer, 1979; Darlington et al, 1980; Darlington et al, 1982). These products have included fibrinogen, transferrin, albumin, prealbumin, α_1 -antitrypsin, α_2 -haptoglobin, α_2 -macroglobin, alpha-fetoprotein and the third component of complement.

A clue to the mechanism of extinction and therefore possibly activation was recently reported by Papaconstantinou et al (1982). They reported a series of hybrids from a mouse hepatoma/rat fibroblast fusion and found extinction of the mouse albumin production, even though all mouse chromosomes were present in the hybrids. This was confirmed by the expression of β -glucuronidase, which is linked to mouse albumin. They then showed, with Southern blotting techniques, that the rat and mouse albumin DNA sequences were present and that the hybrids contained little albumin mRNA, as determined by cDNA-RNA reassociation kinetics. They concluded that the mechanism of extinction in these hybrids was specific for albumin and that the block was at the level of transcription.

The shut off of albumin expression in these experiments could have been accomplished in at least two ways. Diffusible substances have been suggested as a regulatory mechanism (Britten and Davidson, 1969; Tomkins et al, 1969; Malawista and Weiss, 1974; Brown and Weiss, 1975; Kahn et al, 1981). Alternatively, DNA conformational modification could also influence gene activity (Weintraube and Groudine, 1976). The exact mechanism remains unknown although experiments as described above continue to elucidate possibilities for extinction in hybrids.

Other possible factors influencing expression in hybrids are gene dosage relationships of the parental lines' genomes (Brown and Weiss, 1975; Malawista and Weiss, 1974). When a cell line which produces a differentiated function is fused with one that does not, the activation or extinction of those functions may depend on the ratio of parental chromosomes retained

in the hybrids. However, another study (Darlington et al, 1982b) did not demonstrate a gene dosage effect. Rather, it was found that the histogenetic state of the human cells influenced the frequency of hepatic gene expression.

In the studies to be presented, hybrids between a rat hepatoma cell line and a cell line from human fetal liver were produced and production of human extracellular proteins investigated with the goal of determining which human chromosomes are involved in the expression of liver specific protein synthetic functions. With the hybridization of two cell lines with a similar histologic background, we hoped to increase the likelihood of obtaining hybrid cells capable of producing human liver specific proteins. For example, alpha-1-antitrypsin (AAT) has been detected in cultures of human fetal hepatocytes (Eriksson, 1978). The identification and characterization of human AAT produced by hybrid cell lines forms the basis of this study.

2. α_1 -Antitrypsin. AAT, the major protease inhibitor secreted by the liver, is one of the most polymorphic gene products known. Over twenty-six allelic variants have been identified (Morse, 1978). Variants observed fit a model of multiple autosomal codominant alleles at one locus (Fagerhol and Cox, 1981). The glycoprotein product is a monomer with a molecular weight of about 54,000 and has four carbohydrate side chains containing N-acetyl-glucosamine, mannose, galactose and sialic acid. About forty percent of the enzyme is found in plasma and sixty percent in the extravascular space (Fagerhol and Cox, 1981).

Functionally, the protein inhibits a variety of proteolytic enzymes including trypsin, chymotrypsin, collagenase, elastase, cathepsin G, renin, urokinase and Hageman-factor cofactor. Therefore, it may play a role in modulating a variety of enzyme systems. Although AAT accounts for about ninety percent of the serum protease inhibitory capacity, disease states

associated with AAT deficiency suggest that inhibition of granulocyte and macrophage elastase, collagenase and cathepsin may be the major physiological function (Fagerhol and Cox, 1981).

The system for naming allelic variants of AAT has been based on their electrophoretic mobility with type Z being slowest, M intermediate and F fastest. Type M is the most common form of the enzyme. Type S, the second most common form, migrates between Z and M. The molecular difference between types Z and M was found to be a single amino acid substitution of a lysine residue for a glutamic acid (Yoshida et al, 1976). Subtypes of M have also been identified with isoelectric focusing (Cox et al, 1980).

A variety of disease states have been associated with AAT deficiency. Pulmonary disease in patients with AAT deficiency type ZZ was first described by Laurell (1963). The most common pulmonary lesion is adult onset diffuse pan-ascinar emphysema with disruption of the bronchial elastica. The relative risk for development of chronic obstructive pulmonary disease in heterozygotes is uncertain; however, one study reported a three-fold increased risk (Cox et al, 1976). Elevated concentrations of unopposed leukocyte proteases in both MZ and ZZ individuals have been proposed as a mechanism of lung damage.

Childhood cirrhosis and a neonatal hepatitis syndrome have been associated with type ZZ (Moroz et al, 1976b). The livers of deficient patients usually contain large amounts of amorphous material that has been identified as an AAT precursor (Jeppsson et al, 1975; Matsubara et al, 1974). The hepatic accumulation of AAT in deficient patients is probably due to difficulties of post-translational modification which do not permit extracellular release of the protein. However, the pathogenesis of hepatic disease is not fully understood. The products of other genes may influence the phenotypic consequences of certain AAT deficiency states.

Other disease states that may be associated with AAT deficiency include membranoproliferative glomerulonephritis (Moroz et al, 1976), rheumatoid arthritis (Cox and Huber, 1980), and chronic active hepatitis and cryptogenic cirrhosis (Hodges et al, 1981). A role in lymphocyte blastogenesis has also been proposed (Lipsky et al, 1979).

The chromosomal location of the AAT locus was uncertain at the initiation of these studies. Mapping the AAT locus could contribute to the understanding of AAT deficiency states and to defining the molecular basis for deficiency.

MATERIALS AND METHODS

1. Cell lines. Rat hepatoma line 7777-14b-aza was derived from a Morris "minimal deviation" hepatocarcinoma originally generated by chemical carcinogenesis and carried by serial transplants in Fisher rats. Tissue from a 7777 tumor was obtained from Dr. Stewart Sell, Department of Pathology, University of California San Diego. Tumor cells were adapted to in vitro culture conditions and selected for growth attached to plastic dishes. Especially at higher cell densities, the cells continue to have a tendency to form clumps and to grow in suspension. Cells were exposed at low density to medium containing $10^{-5}M$ 8-azaguanine (8-AG) and 8-AG resistant colonies were selected that were subsequently tested for sensitivity to hypoxanthine aminopterin thymidine (HAT) medium. Clone 7777-14b-aza, 8-AG resistant and HAT sensitive, was used for further studies. The karyotype of this line was originally near diploid but acquired a number of rearrangements during continuous culture.

Cell strain HFL 101 (human fetal liver cells) was derived from a normal female fetus aborted at 20 weeks gestation. The liver was obtained under sterile conditions and a single cell suspension was prepared by treatment with collagenase (3 mg/ml) for 10 min. Fusion of the original cell suspension of predominantly hepatocytes to 7777-14b-aza did not yield hybrid colonies. Within two weeks, actively growing cells were obtained from fetal liver tissue in culture. These cells had a fibroblastoid appearance and a normal 46,XX female karyotype.

Standard culture conditions for all experiments included incubation at 37°C in a humidified 5% CO₂ atmosphere (Forma Scientific Model 3172). Cells were grown in minimum essential medium (MEME) with 10% fetal calf serum (FCS) and 1% glutamine. Penicillin and streptomycin were added to prevent bacterial contamination. The media were changed every 2-3 days

during expansion of cell lines. Cultures were split and expanded before reaching confluency.

2. Cell fusion. Equal numbers of rat 7777-14b-aza and human HFL 101 cells were plated as mixed confluent cultures in four 50mm dishes (Falcon Plastics). After 24 hr, the cultures were exposed to 44% polyethylene glycol (6,000 M.W.) in serum free MEME for exactly 1 min. Immediately afterwards, the dishes were rinsed four times with serum free MEME containing 10% DMSO and were incubated in MEME with 10% fetal calf serum (FCS). After 24 hr, the four original fusion plates were subcultured at very low density in selective medium consisting of MEME with 10% fetal calf serum, glutamine, penicillin, streptomycin and $2 \times 10^{-7}M$ ouabain as well as the components of the HAT selection system. Both parental lines were treated identically as control cultures. A ouabain concentration of $10^{-7}M$ had previously been shown to inhibit growth of HFL 101 cells, while a higher concentration of $5 \times 10^{-7}M$ effectively killed the cells. The standard HAT medium killed 7777-14b-aza cells. No resistant colonies appeared in the control plate.

Two weeks after the fusion, presumptive hybrid colonies were picked under an inverted phase microscope using an Eppendorf micropipettor with sterile disposable tips. Individual colonies were transferred into wells of microtiter plates containing MEME, 10% FCS and $2 \times 10^{-7}M$ ouabain. Forty-five colonies were picked from 13 different plates representing at least 13 independent fusion events. An initial set of 19 hybrids derived from 10 original plates were selected on the basis of widely divergent cellular morphology. These hybrid lines were expanded for simultaneous chromosome analysis, collection of culture supernatant and cell harvests for DNA and enzyme studies.

3. Counterselection in guanine analogues. Selection in HAT medium yields clones which have retained the human X chromosome. To obtain subclones without the X, hybrid lines were plated in minimal medium containing 8-AG or a combination of 8-AG and 6-thioguanine (6-TG). Colonies resistant to the guanine analogues were subcloned and tested for sensitivity to HAT medium. Fifteen subclones, guanine resistant and HAT sensitive, from three primary HAT selected hybrid lines were expanded for chromosomal and biochemical studies.

4. Chromosome analysis. For chromosome preparation, an in situ method was used. Cells were plated at low density in a Petri dish containing sterile coverslips. After incubation overnight, the cells were monitored during the next day for mitotic figures. If mitotic activity was sufficient, 50 μ l of a stock colcemid solution (Gibco) was added to each dish containing 4 ml of culture media. After 30 min incubation at 37°C, the culture medium was aspirated carefully from the edges of the plate. Warm (37°C) 0.075 M KCl was added slowly, and the dishes were kept undisturbed at room temperature. After 25 to 30 min, 5 ml of fixative was added gently to the hypotonic solution. After 2 min the fluid was aspirated slowly from the edge of the plate. Standard fixative (3 parts methanol: 1 part glacial acidic acid) was added for 30 min initially and was then changed several times. The coverslips were air dried at room temperature. Standard harvesting procedures in suspension were also employed.

Air dried slides, prepared by the standard method, were GTG-banded using the trypsin-Giemsa protocol as described (Francke and Oliver, 1978). In situ fixed chromosomes on coverslips were more resistant to trypsin treatment. Therefore, the trypsin concentration was doubled and the time in trypsin had to be increased to 60-90 sec. After staining in Wright's Giemsa, coverslips were mounted upside down onto microscope slides using

neutral mounting medium. For cytogenetic analysis, 15 to 20 well banded metaphase spreads were located and photographed with a Zeiss photoscope on Kodak SO-115 Technical Film. Photographic prints of metaphase spreads were analyzed for the presence of human chromosomes and karyotypes were prepared for each hybrid. Chromosome studies were repeated at several passages during expansion of the hybrids.

5. Concentration of culture supernatant. Confluent plates of hybrids and parental cell lines were incubated for 24-48 hr in MEME without FCS. Between 80 and 120 ml of serum free medium obtained from each line were centrifuged in a Beckman centrifuge for 30 min and the supernatants stored frozen at -20°C . After thawing the medium, samples were concentrated approximately 10 fold with a Diaflo Ultrafiltration Cell (Model 52, Amicon). UM10 filters were used which exclude molecules greater than 10,000 M.W. Concentration was carried out under nitrogen pressure to reduce the chance of protein degradation. Further concentration of small volume samples was carried out with a B-15 Minicon macrosolute concentrator (Amicon). This apparatus absorbs through a membrane that excludes molecules greater than 15,000 M.W. Combination of the two procedures achieved an approximate 100-fold concentration of the serum free culture media with minimal loss of the proteins of interest. After extraction of the serum-free media and at different stages of clonal expansion, viable cell pellets were frozen and stored at -70°C (Forma Bio-Freeze).

6. Immunoelectrophoresis. The electrophoresis of antigens into an antibody containing gel is a rapid method for the detection and identification of a particular antigen. The sensitivity and specificity of these procedures is dependent, in part, on the antibodies. Commercially available immunoglobulin G fractions of monospecific goat antisera against human α_1 -antitrypsin (AAT), α -fetoprotein (AFP) and albumin (Atlantic

Antibodies) and rabbit antiserum against human transferrin and ceruloplasmin (DAKO) were used. As a positive control, a 3-fold concentrated normal human serum which had been calibrated for many serum proteins was included in all experiments (Atlantic Antibodies). Amniotic fluid calibrated for AFP was obtained from Dr. A. Baumgarten, Dept. of Laboratory Medicine, Yale-New Haven Hospital. Optimal antibody concentrations were determined experimentally for each antigen-antibody combination.

The electrophoresis buffer consisted of 0.01 M barbital (5,5'-diethyl-barbituric acid) and 0.05 M sodium barbital (Sigma) (pH 8.6). At this pH, the migration of immunoglobulins is apparently balanced by a cathodic movement due to electroendosmosis, which also causes slight movement of neutral molecules towards the cathode.

For preparation of 1% agarose (medium electroendosmotic- M 0.18, Sigma type II) gels, the electrophoresis buffer was diluted 1:1 with distilled H₂O. This dilution creates a concentration gradient between the gel and the running buffer which enhances protein migration. The agarose solution was boiled for 5 min on a hot plate and the molten agarose then cooled to 55°C. An appropriate volume of this solution was mixed with a measured amount of antiserum and was then quickly poured onto an agarose pre-coated glass plate resting on a level surface. One ml of molten agarose per square inch of glass plate produced a gel slab between 1 and 2 mm in thickness. Antigen wells were made with a 2 mm vacuum gel punch and spacing template (BioRad). Depending on the experiment, between 5 and 10 μ l of antigen solution was placed into each well. Immuno-electrophoresis was carried out in an electrophoresis unit (Accurate) cooled to 4°C with a methanol-water circulator (Polyscience), using low resistance cellulose wicks (Ultra Wicks, BioRad) for 12-16 hr at a constant voltage of 20V (ISCO Model 494 power supply).

After completion of electrophoresis, the agarose plates were prepared for staining by first covering them with water-saturated filter paper (Whatman) and then compressing the plates under paper towels and a weight. This procedure absorbs moisture as well as unprecipitated proteins from the gel. Because Coomassie Blue stains all proteins and nonspecific staining may hide a faint precipitate, we further reduced the amount of unreacted proteins in the gels by rehydrating the pressed agarose plates overnight in cold phosphate buffered saline. The plates were then pressed again and air dried with a hair dryer prior to staining. The completely dry plates were immersed in staining solution (45% ethanol, 45% distilled water, 9% glacial acetic acid and 1% Coomassie Brilliant Blue, BioRad) for 15 min and then destained in a 45% ethanol, 45% distilled water and 10% glacial acetic acid solution. The dried plates can be stored indefinitely with only slight loss of color intensity.

A number of immunologic techniques were employed to detect and characterize extracellular proteins present in the culture supernatant from the different cell lines.

Rocket immunoelectrophoresis was carried out following procedures modified from Weeke (1973) and Laurell (1966). The immunoassay consists of one dimensional electrophoresis of antigen into antibody containing agarose gel. This system was used to detect small amounts of a particular protein antigen and to quantitate the production of AAT by certain hybrid cell lines. Two sizes of glass plates were used for this assay, 4" x 4" and 1" x 3" slides. The antigen wells were positioned to allow good wick contact with the gel and maximum distance for protein migration.

Crossed immunoelectrophoresis was carried out following the procedures of Weeke (1973). This procedure demonstrates the homogeneity of antigens in terms of their electrophoretic mobility and antigen specificity. This assay

differs from rocket immunoelectrophoresis in that 4 ml of molten agarose without antibodies were poured onto a 2" x 2" glass slide. Samples were then subjected to electrophoresis at high voltage (100 V) for 4 hr. Half of the gel was cut away and 2 ml of antibody containing gel was poured in its place. A second electrophoresis at 20 V for 12 hr was then done by changing the direction of current flow by 90° in such a way that the proteins migrated into the antibody gel.

Tandem Crossed Immunoelectrophoresis is a variation of the two dimensional electrophoresis system described above. This technique allows direct comparison of an unknown antigen's immunoreactivity with that of a known standard. Preparation and electrophoretic conditions of these plates were similar to the crossed immunoelectrophoresis plates except that two antigen wells were made 5 mm apart, the two samples applied simultaneously and then allowed to diffuse completely into the agarose. The wells were then filled with a drop of molten agarose before electrophoresis. Thus, the antigens migrated simultaneously and produced an overlapping pattern in the antibody containing gel.

Intermediate gel immunoelectrophoresis is a technique similar to the rocket system except that only a portion of the gel contains antibodies. The 1" x 3" microscope slides were used exclusively for this triple layer gel. Molten agarose (1.5 ml) without antibodies was poured onto a slide upon which a brass bar (BioRad) was placed 1 1/2" from the end. After solidifying, the bar was removed and the gel cut back to 1". The second layer was poured in a similar fashion using 3/4 ml agarose, with or without antigen, for 3/4" on the slide. This second layer was then cut back to 1/4". The third layer consisted of 1 3/4 ml of molten agarose plus antibody which covered the remaining 1 3/4" of the plate. Antigen wells were punched in the first layer and electrophoresis was carried out under conditions

similar to those for rocket electrophoresis. A variation of this technique was used to identify small amounts of antigen by leaving an area of antibody-free agarose between the sample wells and agarose with antibody.

7. Immunodiffusion. This technique was carried out as described by Ouchterlony (1953). Molten agarose (4 ml) was poured onto a 2" x 2" glass slide. A number of 2 mm wells were punched and 8 μ l of antigen or antibody solution was delivered to each well. Each plate had one antibody well surrounded by six wells of antigen. The plates were allowed to diffuse for 24 hr at 4°C, and were then pressed, rehydrated and stained as described above. While diffusing, the plates were kept in Petri dishes humidified by moistened filter paper.

8. Cell pellet lysis. Hybrid cell pellets were lysed in Meera Khan's lysis buffer (1971): $5 \times 10^{-3}M$ PO_4 buffer pH 6.4, $1 \times 10^{-3}M$ Na_2EDTA , $1 \times 10^{-3}M$ β mercaptoethanol, and $2 \times 10^{-5}M$ NADP. Viable frozen cell pellets were thawed and spun for 1 min. in an Eppendorf Microfuge. The pellet was resuspended in phosphate buffer, centrifuged again, and the supernatant removed. Cells were then resuspended in 50-100 μ l of lysis buffer and sonicated three times for ten seconds each. Lysates were spun for four minutes in the microfuge and stored frozen at -20°C until use in the rocket immunoelectrophoresis assay and cellulose acetate electrophoresis.

9. Cellulose acetate gel. Electrophoresis of cell lysates on cellulose acetate gel followed by enzyme specific stains allows the identification of enzymes whose chromosomal location is known. This approach is particularly useful as an alternative to karyotype analysis in hybrid cells when certain chromosomes may be difficult to distinguish. Cell lysates were prepared as described above. Standard electrophoresis conditions, buffers, stains and mapping data (Harris and Hopkinson, 1976 and Meera Khan, 1971) were employed to screen for nucleoside phosphorylase

(NP), mapped to chromosome 14; glucose phosphate isomerase (GPI), mapped to chromosome 19, and adenosine deaminase (ADA), mapped to chromosome 20. The latter two enzymes served as markers for their chromosomes since these small human chromosomes were not readily distinguished from those of the rat.

RESULTS

1. α_1 -antitrypsin (AAT)

The first panel of hybrid cell lines (Table I), was initially screened by rocket immunoelectrophoresis for antigen reactive to anti-human AAT serum, after concentrating the culture medium ten-fold. Of the nine hybrid lines only five appeared to excrete a substance reactive with the antibodies. Figure I shows faint rocket shaped precipitates for cell lines 1B, 5C, 8A, 11B and 15C. No reactions are seen with either parental cell line (7777 and 101). In order to detect cell lines possibly producing smaller amounts of antigen and to improve resolution, the media were again concentrated ten-fold to a final concentration of 100 x the original. Repeat screening of these more concentrated media revealed positive reactions for the same hybrid cell lines (Figure II). The parental lines remained negative (lanes 14 and 15). As positive controls, a dilution series of calibrated human serum was run simultaneously (lanes 1 through 4). In addition, normal rat serum (NRS) and fetal calf serum (FCS) (lanes 16 and 17) were included and no specific precipitates were seen.

A second panel, Table I, was then screened by rocket immunoelectrophoresis using hundredfold concentrated media. Figure III shows two strongly positive cell lines (9A and 15B), and the dilution series of human standard (lanes 1-4).

The lower limit of sensitivity of this assay was determined in two ways. The human standard was serially diluted and run in the electrophoresis system. Figure IV shows visible precipitate down to a concentration of 1.4 $\mu\text{g/ml}$. By decreasing the antibody concentration an increase in sensitivity was obtained to 0.7 $\mu\text{g/ml}$ (Figure V). Alternatively, increased sensitivity was obtained by leaving a portion of agarose free of antibody between the antigen wells and the agarose containing antibody. With

electrophoresis, antibody molecules travel slowly with the electroendosmotic flow towards the wells, thereby creating a concentration gradient. This facilitates visualization of small precipitates. Figure VI illustrates this approach with a dilution series of human standard. A similar lower limit of sensitivity ($0.7 \mu\text{g/ml}$) was obtained.

Detection of low levels of antigen produced by certain hybrid lines was accomplished as described above. Figure VII shows the detection of a positive reaction for hybrid ID of the first panel. The two left sections also demonstrate that other cell lines negative by the rocket technique remained negative at the sensitivity limits of these assays. In the right section, antibody concentration was decreased ten-fold and a small precipitate can be seen for ID. The second panel was tested for low producers by decreasing antibody concentration, Figure VIII. Cell lines, 1A, 5A, 14A 18A and 11E showed small precipitates. Lanes 15 and 16 contained media from an earlier fusion experiment of 7777 hepatoma cells to human skin fibroblasts.

The hypothetical possibility that a cell line was producing extremely large amounts of antigen and thus escaping detection was excluded by increasing the antibody concentration ten-fold. This maneuver revealed no high producing clones (data not shown). The compilation of data from rocket immunoelectrophoretic screening with anti-human AAT antibodies is seen in Table II.

Although a subset of hybrid cell lines were clearly producing an antigen reactive with anti-human AAT antibodies, the nature of this antigen needed to be clarified. There are three possibilities that could explain the positive reactions seen. First, the antigen was in fact human AAT. Second, the antigen was rat protein extensively cross-reactive with anti-human antibodies or third, there was a mixture of reactive human and rat protein. To investigate these possibilities other immunoelectrophoretic techniques were used.

Crossed immunoelectrophoresis of culture media from producer hybrids demonstrated that the reactive antigen was not a heterogeneous mixture. Figure IX shows an experiment with three producing hybrids from the first panel after ten-fold media concentration and the human standard. Figure X demonstrates that the characteristics of the precipitate were not altered by concentration up to 100-fold. The symmetrical peaks, without shoulders, indicate an electrophoretically homogeneous antigen. Further, the antigens migrated essentially the same distance from the origin as the human standard suggesting molecules of similar weight and/or charge.

The immunologic identity of the antigen was investigated by use of immunodiffusion (Ouchterlony technique) and tandem crossed immunoelectrophoresis. Both methods demonstrate the immunologic relationship between two antigens by the juxtaposition of their precipitates. The interface of these precipitates can then be analyzed. Three basic patterns have been described. A reaction of identity produces a continuous arc between the precipitates. In the case of partial identity a spur extends beyond an otherwise continuous line. A reaction of nonidentity produces precipitates that cross in both directions.

Immunodiffusion of producer hybrid lines from the first panel is shown in Figure XI. Wells containing human standard were interspersed among wells with culture media, FCS and NRS in plates 1-3. In all cases lines of identity were produced between the human standard and culture medium precipitates. No precipitates were seen with FCS or NRS. Plate 4 of Figure XI demonstrates that precipitates from culture media not only produced lines of identity with the human standard but with each other as well. Immunodiffusion of nonproducing lines, as determined by rocket assay, showed no identifiable precipitates (Figure XII). A precipitate from the low producing hybrid line, ID₁, could not be seen. This demonstrates the increased sensitivity of the electroimmunoassay.

Tandem crossed immunoelectrophoresis allowed simultaneous evaluation of the electrophoretic mobility and immunologic identity of two antigens. In the first dimension, culture media from producing hybrid lines and the human serum standard were applied to separate wells next to each other and were co-migrated into antibody-free agarose. The antigens were then co-migrated into agarose with antibody in the second dimension. Figure XIII shows the results with ten-fold concentrated media of selected hybrid lines from the first panel. All hybrids produced lines of identity with the human standard. Similar lines of identity were produced after 100-fold media concentration (Figure XIV). No lines of nonidentity or partial identity were seen.

The possibility that an extensively crossreactive rat serum protein, activated by hybridization, confounded the results was excluded by an intermediate gel absorption technique. This method demonstrates the presence of crossreactive proteins and can approximate the degree of crossreactivity. A three layered gel was poured with antibody free agarose, containing the antigen wells in the first layer. The second layer contained agarose with either NRS, normal saline or human standard, and the third layer agarose with antibody. On electrophoresis the potentially crossreactive antigens in the middle layer migrate as a front into the antibody layer and absorb any crossreactive antibodies. This lowers the antibody concentration encountered by antigens simultaneously migrating from the wells in the first layer. Since the height of the rocket is inversely related to antibody concentrations, higher peaks will be produced, when the middle layer contains a crossreactive antigen, as compared to a normal saline control.

Precipitates produced by culture media of hybrid lines with NRS in the middle layer (Figure XV plates A and C) were compared to plates B and D where the intermediate gel contained normal saline. The similar heights of

the peaks indicate that NRS did not reduce the antibody concentration and, therefore, did not contain crossreactive antigens. Figure XVI confirms the low-level production of a human protein by hybrid line ID. Plate 1 contained NRS, plate 2 normal saline and plate 3 human serum in the middle layers. No rockets are seen in plate 3 since all the antibodies reactive with human AAT were absorbed. A line of antigen/antibody equivalence can be seen.

From the extensive studies of the first panel it was concluded that the protein produced by our hybrid cell lines was homogeneous and immunologically indistinguishable from human AAT and that the detection systems used were specific for human AAT. For this reason, the second panel was not tested as exhaustively as the first.

Rocket immunoelectrophoresis of hybrid cell pellet lysates, run with antihuman AAT and antihuman albumin antibody, were difficult to interpret due to large amounts of nonspecific staining. No cell line, negative by immunoelectrophoresis of culture media, was clearly positive for AAT or albumin by this technique (data not shown). These results argue against the possibility that large quantities of normally extracellular protein were being sequestered intracellularly because of defects in transport through the cell membrane.

2. Albumin

The production of other liver specific extracellular proteins was screened using rocket immunoelectrophoresis. Using anti-human albumin antibody, the first hybrid panel figure (XVII) showed only one strongly positive reaction; in hybrid 15C. A low producing cell line, 8A was identified using the modified rocket technique with an antibody free agarose layer (Figure XVIII). The second panel (Figure XIX) contained four strongly positive hybrid lines; 9A, 14A, 18A and 11E. The tall faint peaks, seen especially

in 15B, are typical of weakly crossreactive proteins. The identity of those proteins was not investigated. However, tandem crossed immunoelectrophoresis of the second panel's positive cell lines (Figure XX) revealed lines of identity with the human serum standard.

3. Transferrin

Screening of the first panel for human transferrin identified only one hybrid line possibly producing the protein, 8A (Figure XXI plate 2). The lower limit of sensitivity was defined in plate 1 as being $> 1.5 \mu\text{g/ml}$. The second panel was entirely negative for transferrin (Figure XXII).

4. Alpha-fetoprotein (AFP)

Alpha-fetoprotein could not be detected in any culture medium from the first panel (Figure XXIII) or second panel (Figure XIV) at levels $\geq 5 \mu\text{g/ml}$.

5. Ceruloplasmin

Human ceruloplasmin could not be detected in any culture media from the first hybrid panel (Figure XXV) or second panel (Figure XXVI) at levels $\geq 0.5 \mu\text{g/ml}$. A summary of the immunoelectrophoresis data for all hybrid cell lines and extracellular proteins studied is shown in Table II.

6. Chromosomal analysis

Karyotyping of the hybrid cell lines was carried out concurrently with the immunoelectrophoresis studies in order to determine which human chromosomes were necessary for human liver specific protein production. Figure XXVII shows a karyotype of the rat hepatoma parental line. The majority of chromosomes are acrocentric and are readily distinguished from human chromosomes. The human fetal liver parental line had a normal 46,XX karyotype. The combination of chromosomal morphology and banding pattern allowed identification of the human chromosomes in the hybrid karyotypes.

A representative hybrid karyotype of cell line ID (Figure XXVIII) shows

a double/triple set of rat chromosomes and intact human chromosomes 3, 6, 7, 9, and X. Changes in ploidy and rearrangements of rat chromosomes were frequently seen in the hybrid cell lines.

Figure XXIX shows the karyotype of hybrid line 15B. Human chromosomes 1, 5, 8, 14, 21, X and rearrangements of 7 were identified.

For each hybrid cell line 15 to 20 informative karyotypes were scored for the presence of human chromosomes and the average frequency of each human chromosome was calculated. The pattern of presence or absence of each human chromosome was compared with the pattern of AAT production and the discordancy ratio tabulated (Table III). Discordancy was defined as chromosome present-protein absent or chromosome absent-protein present.

Cellulose acetate investigation of nucleoside phosphorylase (NP) for confirmation of the presence of chromosome 14 in producer hybrids was unsuccessful for technical reasons. Identification of human NP in hybrid cells depended on good electrophoretic separation of the parental lines' isoenzymes. This was not accomplished since the rat and human enzymes migrated similar distances. Therefore intermediate bands of recombined subunits of this trimeric enzyme could not be evaluated. Extensive experimentation with different buffers and electrophoresis parameters did not lead to improved resolution. Cellulose acetate studies of GPI (on human chromosome 19) and ADA (on human chromosome 20) determined the presence of these chromosomes in the hybrids.

7. Counterselection in guanine analogues

Because of HAT selection, all hybrid cell lines had retained the human X chromosome. To test for the possibility that the X chromosome carries a gene also necessary for AAT expression, hybrids were counterselected in guanine analogue containing medium. This medium selects against the presence of the human X. Figure XXX shows the rocket immunoelectrophoresis

of counterselected hybrid lines run simultaneously with the HAT selected lines. Clone 8A HAT and four subclones counterselected in 8-azaguanine produced human AAT. HAT selected lines 18A and 8B and their subclones did not.

DISCUSSION

These studies have identified an extracellular product of human fetal liver cell/rat hepatoma somatic cell hybrids that was immunochemically indistinguishable from human AAT. It was also shown by intermediate gel absorption experiments that no protein found in normal rat serum cross-reacted with anti-AAT serum. Therefore, the protein detected was considered to be of human origin. No cell line was shown to be sequestering significant quantities of AAT intracellularly. Therefore, the production of AAT was reflected by its presence in the culture medium.

Functional studies (i.e. measurements of antitryptic activity) were not performed with the culture media. The rat was assumed to possess an antitrypsin-like enzyme, and recently Urban (1982) characterized a rat liver specific alpha globulin similar to human AAT. A functional assay would not be able to distinguish human or rat enzymatic activity.

Since AAT could not be detected in the supernatant of either parental cell line, it must be concluded that activation of the human locus occurred in the hybrid cell lines. The mechanism of activation was not studied. Activation may have been facilitated by the histological origin or gestational age of the human parental cells.

These results demonstrate concordant segregation of the human AAT positive phenotype and human chromosome 14. All other chromosomes are excluded by discordant cell lines. Only two possibly discordant hybrid lines were found. Hybrid lines 1D and 14A appeared to produce low levels of human AAT but no human 14 was identified by karyotyping. The three most probable explanations are: 1) that chromosome 14 was present at very low frequency and thus escaped detection in the 15 to 20 karyotypes analyzed; 2) that rearranged fragments of 14, present at low frequency, were not identified;

or 3) that pieces of 14 were present in the unidentifiable genetic material, called markers, consistently found in hybrid cells.

The very low frequency hypothesis is supported by the case of hybrid line 18A. This line initially appeared to produce AAT in quantities similar to 1D and 14A. However, with expansion of this line, before counterselection in guanine analogues, expression was lost. In addition, the approximate correlation of gene dosage and amount of AAT produced supports the low frequency explanation.

Subclones of 8A, 8B and 18A counterselected in guanine analogues demonstrated that elimination of the human X chromosome did not stimulate expression. Therefore, the human X was not necessary for maintaining expression, nor was its presence inhibiting production. It may be concluded that chromosome 14 is both necessary and sufficient for expression of human AAT and therefore carries the structural gene.

The localization of the AAT locus to chromosome 14 is consistent with the results of other studies. Gedde-Dahl (1972) established the close linkage of the gene for human AAT, the PI locus, with GM, locus for the immunoglobulin heavy chain constant region. The most recent lod score being above 20 (Gedde-Dahl, 1981). A number of conflicting studies had mapped the GM locus to 6 (Smith and Hirschhorn, 1978), 8 (Bennick et al, 1978), 12 (Noades and Cook, 1976), and 14 (Croce et al, 1979; Hobart et al, 1981). Previous somatic cell hybridization studies had assigned the PI locus to chromosome 9 (Turner and Turner, 1980) or chromosome 14 (Darlington et al, 1982a). More recently, cytogenetic studies of two families with chromosome 14 abnormalities have now mapped GM to 14 q at band q 32.2 and the PI locus to a position between bands q 24.3 and q 32.1 (Cox et al, 1982). The present study, conducted simultaneously with those of Cox et al (1982) and

Darling et al (1982a), confirms the assignment of PI to human chromosome 14 by cell hybridization methodologies.

In addition, these studies have used genetic analysis to characterize a series of somatic cell hybrids in which the human loci for various extracellular liver specific proteins have been activated. Further investigations with these hybrids, using cloned probes of the respective genes, may provide insight into the regulation of gene expression, an area of inquiry which remains central to molecular and developmental biology.

Table I: Hybrid Cell Lines and Controls by Panel

Panel 1: 1B, 1D, 2C, 2G, 5C, 8A, 11B, 12C, 15C,
7777-14b-aza, HFL 101

Panel 2: 1A, 5A, 9A, 14A, 18A, 8B, 15B, 11D, 11E, 2F

Table II: Immunoelectrophoresis of Hybrid Cell Lines,
Detection of Human Extracellular Proteins

Hybrid line	AAT	Antiserum		AFP	Ceruloplasmin
		Albumin	Transferrin		
1A	+	-	-	-	-
1B	+	-	-	-	-
1D	+	-	-	-	-
2C	-	-	-	-	-
2F	-	-	-	-	-
2G	-	-	-	-	-
5A	+	-	-	-	-
5C	+	-	-	-	-
8A	+	+	+	-	-
8B	-	-	-	-	-
9A	+	+	-	-	-
11B	+	-	-	-	-
11D	-	-	-	-	-
11E	+	+	-	-	-
12C	-	-	-	-	-
14A	+	+	-	-	-
15B	+	-	-	-	-
15C	+	+	-	-	-
18A	+	+	-	-	-

Table III: Series XXII: Rat Hepatoma 7777-14b-aza x Human Fetal Liver Cell Hybrids and ATT Expression

Hybrid Clone	Human Chromosomes (mean number of copies/cell)																						X	AAT	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22			
XXII-1A	0	0	1.0	0	0	.4	.5	0	.5	0	0	0	0	0	0	0	0	0	0	0	0	.1	.2	.8	+
XXII-1B	.5	0	.6	.7	.5	1.1	1.2	.3	0	.2	0	.9	0	.6	.1	.4	0	0	0	.2	0	.7	.8	.5	+
XXII-1D	0	0	.8	0	0	.9	1.0	0	.7	0	0	0	0	0	0	0	0	0	0	0	0	.7	.5	.8	+
XXII-2C	0	0	0	0	0	.2	1.2	0	.1	.1	0	0	0	0	0	.1	0	0	0	0	0	0	0	1.1	-
XXII-2F	0	0	0	0	0	.2	1.5	0	0	.4	0	0	0	0	0	.3	0	0	0	0	0	0	0	.9	-
XXII-2G	0	0	0	0	0	.2	1.1a	0	.3	1.1	0	0	0	.5	0	.1	0	0	0	0	0	0	0	.9	-
XXII-5A	1.0	0	1.0	0	0	0	0	.6	.8	0	0	0	0	1.0	0	0	0	0	0	0	0	.7	.3	.9	+
XXII-5C	.9	0	1.0	0	.7	0	0	.6	.7	0	0	0	.9	1.0	0	0	0	0	0	0	0	.1	.2	.9	+
XXII-8A	1.2	.6	1.4	.8	.3	.7	2.5	.3	.6	1.1	.7	1.1	0	1.0	.2	0	0	0	.2	0	0	.4	.2	.8	+
XXII-8B	0	0	0	0	0	0	0	.4	0	0	.3	.6	0	0	0	0	0	0	0	0	0	.2	0	1.0	-
XXII-9A	0	0	0	.1	0	0	0	0	0	0	0	.6	0	.3	0	0	0	0	0	0	0	.4	0	.6	+
XXII-11B	0	0	0	0	0	.7	.7	.1	0	.5	.9	0	.1	.5	.6	.4	.2	0	0	0	0	0	0	.3	+
XXII-11D	0	0	.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.8	-
XXII-11E	0	1.0	0	0	0	0	0	0	0	0	0	0	0	.1b	.1	0	0	0	0	0	0	0	0	.8	+
XXII-12C	0	0	.4	0	0	0	0	0	0	0	0	0	0	0	.1	0	0	0	0	0	0	0	0	1.4	+
XXII-14A	0	0	0	0	.5	0	0	0	0	0	1.0	.2	0	0	0	.4	0	0	0	0	0	.4	0	.8	-
XXII-15B	1.0	0	0	0	1.1	1.0	1.5	1.0	.6	.2	.2	1.5	0	0	.9	.4	.4	0	0	0	0	.4	0	1.1	+
XXII-15C	.5	.4	.4	.9	.4	1.0	1.0	.8	0	0	0	0	0	.8	0	0	.3	0	0	0	0	.3	0	1.8	+
XXII-18A	0	0	0	0	0	0	.7	.2	0	.7	1.0	.9	0	.4	.3	0	.4	.2	0	0	.1	.6	.2	.9	+
Discordant Total	6/19	10/19	3/19	7/19	7/19	8/19	7/19	3/19	7/19	10/19	10/19	8/19	10/19	2/19	6/19	12/19	8/19	10/19	11/19	9/19	9/19	2/19	3/19	7/19	±
*Discordant Total	4/17	9/17	2/17	5/17	6/17	8/17	7/17	2/17	7/17	9/17	9/17	7/17	8/17	0/17	5/17	11/17	7/17	8/17	9/17	8/17	2/17	2/17	7/17	7/17	+

a = $i(7p)$

b = 14 translocation

* Calculated discordancy ratio excluding line 1D and 14A. 18A scored as (-) (see discussion).

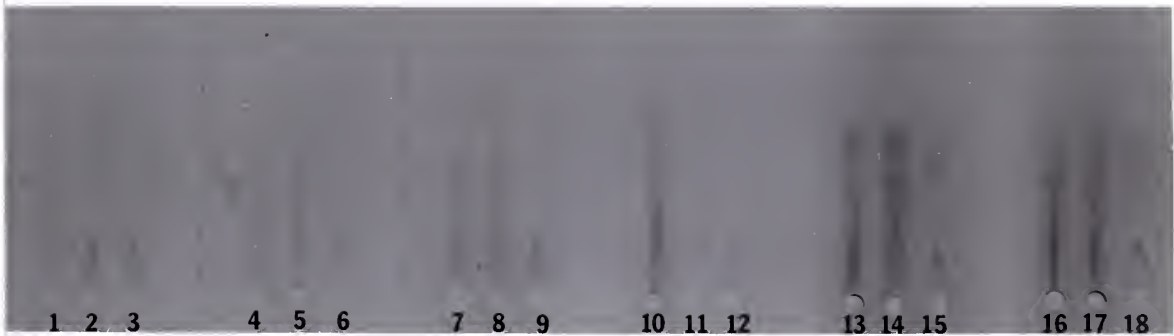


Figure I.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	12C	7	15C	13	11B
2	1B	8	1D	14	5C
3	STD	9	STD	15	STD
4	8A	10	7777	16	7777
5	2G	11	101	17	2C
6	STD	12	STD	18	STD

Immunoelectrophoresis of ten-fold concentrated culture media from first hybrid panel, parental cell lines and calibrated human standard (STD), 35 $\mu\text{g}/\text{ml}$. Performed with 5 μl samples. Antiserum: anti-AAT 2 $\mu\text{l}/3\text{ml}$ of 1% agarose.

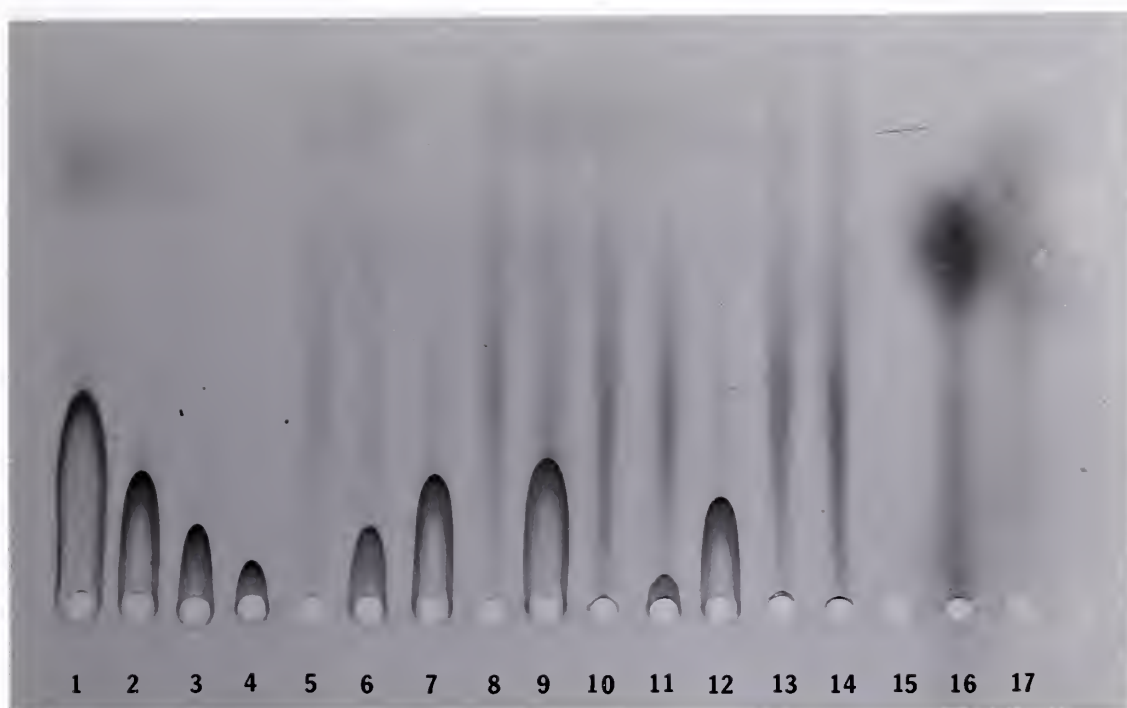


Figure II.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	88 $\mu\text{g/ml}$ STD	7	8A	13	2C
2	44 $\mu\text{g/ml}$ STD	8	2G	14	7777
3	22 $\mu\text{g/ml}$ STD	9	15C	15	101
4	11 $\mu\text{g/ml}$ STD	10	1D	16	NRS
5	12C	11	11B	17	FCS
6	1B	12	5C		

Immunoelectrophoresis of one hundredfold concentrated culture media from first hybrid panel, parental cell lines, calibrated human standard, normal rat serum (NRS) and fetal calf serum (FCS). Performed with 5 μl samples. Antiserum: anti-AAT 60 $\mu\text{l}/20\text{ ml}$ of 1% agarose.

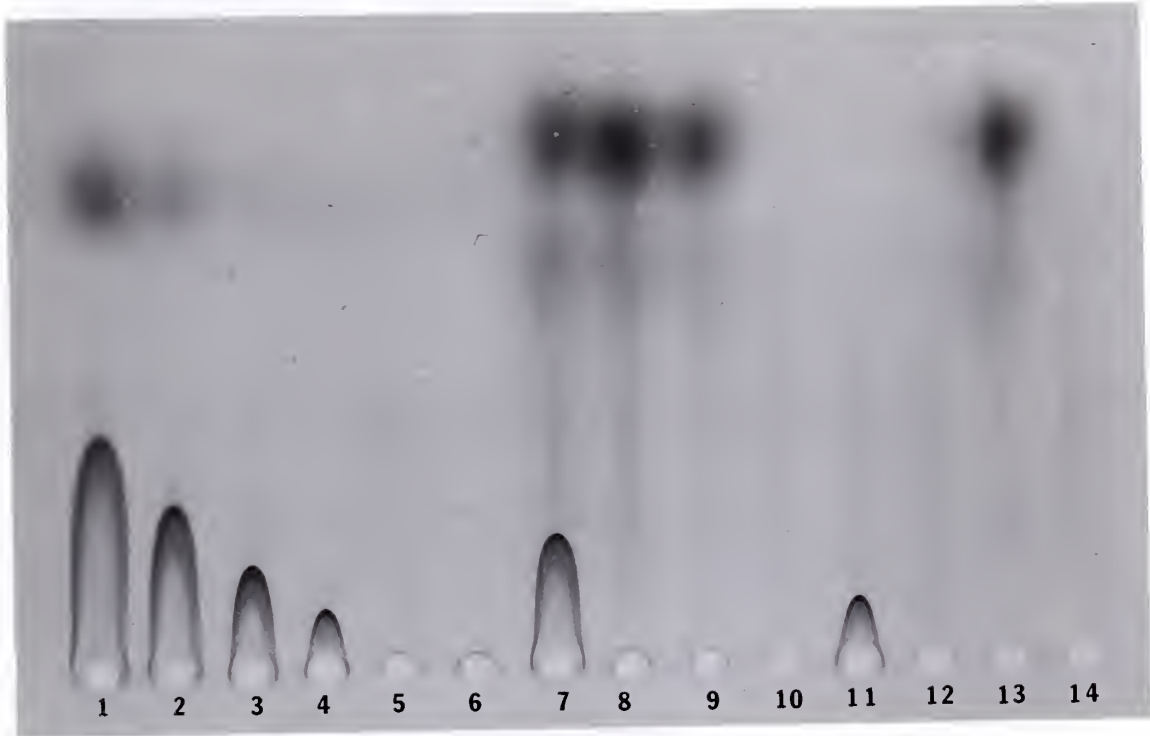


Figure III.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	88 $\mu\text{g/ml}$ STD	6	5A	11	15B
2	44 $\mu\text{g/ml}$ STD	7	9A	12	11D
3	22 $\mu\text{g/ml}$ STD	8	14A	13	11E
4	11 $\mu\text{g/ml}$ STD	9	18A	14	2F
5	1A	10	8B		

Immunoelectrophoresis of one hundredfold concentrated culture media from second hybrid panel and calibrated human standard. Performed with 5 μl samples. Antiserum: anti-AAT 60 $\mu\text{l}/20\text{ ml}$ of 1% agarose.

Limits of sensitivity by decreasing antibody concentration



Figure IV

Calibrated human standard 5 μ l samples. Antiserum: anti-AAT
40 μ l/20 ml of 1% agarose.

<u>Lane</u>		<u>Lane</u>	
1	44 μ g/ml STD	4	5.5 μ g/ml
2	22 μ g/ml	5	2.8 μ g/ml
3	11 μ g/ml	6	1.4 μ g/ml

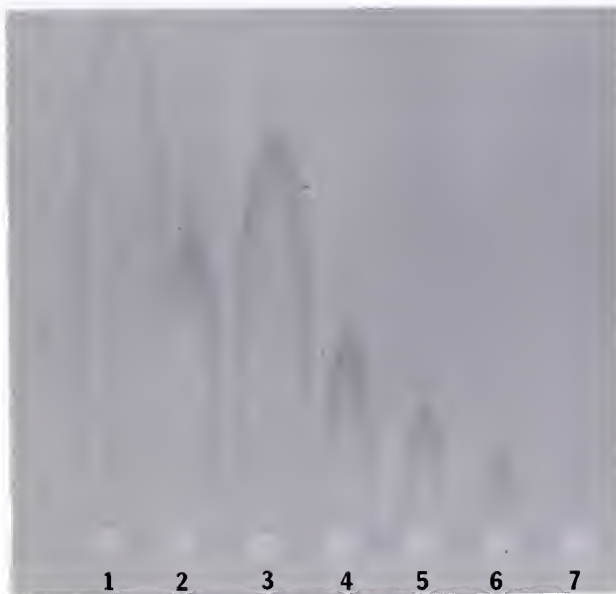


Figure V

Calibrated human standard 5 μ l samples. Antiserum: anti-AAT
20 μ l/20 ml of 1% agarose.

<u>Lane</u>		<u>Lane</u>	
1	44 μ g/ml STD	5	2.8 μ g/ml
2	11 μ g/ml	6	1.4 μ g/ml
3	22 μ g/ml	7	0.7 μ g/ml
4	5.5 μ g/ml		

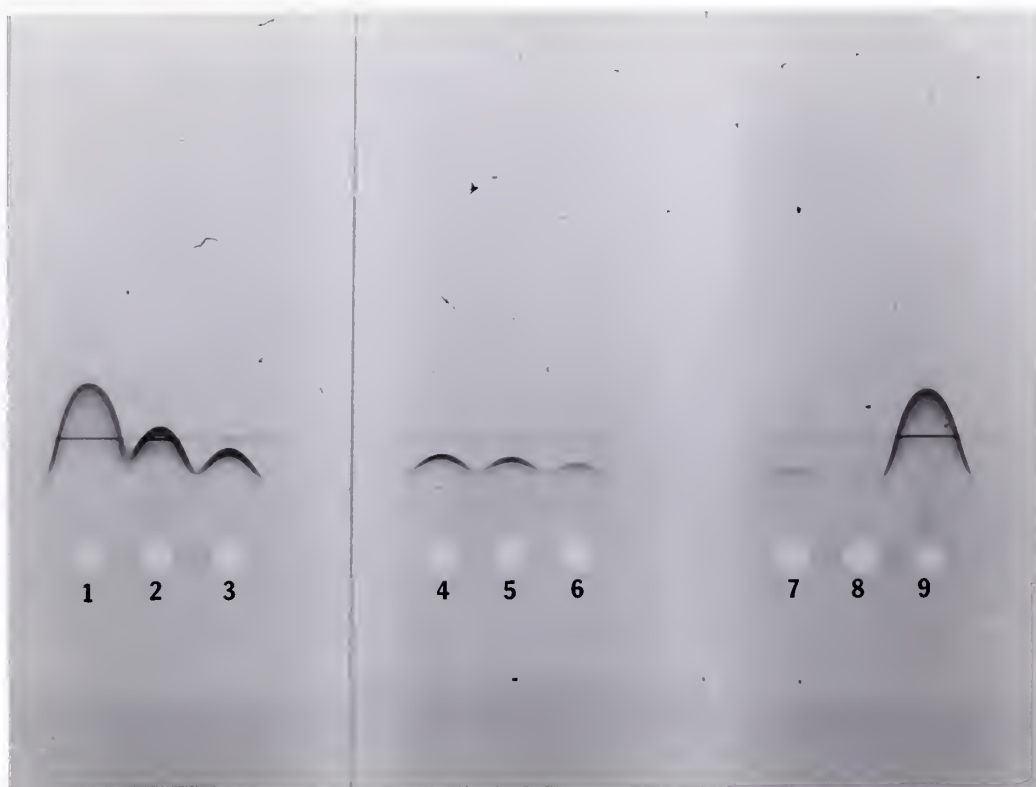


Figure VI.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	44 $\mu\text{g/ml}$ STD	4	5.5 $\mu\text{g/ml}$	7	1.4 $\mu\text{g/ml}$
2	22 $\mu\text{g/ml}$	5	3.5 $\mu\text{g/ml}$	8	0.7 $\mu\text{g/ml}$
3	11 $\mu\text{g/ml}$	6	2.8 $\mu\text{g/ml}$	9	44 $\mu\text{g/ml}$

Lower limits of sensitivity in a two layered agarose gel with calibrated human standard. Antiserum: anti-AAT 4 $\mu\text{l/ml}$ of 1% agarose.

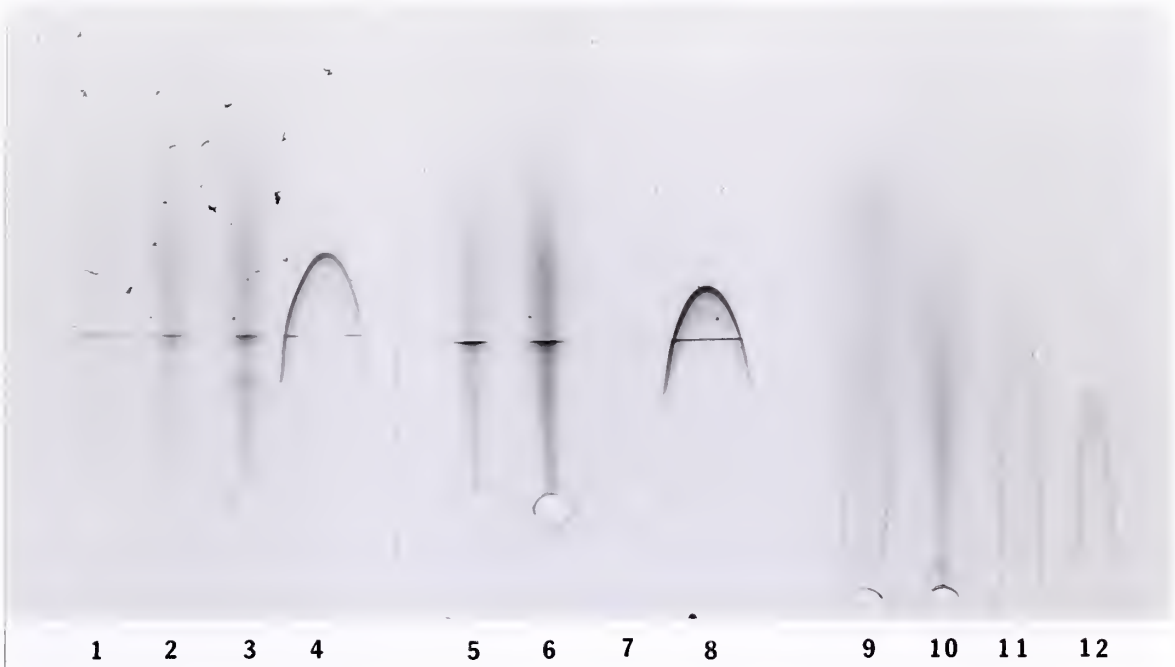


Figure VII.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	12C	5	2C	9	X
2	2B	6	7777	10	1D
3	1D	7	101	11	44 µg/ml STD
4	44 µg/ml STD	8	44 µg/ml STD	12	35 µg/ml STD

Detection of low level antigen in one hundred fold concentrated culture media from first hybrid panel by two layer gel and decreased antibody concentration techniques. Performed with 5 µl samples. Lanes 1-8; Antiserum: anti-AAT 4 µl/ml of 1% agarose, Lanes 9-12; antiserum: anti-AAT 1 µl/ml of 1% agarose. Lane 9 contains media from a different fusion experiment (X).

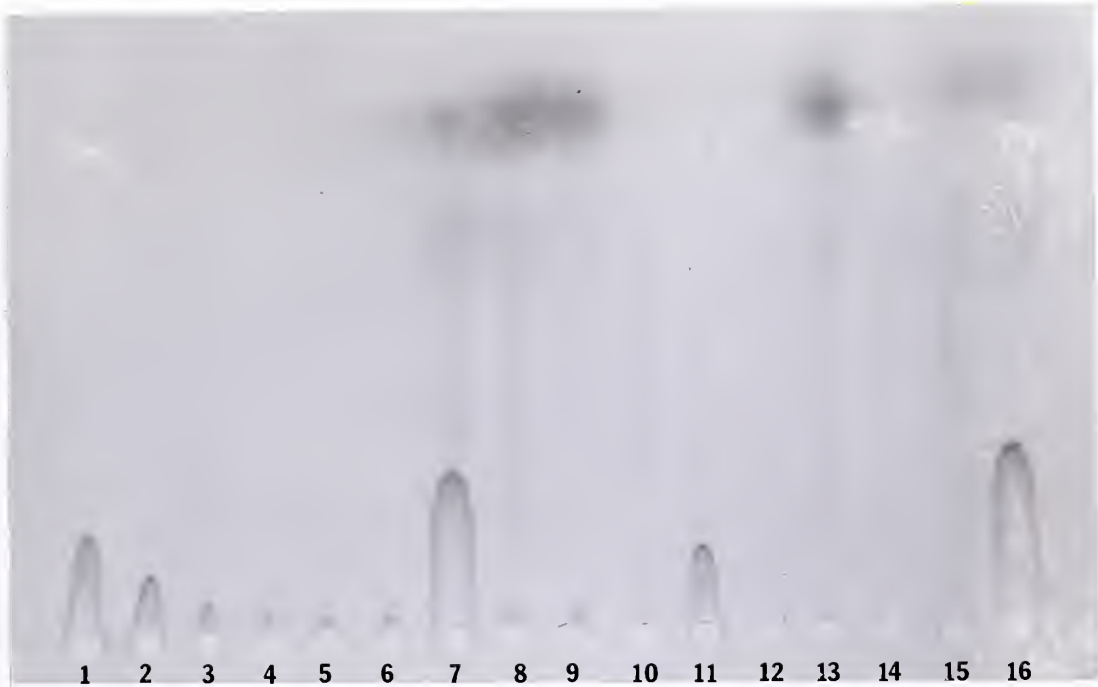


Figure VIII.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	11 µg/ml STD	6	5A	11	15B
2	5.5 µg/ml STD	7	9A	12	11D
3	2.8 µg/ml STD	8	14A	13	11E
4	1.4 µg/ml STD	9	18A	14	2F
5	1A	10	8B	15	X
				16	Y

Detection of low level antigen in one hundredfold concentrated culture media from second hybrid panel by decreased antibody concentration technique with calibrated human standard. Performed with 5 µl samples. Antiserum: anti-AAT 30 µl/20 ml of 1% agarose. Lanes 15 and 16 contain media from a different fusion experiment (X and Y).

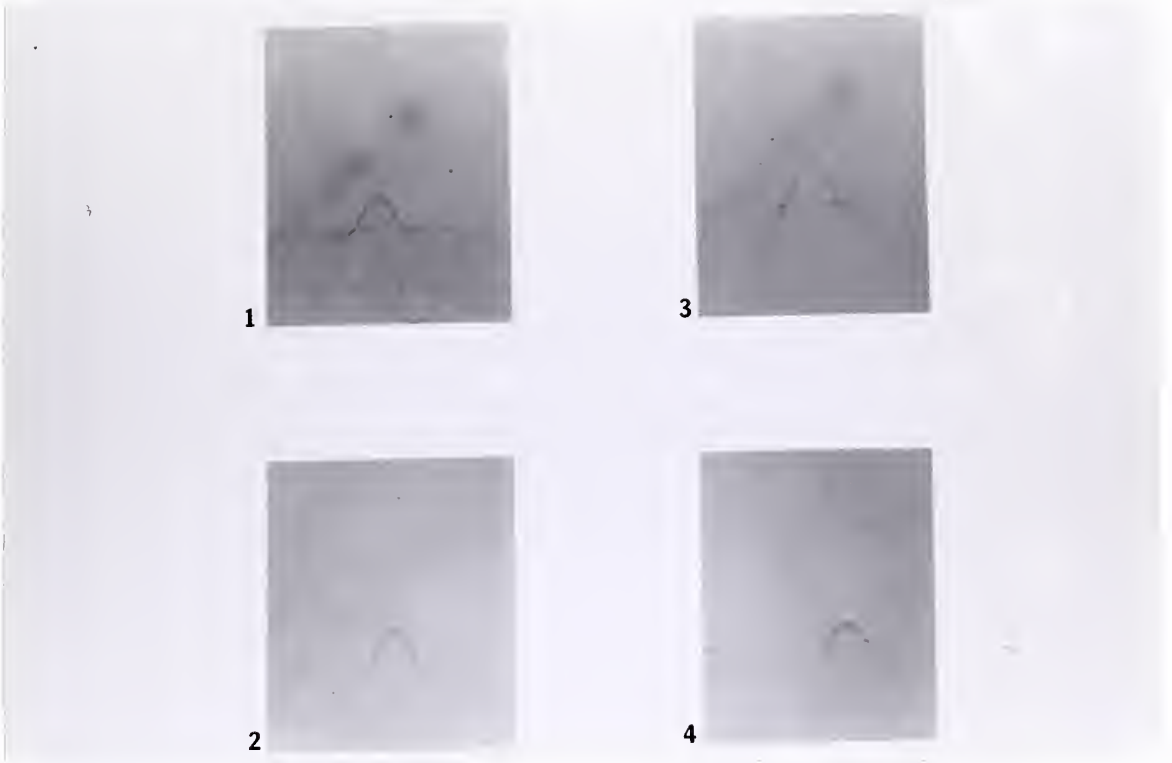


Figure IX.

Plate

1 8A
2 5C

Plate

3 15C
4 35 $\mu\text{g}/\text{ml}$ STD

Crossed immunoelectrophoresis of ten-fold concentrated culture media from first hybrid panel and calibrated human standard. Performed with 7 μl samples. Antiserum: anti-AAT 1 $\mu\text{l}/\text{ml}$ of 1% agarose.

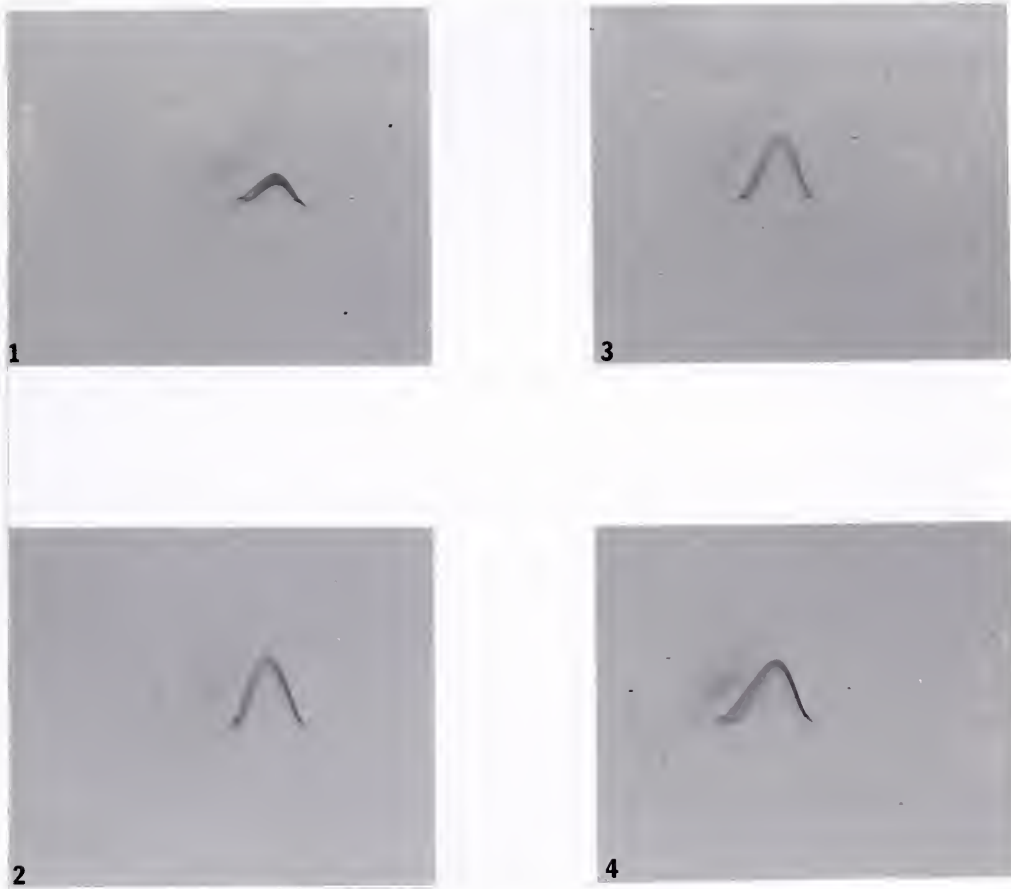


Figure X.

Plate

1	1B
2	5C

Plate

3	8A
4	15C

Crossed immunoelectrophoresis of one hundred-fold concentrated culture media from first hybrid panel. Performed with 6 μ l samples.
Antiserum: anti-AAT 4 μ l/ml of 1% agarose.



Figure XI.

Immunodiffusion of one hundred fold concentrated culture media from first hybrid panel, calibrated human standard (44 $\mu\text{g/ml}$), fetal calf serum (FCS) and normal rat serum (NRS). Performed with 5 μl samples. Antiserum: anti-AAT, 5 μl in center wells.



Figure XII.

Immunodiffusion of one hundred fold concentrated culture media from first hybrid panel not producing AAT, parental cell lines, and calibrated human standard ($44 \mu\text{g/ml}$). Performed with $5 \mu\text{l}$ samples. Antiserum: anti-AAT, $5 \mu\text{l}$ in center wells.

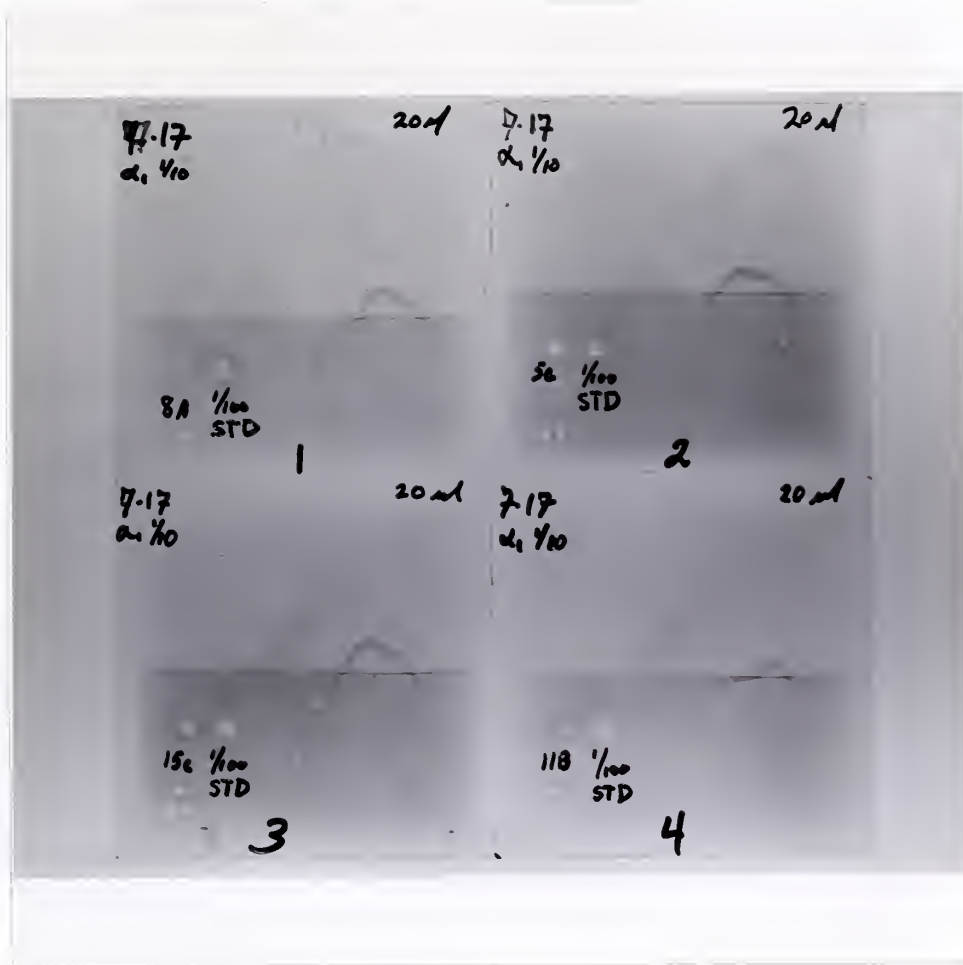


Figure XIII.

Tandem crossed immunoelectrophoresis of ten-fold concentrated culture media from first hybrid panel with calibrated human standard ($35 \mu\text{g/ml}$). Performed with $7 \mu\text{l}$ samples. Antiserum: anti-AAT, $1 \mu\text{l/ml}$ of 1% agarose.

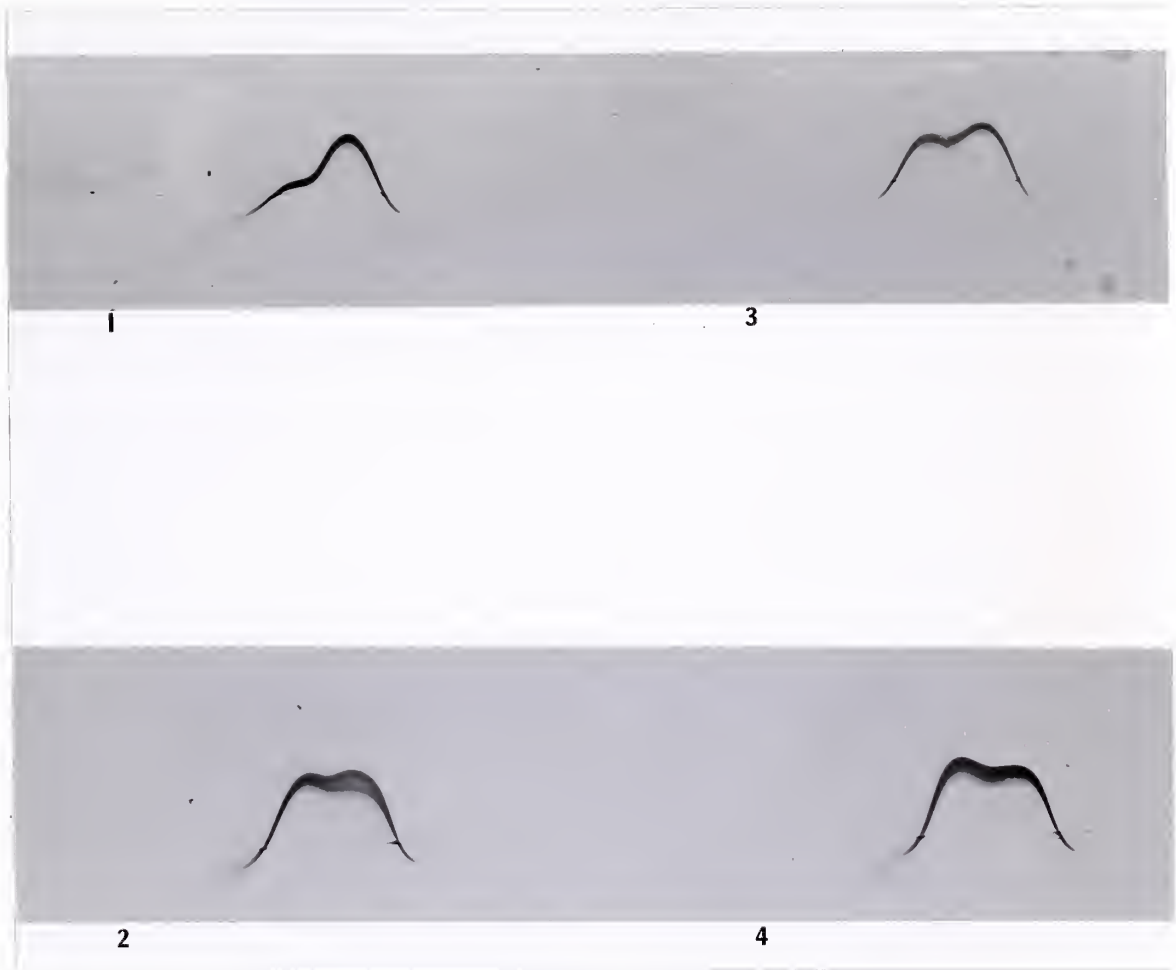


Figure XIV.

<u>Plate</u>		<u>Plate</u>	
1	1B and STD	3	8A and STD
2	5C and STD	4	15C and STD

Tandem crossed immunoelectrophoresis of one hundred-fold concentrated culture media from first hybrid panel with calibrated human standard (44 $\mu\text{g}/\text{ml}$). Performed with 5 μl samples. Antiserum: anti-AAT 4.5 $\mu\text{l}/\text{ml}$ of 1% agarose.

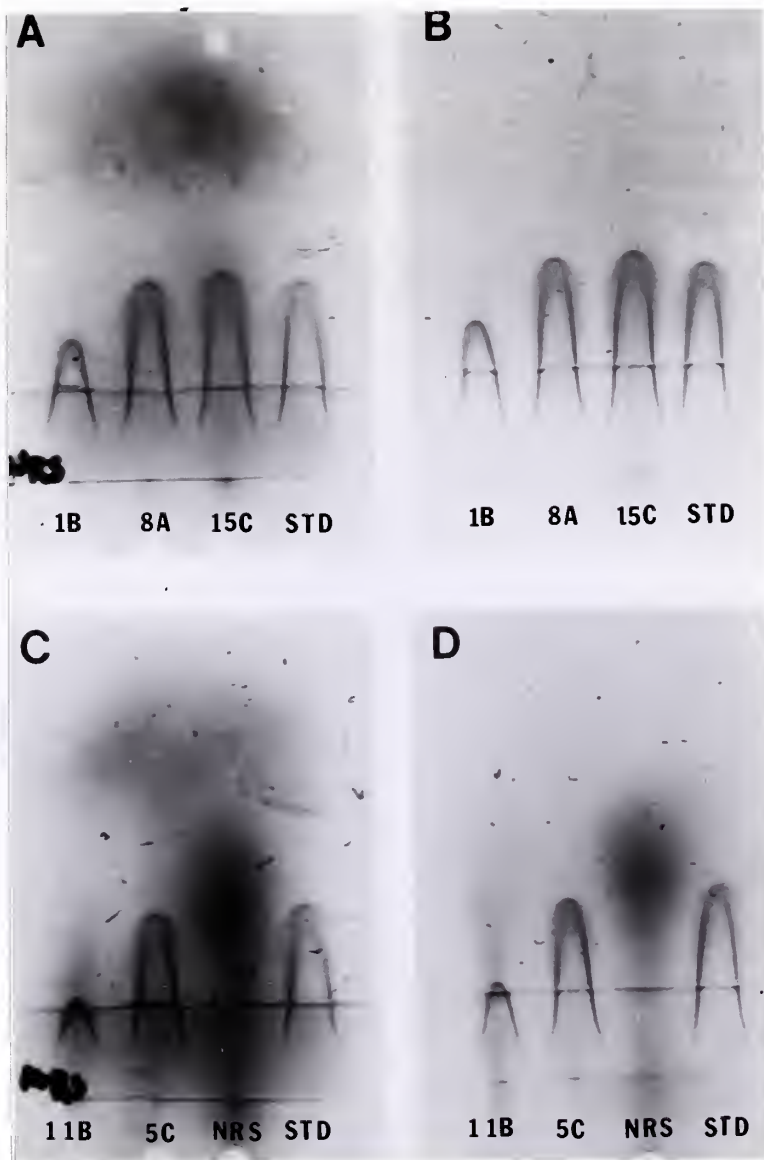


Figure XV.

Intermediate gel absorption with normal rat serum (NRS), calibrated human standard (STD) 44 $\mu\text{g}/\text{ml}$, and one hundred-fold concentrated culture media from first hybrid panel. Performed with 5 μl samples. Antiserum: anti-AAT, 4.5 $\mu\text{l}/\text{ml}$ of 1% agarose. Intermediate layer plates A and C - NRS, plates B and D - normal saline. Plates A/B and C/D are compared.

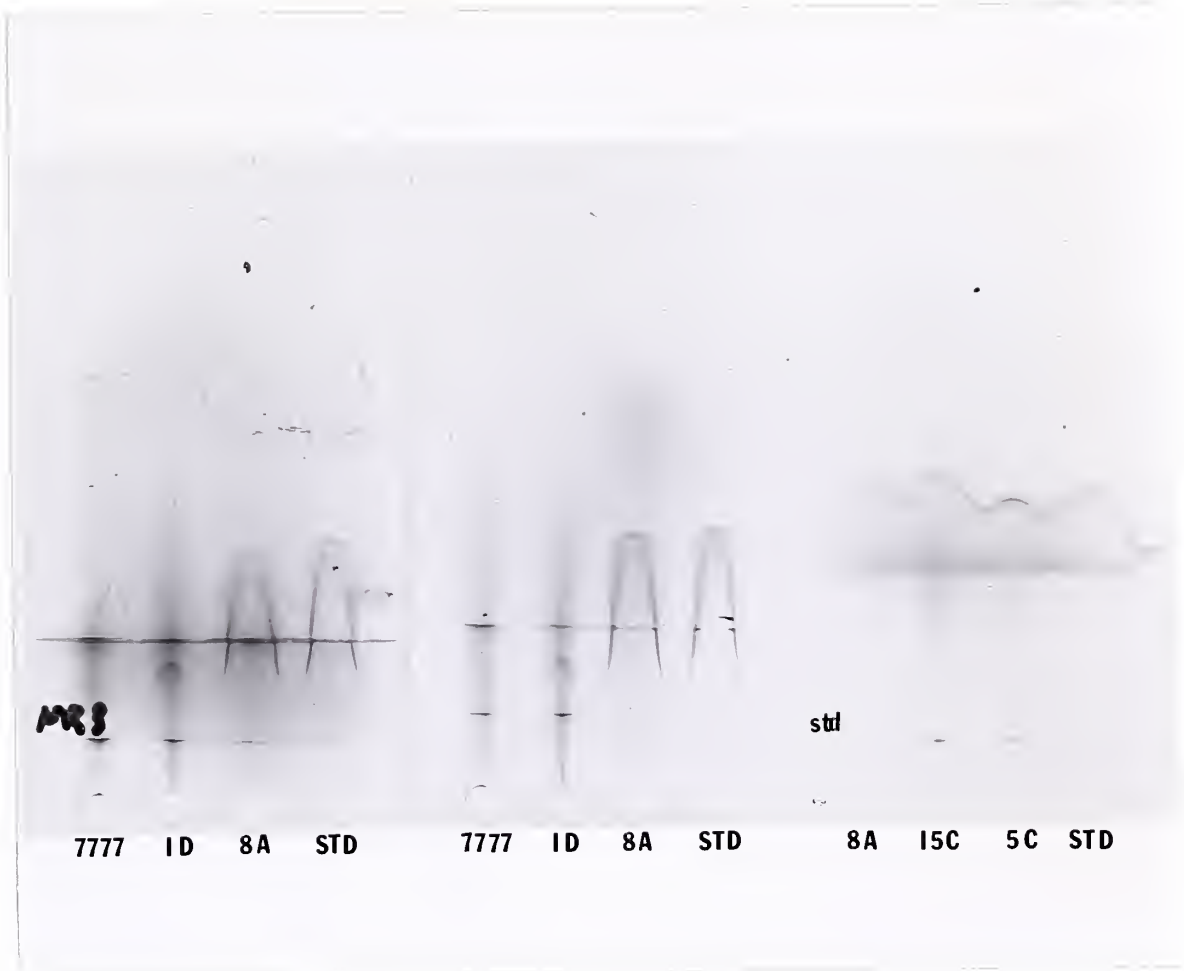


Figure XVI.

Intermediate gel absorption with normal rat serum (NRS), one hundred-fold concentrated culture media from hybrid lines 1D, 15C, 5C and 8A, parental line 7777 and calibrated human standard (STD) 44 $\mu\text{g/ml}$. Performed with 5 μl samples. Antiserum: anti-AAT, 4.5 μml of 1% agarose. Intermediate layer plate 1 - NRS, plate 2 - normal saline, plate 3 - STD.

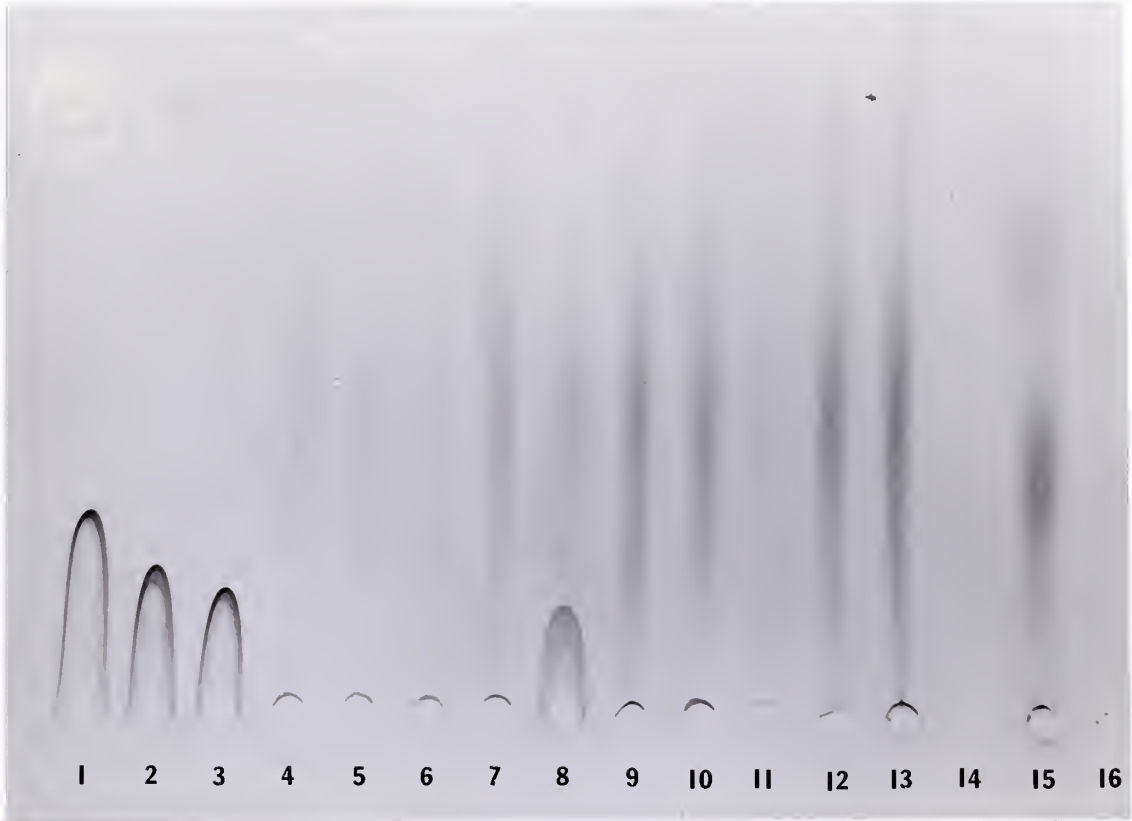


Figure XVII.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	370 $\mu\text{g/ml}$ STD	7	2G	12	2C
2	230 $\mu\text{g/ml}$ STD	8	15C	13	7777
3	180 $\mu\text{g/ml}$ STD	9	1D	14	101
4	12C	10	11B	15	NRS
5	1B	11	5C	16	FCS

Immunoelectrophoresis of one hundred-fold concentrated culture media from first hybrid panel with calibrated human standard, parental cell lines, normal rat serum (NRS) and fetal calf serum (FCS). Performed with 5 μl samples. Antiserum: anti human albumin. 1 $\mu\text{l/ml}$ of 1% agarose.

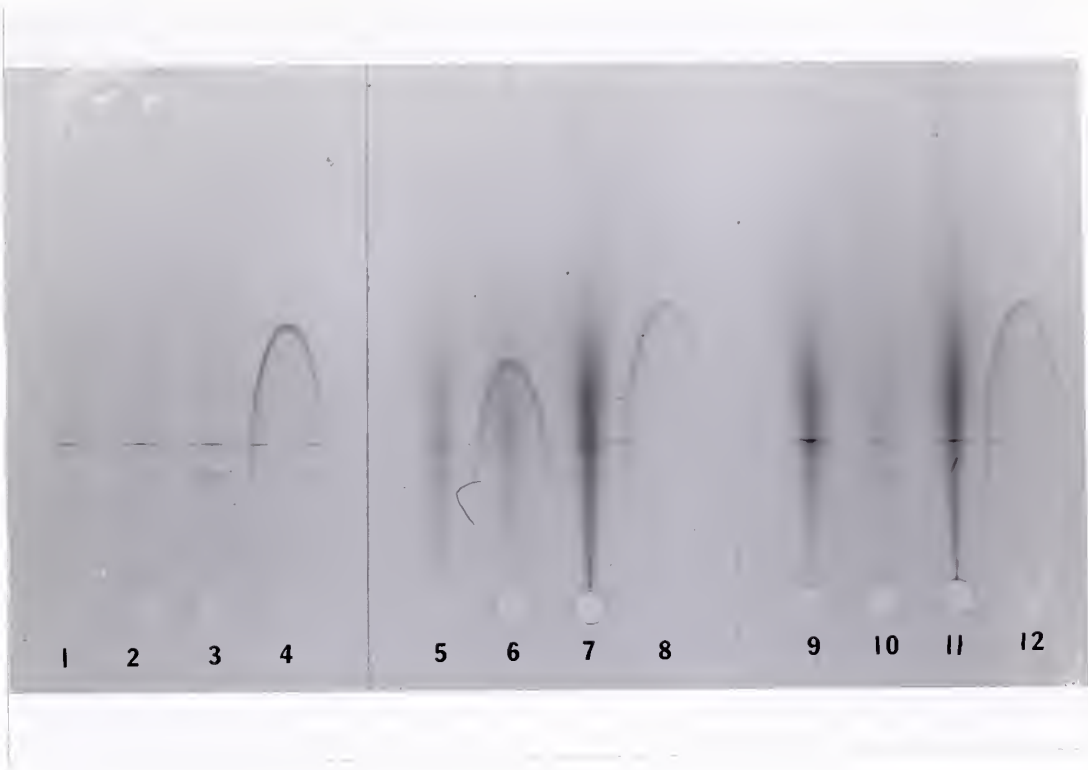


Figure XVIII.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	12C	5	2G	9	11B
2	1B	6	15C	10	5C
3	8A	7	1D	11	2C
4	230 μ g/ml STD	8	STD	12	STD

Detection of low level albumin production in one hundred-fold concentrated culture from first hybrid panel by two layer gel technique. Performed with 5 μ l samples. Antiserum: anti-human albumin 1 μ l/ml of 1% agarose.

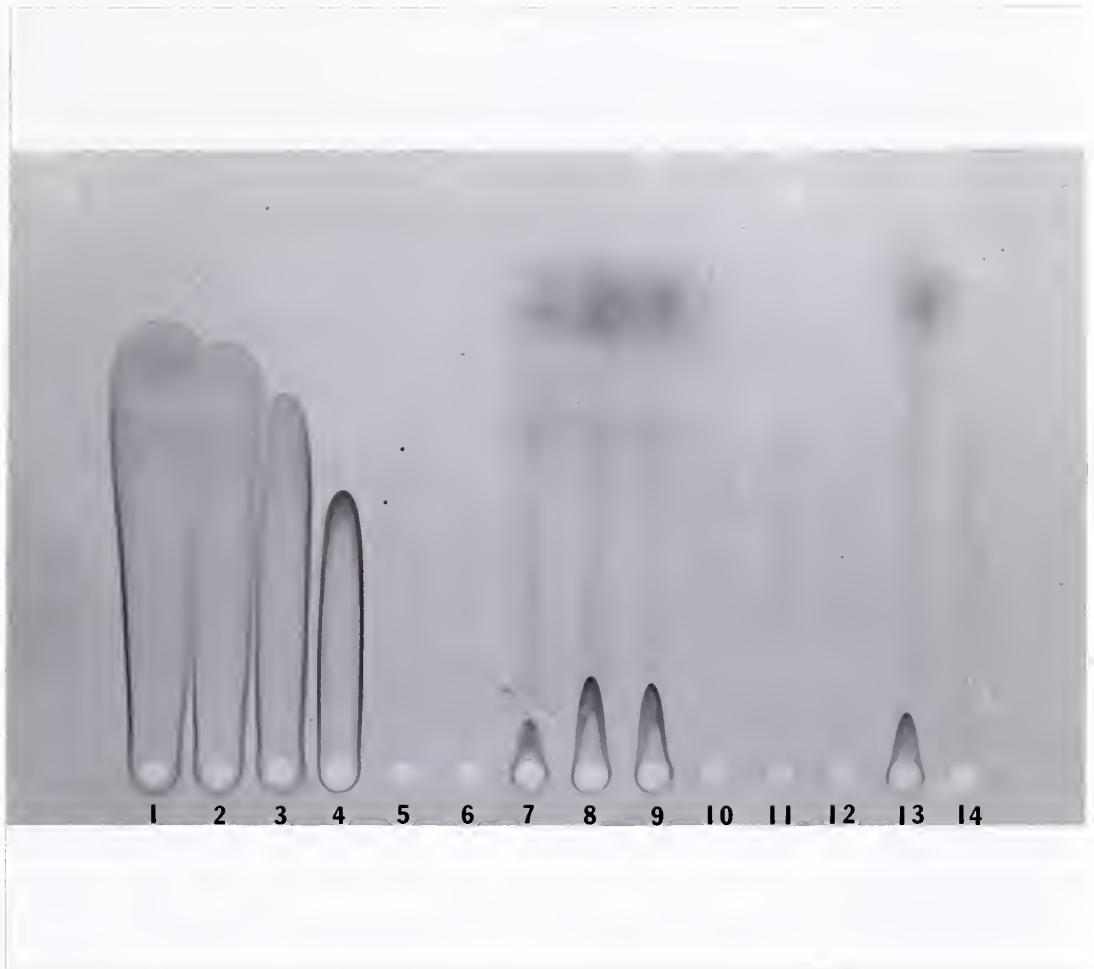


Figure XIX.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	1.5 mg/ml STD	6	5A	11	15B
2	750 μ g/ml STD	7	9A	12	11D
3	375 μ g/ml STD	8	14A	13	11E
4	180 μ g/ml STD	9	18A	14	2F
5	1A	10	8B		

Immunelectrophoresis of one hundred-fold concentrated culture media from record hybrid panel and calibrated human standard. Performed with 5 μ l samples. Antiserum: anti-human albumin 2 μ l/ml of 1% agarose.

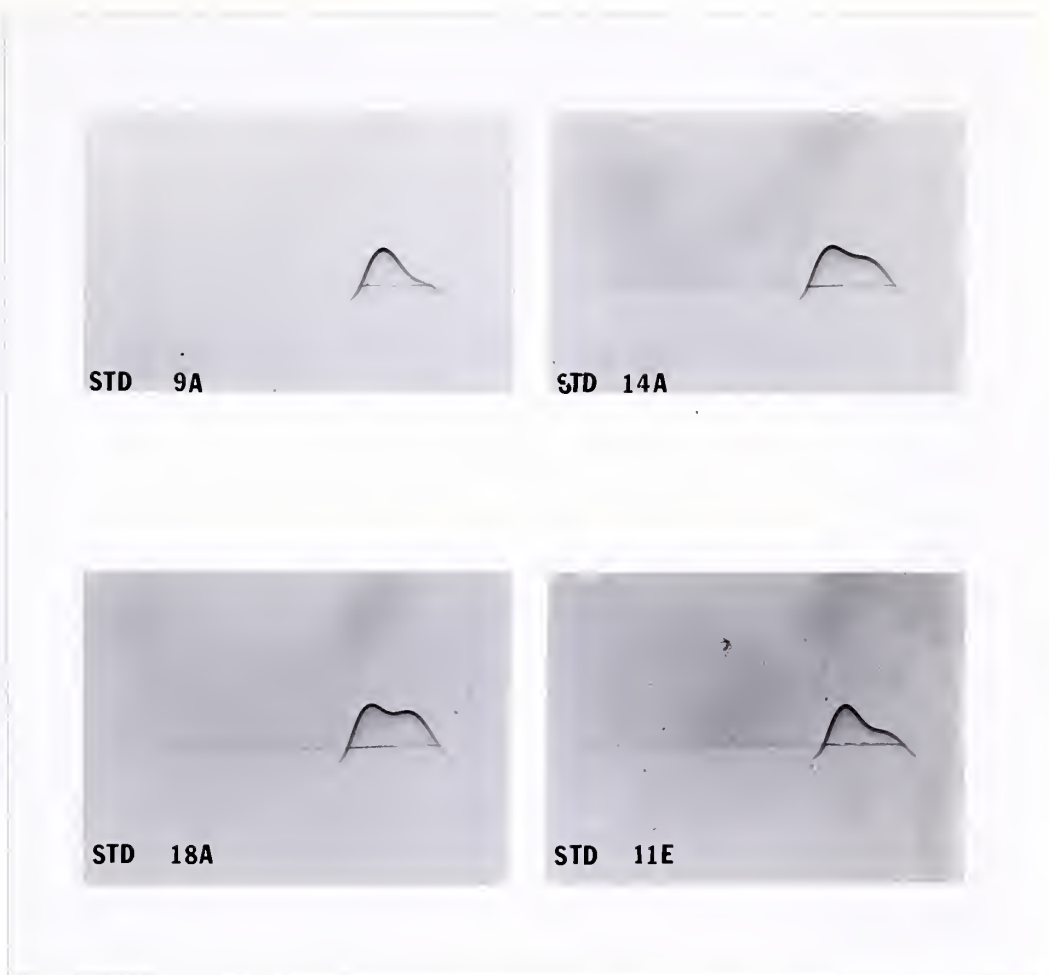


Figure XX.

Tandem crossed immunoelectrophoresis of one hundred fold concentrated culture media from second hybrid panel with calibrated human standard (46 $\mu\text{g}/\text{ml}$). Performed with 6 μl samples. Antiserum: anti-human albumin, 2 $\mu\text{l}/\text{ml}$ of 1% agarose.

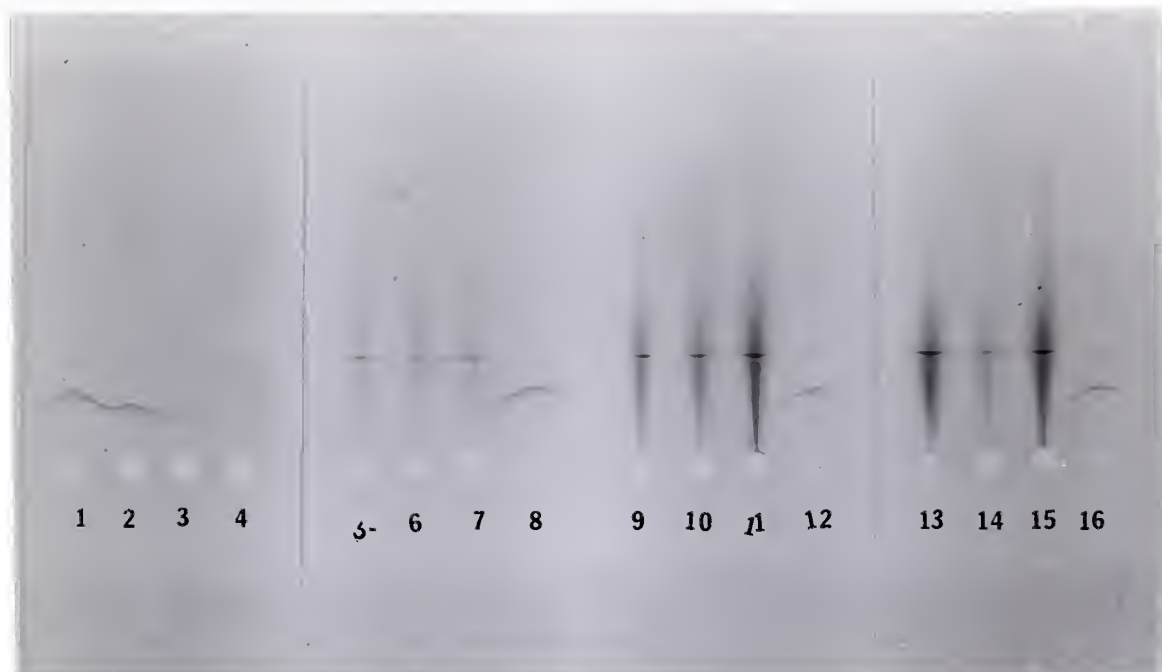


Figure XXI.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	12 µg/ml STD	5	12C	9	2G	13	11B
2	6 µg/ml STD	6	1B	10	15C	14	5C
3	3 µg/ml STD	7	8A	11	1D	15	2C
4	1.5 µg/ml STD	8	12 µg/ml STD	12	12 µg/ml STD	16	12 µg/ml STD

Detection of low level transferrin production, by two layer gel technique, in one hundred-fold concentrated culture media from first hybrid panel and determination of limits of sensitivity. Performed with 5 µl samples. Antiserum: anti-human transferrin 3 µl/ml of 1% agarose.

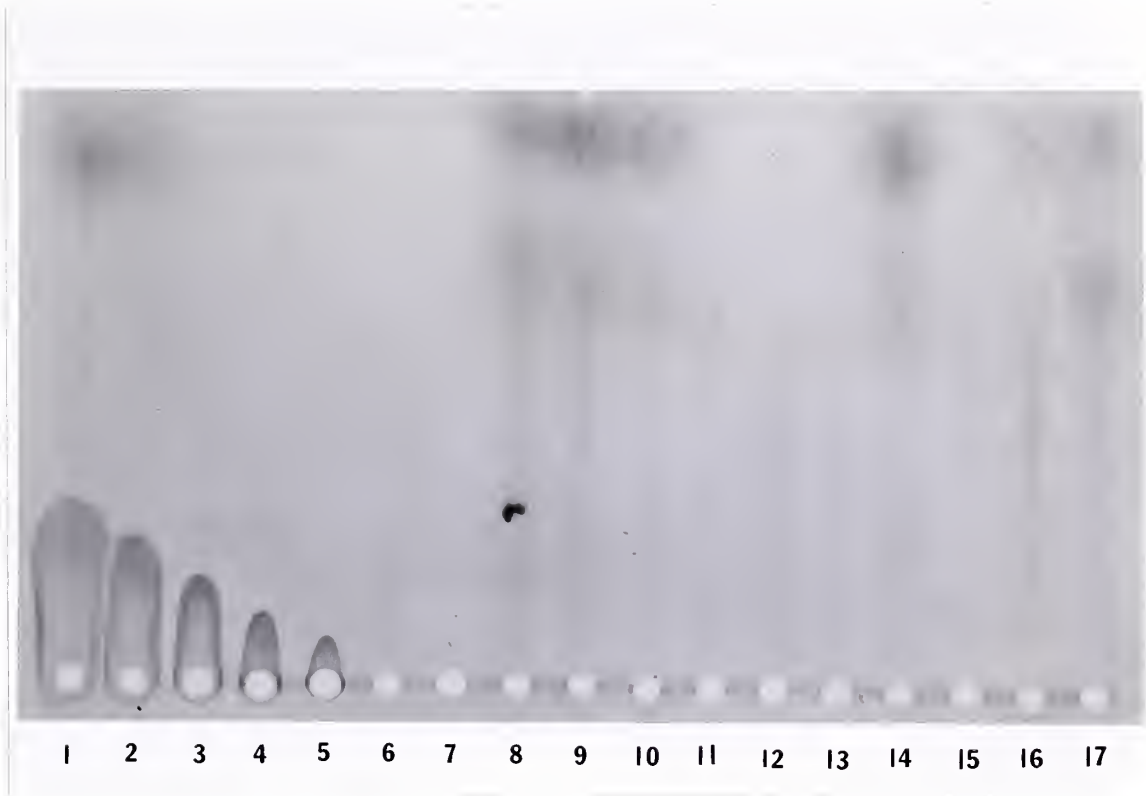


Figure XXII.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	200 µg/ml STD	6	1A	10	18A	14	11E
2	100 µg/ml STD	7	5A	11	8B	15	2F
3	50 µg/ml STD	8	9A	12	15B	16	7777
4	25 µg/ml STD	9	14A	13	11B	17	101
5	12 µg/ml STD						

Immunoelectrophoresis of one hundred-fold concentrated culture media from second hybrid panel with calibrated human serum and parental cell lines. Performed with 5 µl samples. Antiserum: anti-human transferrin 2 µl/ml of 1% agarose.

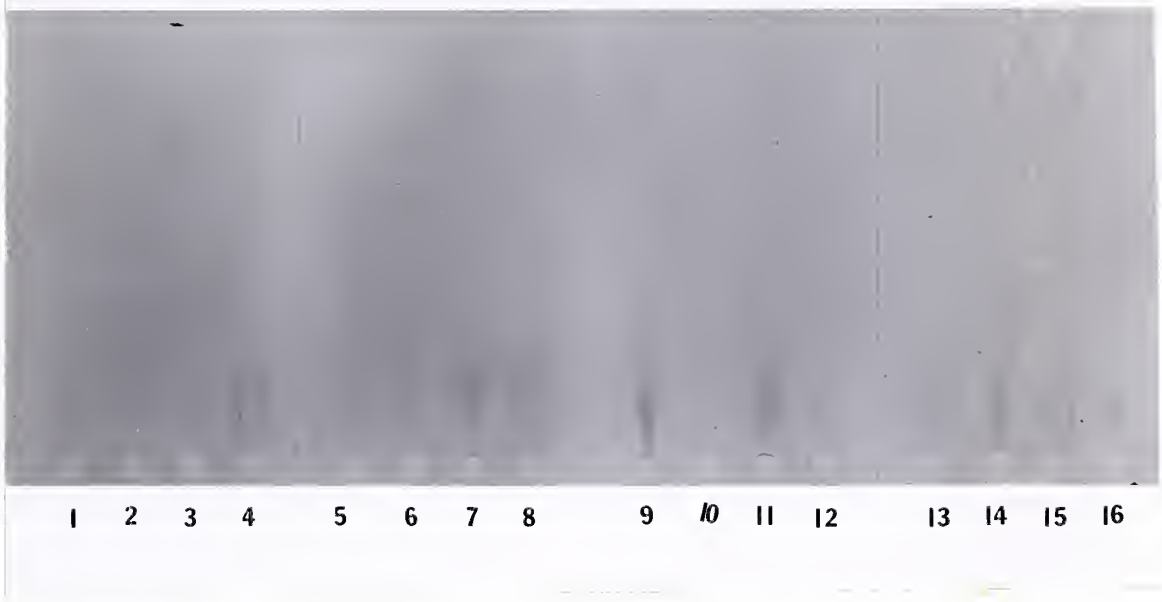


Figure XXIII.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	12A	5	2G	9	7777	13	5C
2	1B	6	15C	10	101	14	2C
3	8A	7	1D	11	11B	15	11 $\mu\text{g/ml}$
4	11 $\mu\text{g/ml}$ STD	8	11 $\mu\text{g/ml}$ STD	12	11 $\mu\text{g/ml}$ STD	16	5 $\mu\text{g/ml}$

Immunoelectrophoresis of ten-fold concentrated culture media from first hybrid panel, parental cell lines and calibrated amniotic fluid. Performed with 7 μl samples. Antiserum: anti-human alpha-fetoprotein 10 $\mu\text{l/ml}$ of 1% agarose.

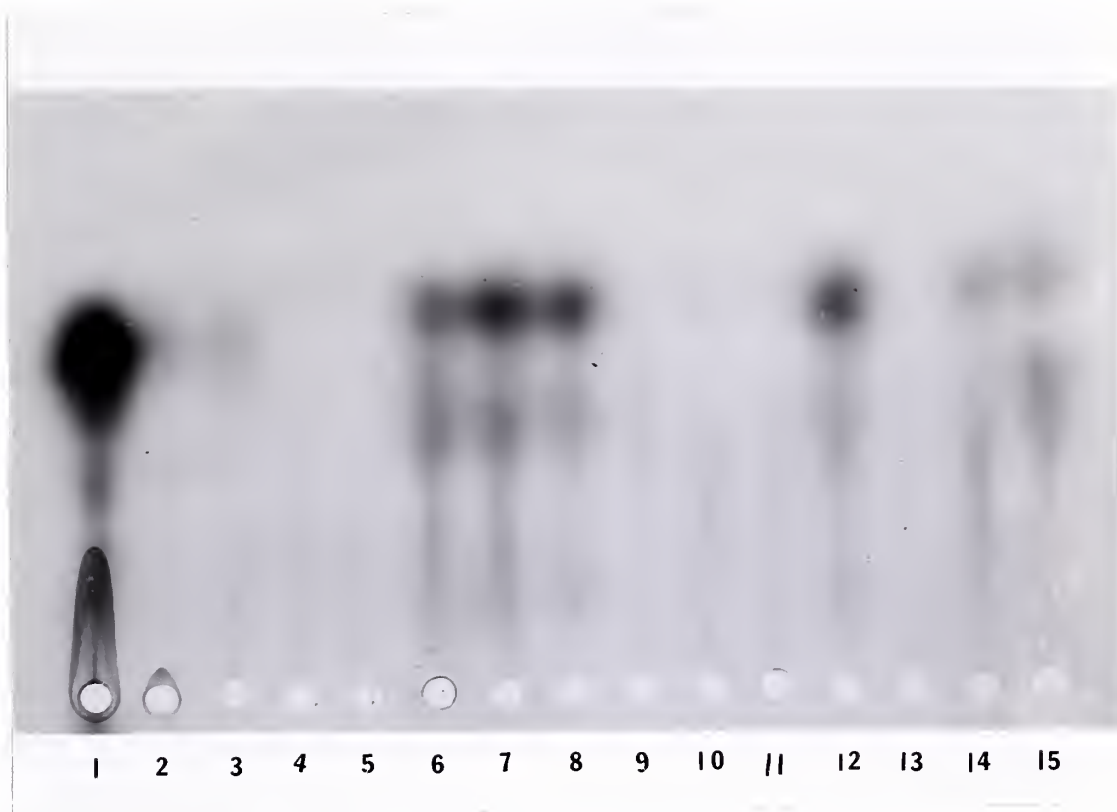


Figure XXIV.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	28 $\mu\text{g/ml}$ STD	6	9A	11	11B
2	12 $\mu\text{g/ml}$ STD	7	14A	12	11E
3	HSS	8	18A	13	2F
4	1A	9	8B	14	7777
5	5A	10	15B	15	101

Immunoelectrophoresis of one hundred-fold concentrated culture media from a second hybrid panel, parental cell lines, calibrated amniotic fluid and human serum standard (HSS). Performed with 7 μl samples. Antiserum: anti-human alpha-fetoprotein 10 $\mu\text{l/ml}$ of 1% agarose.

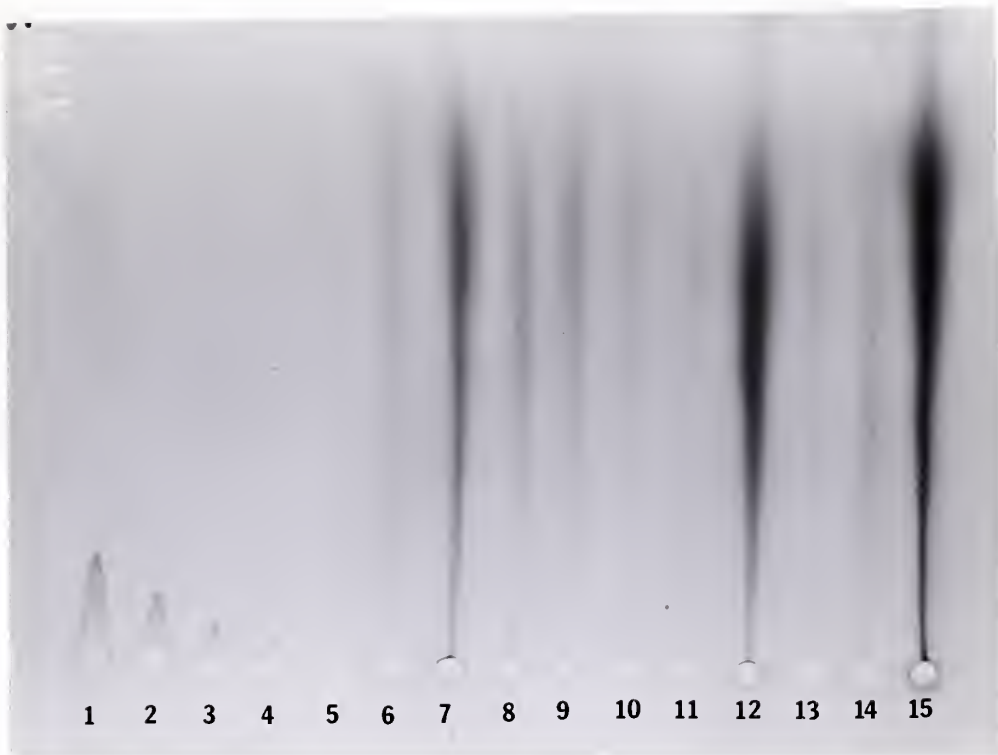


Figure XXV.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	4 µg/ml STD	6	1D	11	11B
2	2 µg/ml STD	7	2C	12	12C
3	1 µg/ml STD	8	2G	13	15C
4	0.5 µg/ml STD	9	5C	14	7777
5	1B	10	8A	15	101

Immunoelectrophoresis of one hundred-fold concentrated culture media from first hybrid panel, parental cell lines and calibrated human standard. Performed with 5 µl samples. Antiserum: anti-human ceruloplasmin 4 µl/ml of 1% agarose.

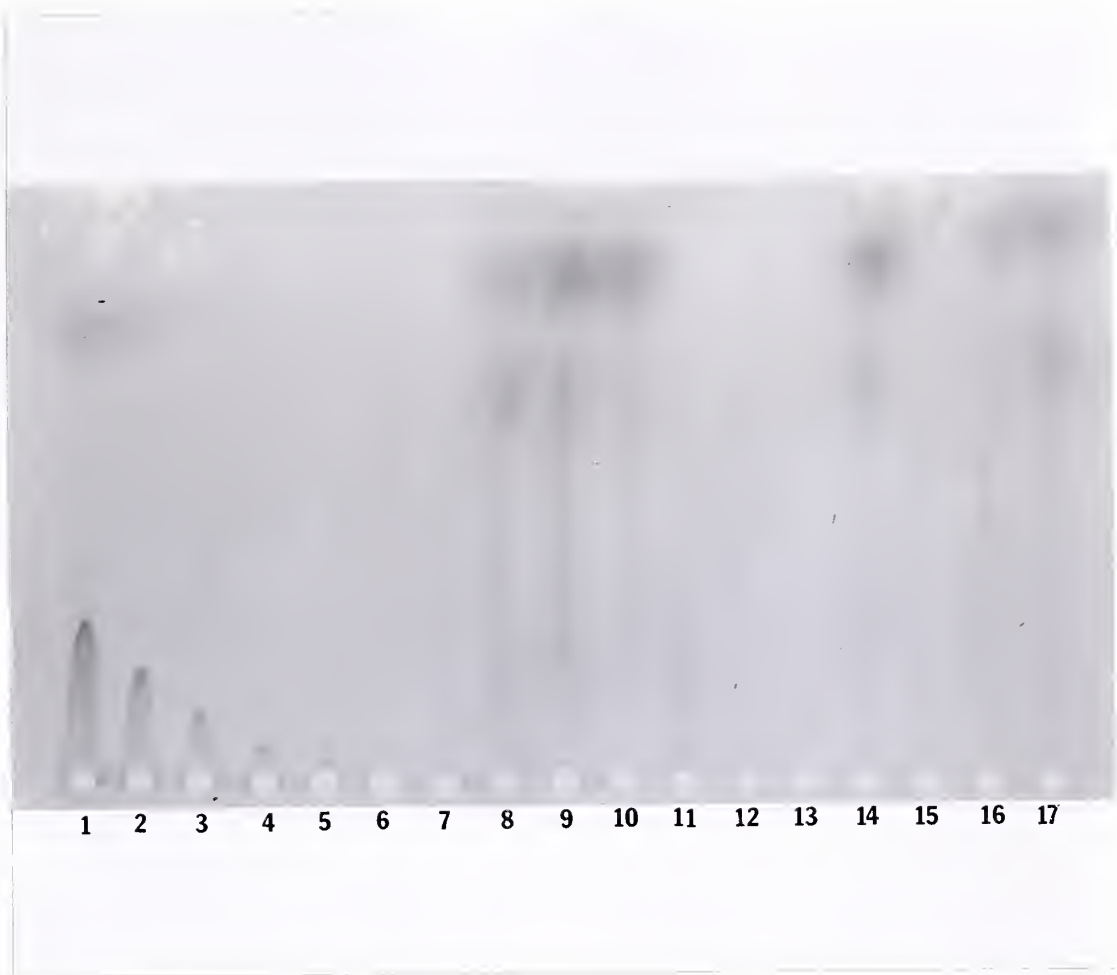


Figure XXVI.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	18 $\mu\text{g/ml}$ STD	6	1A	10	18A	14	11E
2	9 $\mu\text{g/ml}$ STD	7	5A	11	8B	15	2F
3	4 $\mu\text{g/ml}$ STD	8	9A	12	15B	16	7777
4	2 $\mu\text{g/ml}$ STD	9	14A	13	11B	17	101
5	1 $\mu\text{g/ml}$ STD						

Immunoelectrophoresis of one hundred-fold concentrated culture media from second hybrid panel, parental cell lines, and calibrated human standard. Performed with 5 μl samples. Antiserum: anti-human ceruloplasmin 3 $\mu\text{l/ml}$ of 1% agarose.

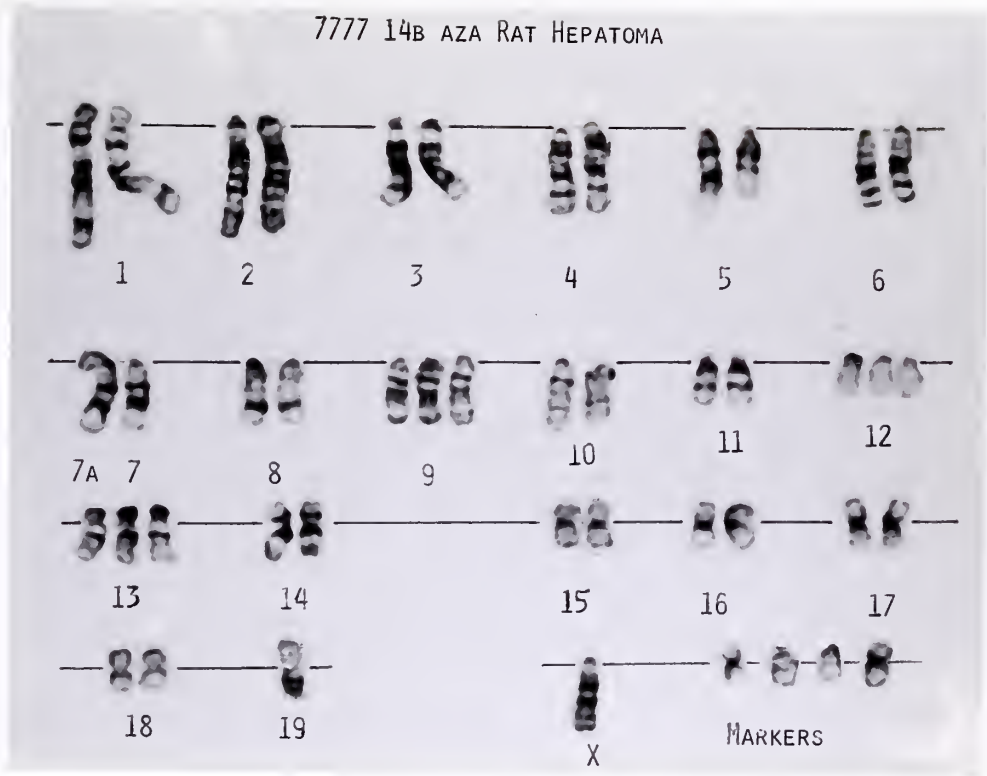


Figure XXVII.

Karyotype of GTG-banded metaphase from rat hepatoma parental cell line, 7777-14a aza.

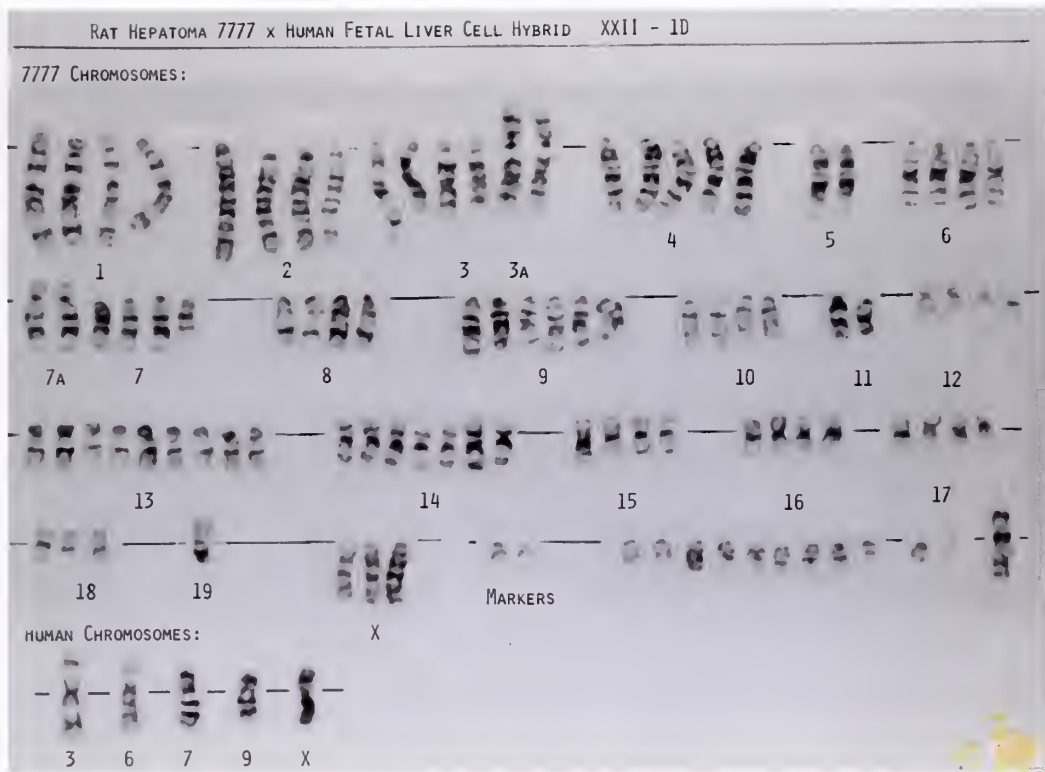


Figure XXVIII.

Karyotype of GTG-banded metaphase from rat hepatoma-human fetal liver hybrid line 1D.

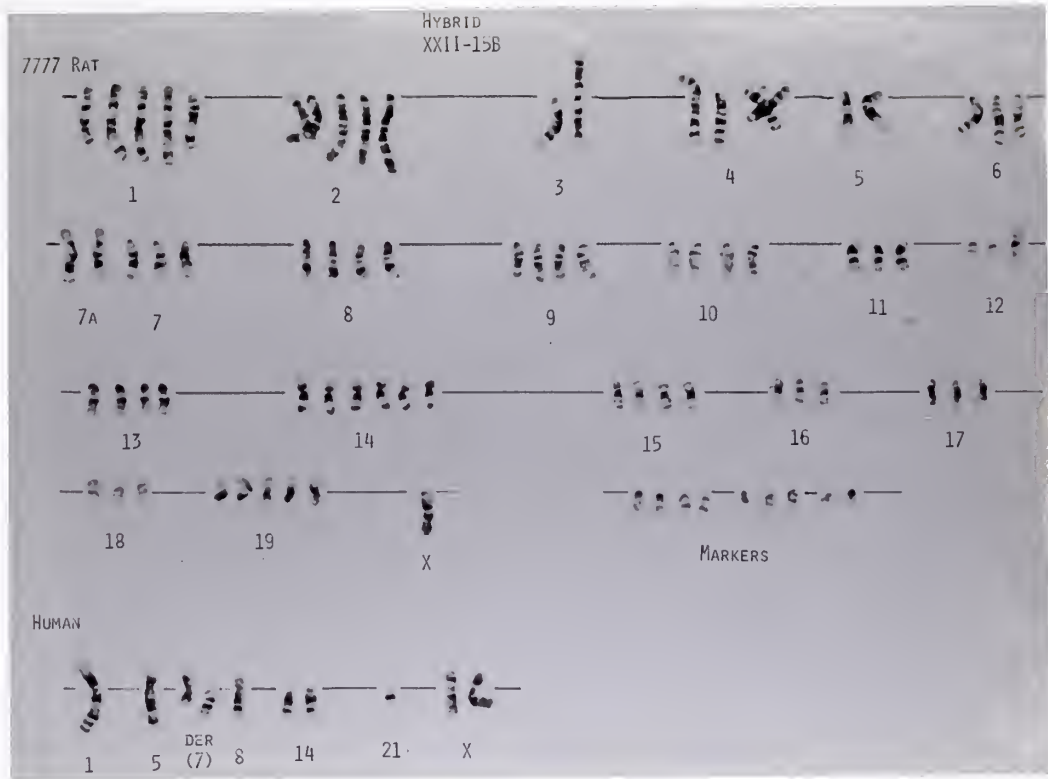


Figure XXIX.

Karyotype of GTG-banded metaphase from rat hepatoma-human fetal liver hybrid line 15B.

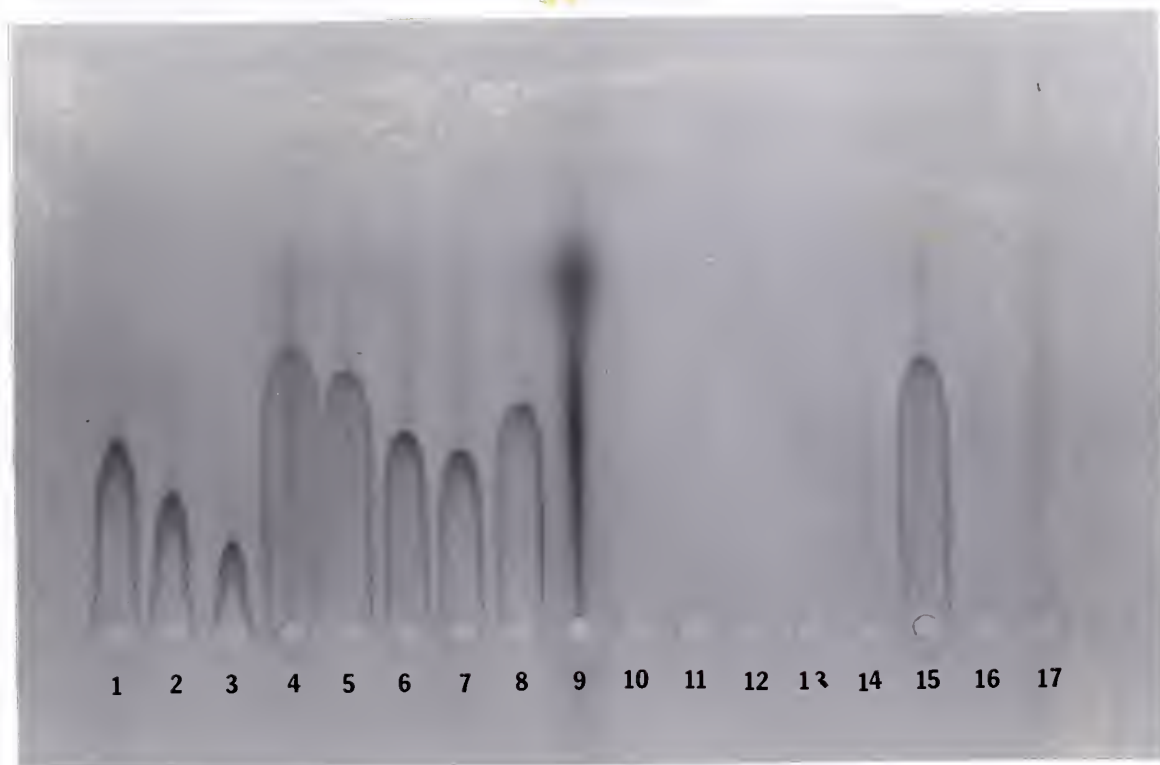


Figure XXX.

<u>Lane</u>		<u>Lane</u>		<u>Lane</u>		<u>Lane</u>	
1	44 $\mu\text{g/ml}$ STD	6	4d/8A	10	1b/18A	14	3d/18A
2	22 $\mu\text{g/ml}$ STD	7	5a/8A	11	2b/18A	15	8A HAT
3	11 $\mu\text{g/ml}$ STD	8	5b/8A	12	3b/18A	16	8B HAT
4	4b/8A	9	1b/8B	13	3c/18A	17	18A HAT
5	4c/8A						

Immunoelectrophoresis of concentrated culture media from hybrid lines counterselected in guanine analogues, hybrid lines selected in HAT media and calibrated human standard. Performed with 5 μl samples. Antiserum: anti-AAT 1 $\mu\text{l/ml}$ of 1% agarose.

Lanes 4-8 counterselected lines from HAT line 8A
 Lane 9 counterselected line from HAT line 8B
 Lanes 10-14 counterselected lines from HAT line 18A
 Lanes 15-17 HAT lines

REFERENCES

- Allderdice P.W., Miller O.J., Pearson P.L., Klein G., Harris H. Human Chromosomes in 18 Man-Mouse Somatic Cell Hybrids Analyzed by Quinacrine Fluorescence. *J Cell Sci.* 1973; 12:809-830.
- Axelsen N.H. (Ed). Quantitative Immunoelectrophoresis. *Scandinavian Journal of Immunology (Suppl 2)*. Universitetsforlaget, Oslo, 1975.
- Baker R.M., Brunette D.M., Mankovitz R., Thompson L.H., Whitmore G.F., Siminovitch L., Till J.E. Ouabain Resistant Mutants of Mouse and Hamster Cells in Culture. *Cell.* 1974; 1:9-21.
- Barski G., Sorieul S., Cornefert F. Production Dans des Cultures in Vitro de Deux Souches Cellulaires en Association de Cellules Character "Hybride". *Comp Rend.* 1960; 251:1825.
- Bennick A., Gedde-Dahl T., Brogger A. Mapping with B-Cell Hybrids. *Cytogenet Cell Genet.* 1978; 22:661-665.
- Britten R.J., Davidson E.H. Gene Regulation for Higher Cells: A Theory. *Science.* 1969; 165:349-357.
- Brown J.E., Weiss M.C. Activation of Production of Mouse Liver Enzymes in Rat Hepatoma-Mouse Lymphoid Cell Hybrids. *Cell.* 1975; 6:481-495.
- Cox D.W., Hoepfner V.H., Levison H. Protease Inhibitors in Patients with Chronic Obstructive Pulmonary Disease: The Alpha-1-Antitrypsin Heterozygote Controversy. *Am Rev Res Dis.* 1976; 113:601-606.
- Cox D.W., Johnson A.M., Fagerhol M.K. Report on Nomenclature Meeting for α_1 -Antitrypsin. *Hum Genet.* 1980; 53:429-433.
- Cox D.W., Huber O. Association of Severe Rheumatoid Arthritis with Heterozygosity for α_1 -Antitrypsin Deficiency. *Clin Genet.* 1980; 17:153-160.
- Cox D.W., Markovic V.D., Teshima I.E. Genes for the Immunoglobulin Heavy Chain and for α_1 -Antitrypsin are Localized to Specific Regions of Chromosome 14q. *Nature.* 1982; 297:428-430.
- Croce C.M., Knowles B.B., Koprowski H. Preferential Retention of the Human Chromosome C-7 in Human (Thymidine Kinase Deficient) Mouse Hybrid Cells. *Exptl Cell Res.* 1973; 82:457-461.
- Croce C.M., Shander M., Martinis J., Cicurel L., Dolly T.W., Koprowski H. Chromosomal Location of the Genes for Human Immunoglobulin Heavy Chains. *Proc Natl Acad Sci USA.* 1979; 76:3416-3419.
- Darlington G.J., Bernhard H.P., Ruddle F.H. Human Serum Albumin Phenotype Activation in Mouse Hepatoma-Human Leukocyte Cell Hybrids. *Science.* 1974; 185:859-862.

- Darlington G.J., Astrin K.H., Muirhead S.P., Desnick R.J., Smith M. Assignment of Human α_1 -Antitrypsin to Chromosome 14 by Somatic Cell Hybrid Analysis. *Proc Natl Acad Sci USA*. 1982a; 79:870-873.
- Darlington G.J., Bernard H.P., Miller R.A., Ruddle F.H. Expression of Liver Phenotypes in Cultured Mouse Hepatoma Cells. *JNCI*. 1980; 64:809-819.
- Darlington G.J., Rankin J.K., Schlanger G. Expression of Human Hepatic Genes in Somatic Cell Hybrids. *Somatic Cell Genet*. 1982b; 8:403-412.
- Davidson R. Gene Expression in Somatic Cell Hybrids. *A Rev Genet*. 1974; 8:195-217.
- Davidson R., Ephrussi B., Yamamoto K. Regulation of Melanin Synthesis in Mammalian Cells as Studied by Somatic Hybridization. I. Evidence for Negative Control. *J Cell Physiol*. 1968; 72:115-128.
- Davidson R., Ephrussi B., Yamamoto K. Regulation of Pigment Synthesis in Mammalian Cells as Studied by Somatic Cell Hybridization. *Proc Natl Acad Sci USA*. 1966; 60:894-901.
- Davis F.M., Adelberg E.A. Use of Somatic Cell Hybrids for Analysis of the Differential State. *Bacteriol Rev*. 1973; 37:197-214.
- Enders J.F., Peebles T.C. Propagation in Tissue Cultures of Cytopathogenic Agents from Patients with Measles. *Proc Soc Exp Biol Med*. 1954; 86:277-286.
- Engle E., McGee B.J., Harris H. Cytogenetic and Nuclear Studies on Aq and B₉₂ Cells Fused Together by Sendi Virus, The Early Phase. *J Cell Sci*. 1969; 5:93-119.
- Eriksson S., Ragner A., Astedt B. Organ Cultures of Human Fetal Hepatocytes in the Study of Extra and Intracellular α_1 -Antitrypsin. *Biochim Biophys Acta*. 1978; 542:496-505.
- Fagerhol M.K., Cox D.W. The Pi Polymorphism: Genetic, Biochemical and Clinical Aspects of Human α_1 -Antitrypsin. Advances in Human Genetics (Chap 1). Harris H. (Ed). Plenum Press, New York, 1981.
- Francke U., Oliver N. Quantitative Analysis of High Resolution Trypsin-Giemsa Bands on Human Prometaphase Chromosomes. *Hum Genet*. 1978; 45:137-165.
- Gedde-Dahl T., Fagerhol M.K., Cook P.J., Noades J. Autosomal Linkage Between the Gm and Pi Loci in Man. *Ann Hum Genet*. 1972; 35:393-399.
- Gedde-Dahl T., Frants R.R., Olaisen B., Eriksson A.W., Loghern E., Lamm L. The Gm- Pi Linkage Heterogeneity in View of Pi M Subtypes. *Ann Hum Genet*. 1981; 45:143-153.
- Harris H. Cell Fusion. Harvard University Press, Cambridge, Massachusetts, 1970.

- Harris H., Watkins J.F., Ford C.E., Schoefl G.I. Artificial Heterokaryons of Animal Cells from Different Species. *J Cell Sci.* 1966; 1:1-57.
- Harris H., Hopkinson D.A. Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland Pub Co. Amsterdam, 1976.
- Harris H., Watkins J.F. Hybrid Cells Derived from Mouse and Man: Artificial Heterokaryons of Mammalian Cells from Different Species. *Nature* 1965; 205:640-646.
- Henle G., Deinhardt F., Girardia A. Cytologic Effects of Mumps Virus in Tissue Cultures of Epithelial Cells. *Proc Soc Exp Biol Med.* 1954; 87:386-393.
- Hobart M.J., Rabbitts T.H., Goodfellow P.N., Soloman E., Chambers S., Spurr N., Povey S. Immunoglobulin Heavy Chain Genes are Located on Chromosome 14. *Ann Hum Genet.* 1981; 45:331-335.
- Hodges J.R., Millward-Sadler G.H., Barbatis C., Wright R. Heterozygous MZ Alpha-1-Antitrypsin Deficiency in Adults with Chronic Active Hepatitis and Cryptogenic Cirrhosis. *N Engl J Med.* 1981; 304:557-560.
- Jeppsson J.O., Larrsson C., Eriksson S. Characterization of α_1 -Antitrypsin in the Inclusion Bodies of the Liver in α_1 -Antitrypsin Deficiency. *N Engl J Med.* 1975; 293:576-579.
- Johnson R.T., Harris H. DNA Synthesis and Mitosis in Fused Cells. I. HeLa Homokaryons. *J Cell Sci.* 1969; 5:603-624.
- Johnson R.T., Harris H. DNA Synthesis and Mitosis in Fused Cells. II. HeLa-Chick Erythrocyte Heterokaryons. *J Cell Sci.* 1969; 5:625-644.
- Johnson R.T., Harris H. DNA Synthesis and Mitosis in Fused Cells. III. HeLa-Ehrlich Heterokaryons. *J Cell Sci.* 1969; 5:645-698.
- Kahn R.C., Bertolotti R., Ninio M., Weiss M.C. Short-lived Cytoplasmic Regulators of Gene Expression in Cell Cybrids. *Nature.* 1981; 290:717-720.
- Kaighn M.E., Prince A.M. Production of Albumin and Other Serum Proteins by Clonal Cultures of Normal Human Liver. *Proc Natl Acad Sci USA.* 1971; 68:2396-2400.
- Kielty C.M., Povey S., Hopkinson D.A. Regulation of Expression of Liver-Specific Enzymes. *Ann Hum Genet.* 1981; 45:341-356.
- Kirsch I.R., Morton C.C., Nakahara K., Leder P. Human Immunoglobulin Heavy Chain Genes Map to a Region of Translocations in Malignant B Lymphomas. *Science.* 1982; 216:301-303.
- Kucherlapati R.S., Ruddle F.H. Mammalian Somatic Hybrids and Human Gene Mapping. *Ann Int Med.* 1975; 83:553-560.

- Kueppers F. Antigenic Similarity of Human α_1 -Antitrypsin to a Corresponding Protein in the Serum of Non-Human Primates. *Experientia*. 1968; 24:1277-1278.
- Lambert R.A. The Production of Foreign Body Giant Cells in Vitro. *J Exp Med*. 1912; 15:510. (Sited by Harris H., 1970).
- Laurell C.B. Quantitative Estimation of Proteins by Electrophoresis in Agarose Gel Containing Antibodies. *Analyt Biochem*. 1966; 15:45-52.
- Laurell C.B., Eriksson S. The Electrophoretic α_1 -Globulin Pattern of Serum in α_1 -Antitrypsin Deficiency. *Scand J Clin Invest*. 1963; 15:132-140.
- Lewis W.H. The Formation of Giant Cells in Tissue Cultures and their Similarity to those in Tuberculous Lesions. *Am Rev Tuberc Pulm Dis*. 1927; 15:616-628.
- Lipsky J.J., Berninger R.W., Hyman L.R., Talamo R.C. Presence of Alpha-1-Antitrypsin on Mitogen Stimulated Human Lymphocytes. *J Immun*. 1979; 122:24-26.
- Littlefield J.W. The Selection of Hybrid Mouse Fibroblasts. *Cold Spring Harb Quant Biol*. 1964; 29:161-166.
- Littlefield J.W. Selection of Hybrids from Matings of Fibroblasts in Vitro and their Presumed Recombinants. *Science*. 1964; 145:709.
- Littlefield J.W. The Use of Drug Resistant Markers to Study the Hybridization of Mouse Fibroblasts. *Expl Cell Res*. 1966; 41:190-199.
- Malawista S.E., Weiss M.C. Expression of Differentiated Functions in Hepatoma Cell Hybrids: High Frequency of Induction of Mouse Albumin Production in Rat Hepatoma-Mouse Lymphoblast Hybrids. *Proc Natl Acad Sci USA*. 1974; 71:927-931.
- Marston R.Q. Cytopathogenic Effects of Haemadsorption Virus Type I. *Proc Soc Exp Biol Med*. 1958; 98:853-856.
- Matsubara S., Yoshida A., Liberman J. Material Isolated from Normal and Variant Human Liver that Immunologically Cross-reacts with Alpha-1-Antitrypsin. *Proc Natl Acad Sci USA*. 1974; 71:3334-3337.
- Matsuya Y., Green H. Somatic Cell Hybrid Between the Established Human Line D98 (Presumptive HeLa) and 3T3. *Science*. 1969; 163:697-698.
- Meera Khan P. Enzyme Electrophoresis on Cellulose Acetate Gel: Zymogram Patterns in Man-Mouse and Man-Chinese Hamster Somatic Cell Hybrids. *Arch Biochem Biophys*. 1971; 145:470-483.
- Migeon B.R., Miller C.S. Human-Mouse Somatic Cell Hybrids with Single Human Chromosome (Group E), Link with Thymidine Kinase Activity. *Science*. 1968; 162:1005-1009.
- Moroz S.P., Cutz E., Balfe J.W., Sass-Kortsak A. Membranoproliferative Glomerulonephritis in Childhood Cirrhosis Associated with Alpha-1-Antitrypsin Deficiency. *Pediatrics*. 1976; 57:232-238.

- Moroz S.P., Cutz, E., Cox D.W., Sass-Kortsak A. Liver Disease Associated with Alpha-1-Antitrypsin Deficiency in Childhood. *Pediatrics*. 1976; 88:19-25.
- Morse J.O. Alpha-1-Antitrypsin Deficiency. *N Engl J Med*. 1978; 299:1045-1048.
- Nabholz M., Miggiano V., Bodmer W. Genetic Analysis with Human-Mouse Somatic Cell Hybrids. *Nature*. 1969; 223:358-363.
- Noades J.E., Cook P.J. Family Studies with the Gm:Pi Linkage Group. *Human Gene Mapping*. 1976; 3:341-344.
- Norum R.A., Migeon B.R. Non-random Loss of Human Markers from Man-Mouse Somatic Cell Hybrids. *Nature*. 1974; 251:72-74.
- Okada Y. The Fusion of Ehrlich's Tumor Cells Caused by HVJ Virus in Vitro. *Biken's J*. 1958; 1:103-110.
- Okada Y. Analysis of Giant Polynuclear Cell Formation Caused by HVJ from Ehrlich's Ascites Tumor Cells. I. Microscopic Observation of Giant Polynuclear Cell Formation. *Expl Cell Res*. 1962; 26:98-107.
- Okada Y., Tadokoro J. Analysis of Giant Polynuclear Cell Formation Caused by HVJ from Ehrlich's Ascites Tumor Cells. II. Quantitative Analysis of Giant Polynuclear Cell Formation. *Expl Cell Res*. 1962; 26:108-128.
- Ouchterlony O. Antigen-Antibody Reactions in Gels. *Acta Pathol Microbiol Scand*. 1953; 32:231-240.
- Papaconstantinou J., Wong E., Ratrie H., Szpirer C., Szpirer J. Molecular Mechanism of Extinction of Liver Specific Function in Mouse Hepatoma X Rat Fibroblast Hybrids: Extinction of the Albumin Gene. *Somatic Cell Genet*. 1982; 8:363-376.
- Pontecorvo G. Production of Mammalian Somatic Cell Hybrids by Means of Polyethylene Glycol Treatment. *Somatic Cell Genet*. 1975; 1:397-400.
- Rankin J.K., Darlington G.J. Expression of Human Hepatic Genes in Mouse Hepatoma-Human Amniocyte Hybrids. *Somatic Cell Genet*. 1979; 5:1-10.
- Rao P., Johnson R.T. Mammalian Cell Fusion. Studies on the Regulation of DNA Synthesis and Mitosis. *Nature Lond*. 1970; 225:159-164.
- Reardon P.C. Non Random Human Chromosome Segregation in Human-Mouse Myeloma Cell Hybrids. *Conn Med*. 1981; 45:450-451.
- Riccuiti F., Ruddle F.H. Assignment of Nucleoside Phosphorylase to D-14 and Localization of X-Linked Loci in Man by Somatic Cell Genetics. *Nature New Biology*. 1973; 241:180-182.
- Robin C. Sur l'existence de deux especes nouvelles de elements anatomiques qui se trouvent dans le canal medullaire des os. C.r. Seanc Soc Biol. 1849. (Sited by Harris H., 1970).

- Rokitansky C. Lehrbuchde Pathologischen Anatomie. Vol. I. Braumuller Vienna, 1855. (Sited by Harris H., 1970).
- Rushton A.R. Quantitative Analysis of Human Chromosome Segregation in Man-Mouse Somatic Cell Hybrids. *Cytogenet Cell Genet*. 1976; 17:243-253.
- Ruddle F.H., Creagan R.P. Parasexual Approaches to the Genetics of Man. *A Rev Genet*. 1975; 9:407-486.
- Sharp H.L. Alpha-1-Antitrypsin Deficiency. *Hosp Pract*. 1971; 5:83-96.
- Silagi S. Hybridization of a Malignant Melanoma Cell Line with L Cells in Vitro. *Cancer Res*. 1967; 27:1953-1960.
- Smith M., Hirschhorn K. Location of Genes for Human Heavy Chain Immunoglobulin to Chromosome 6. *Proc Natl Acad Sci USA*. 1978; 75:3367-3371.
- Szpirer J., Szpirer C. Control of Serum Protein Synthesis in Hepatoma-Fibroblast Hybrids. *Cell*. 1975; 6:53-60.
- Szpirer J., Szpirer C. Production of Serum Proteins in Normal Diploid Fibroblast-Hepatoma Cell Hybrids and in A-9 Normal Liver Cell Hybrids. *J Cell Sci*. 1979; 35:267-279.
- Tomkins G.M., Gelehrter T.D., Granner D., Martin D., Samuels H.H., Thompson E.B. Control of Specific Gene Expression in Higher Organisms. *Science*. 1969; 166:1474-1480.
- Turner B.M., Turner V.S. Secretion of α_1 -Antitrypsin by an Established Human Hepatoma Cell Line and by Human/Mouse Hybrids. *Somatic Cell Genet*. 1980; 6:1-14.
- Urban J. Isolation and Properties of Rat α_1 -Antitrypsin and Demonstration of a Liver Specific Form. *Biochemistry International*. 1982; 4:177-185.
- Virchow R. Reizung und Reizbarkeit. *Virchow Arch Path Anat Physiol*. 1858; 1:14. (Sited by Harris H., 1970).
- Warthin A.S. Occurrence of Numerous Large Giant Cells in the Tonsils and Pharyngeal Mucosa in the Prodromal Stage of Measles. *Archs Path*. 1931; 11:864-874.
- Weeke B. (Ed). *A Manual of Quantitative Immunoelectrophoresis*. Scandinavian Journal of Immunology (Suppl 1), Universitetsforlaget, Oslo, 1973.
- Weintraub H., Groudine M. Chromosomal Subunits in Active Genes Have an Altered Conformation. *Science*. 1976; 193:848-856.
- Weiss M.C., Green H. Human-Mouse Hybrid Cell Lines Containing Partial Complements of Human Chromosomes and Functioning Human Genes. *Proc Natl Acad Sci USA*. 1967; 58:1104.

Yamanaka T., Okada Y. Cultivation of Fused Cells Resulting From Treatment of Cells with HVJ. *Biken's J.* 1966; 9:159-175.

Yoshida A., Liberman J., Gaidulis L., Ewing C. Molecular Abnormality of Human Alpha-1-Antitrypsin Variant (Pi ZZ) Associated with Plasma Activity Deficiency. *Proc Natl Acad Sci USA.* 1976; 73:1324-1328.

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