Two-dimensional Polyacrylamide Gel Electrophoresis of Serum Proteins from Diploid and Trisomy-21 Individuals.

by

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Two-dimensional polyacrylamide gel electrophoresis of serum proteins from diploid and trisomy-21 individuals

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Abstract

Serum samples from diploid and trisomy-21 adults were compared, using both Laemmli SDS gel electrophoresis and O'Farrell two-dimensional polyacrylamide gel electrophoresis. Neither system distinguished the serum protein complement of the trisomy-21 group from that of the diploid group. With the O'Farrell system, both groups exhibited polymorphisms, especially in the alpha haptoglobin chains. Serum samples from normal newborn babies and from one trisomy-E baby were not distinguishable from one another in either gel system. None of the infant serum samples contained the alpha or beta haptoglobin chains.

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General Introduction: Literature Review

I. Trisomy-21.

The trisomy-21 syndrome was first described clinically in 1846 by Seguin, who called it furfuraceous idiocy. Langdon Down (1866) compiled his observations on the syndrome, referring to it as "Mongoloid idiocy", a term used in medical references well into the 1960's. Most recently, "Down's syndrome" or "trisomy-21" are the preferred terms.

The observed phenotype is well known and well documented. In 1959, Lejeune, Gautier and Turpin presented cytological evidence that the cause of Down's syndrome was the presence of a third copy of chromosome 21. Other trisomies have since been discovered, but trisomy-21 is the most frequent, occurring about once in every 700 live births. A case of a trisomy which resembles the human 21 trisomy has been reported in another primate, the chimpanzee (McClure, Belden and Pieper, 1969). Several authors have investigated the influence of advanced maternal age (Lowry et al., 1976). The possibility of various influences from environmental factors are also under consideration as causes for the

nondisjunction event which leads to trisomy-21 (Mikkelsen, 1977). As well, it is suspected that certain thyroid diseases may predispose a woman to having a Down's child (Fialkow et al., 1971).

Nearly 150 neurological disorders which lead to mental retardation can be traced to a gene mutation which blocks one or more enzymatic steps (Brady, 1976). Although the gene dosage effect of trisomy-21 wouldn't be expected to produce abnormal enzymes, one could anticipate changes in enzyme activity. And in fact the 1.5 level of enzymatic activity expected in trisomy has been shown for an enzyme mapped to chromosome 21, superoxide dismutase (Sinet et al., 1974).

There may be anywhere from 100 to 1000 genes involved in a cytogenetically recognizable disease such as trisomy-21 (Lejeune, 1977). Products of these genes are likely to effect the dynamics of a number of chemical reactions involved in the morphological and biochemical functions which work together to produce a phenotype. The problem of fitting together what is known and deciding what to look at next can be approached from three aspects:

1. The clinical aspect: a careful description of morphological and pharmacological symptoms.

2. The gene map: use of somatic cell hybrid studies to localize genes to chromosome 21.

3. Biochemistry: systematic investigation of shifts in pathways and protein abnormalities.

This investigation concentrated on the third parameter.

II. Gene dosage effects.

The effects of autosomal aneuploidy, where a portion of the genome is either triplicated or occurs in a monosomic condition, are almost always deleterious. In the genome of the fruit fly Drosophila, there are 57 loci, aneuploidy for which leads to a recognizable effect on the organism (Lindsley et al., 1972). In many ways the effects of aneuploidy in man and in Drosophila are analogous. In both organisms the presence of excess DNA is less deleterious than missing a portion of the chromosome complement. Trisomy 21 involves an excess of 1.8% of

the haploid autosomal complement (Penrose, 1964). The largest viable deficiency on the other hand is about 3.0% of the haploid autosomal complement for either organism (Sandler and Hecht, 1973). Both can tolerate about the same fraction of the haploid genome in three doses, both become more phenotypically distorted as the size of the aneuploid region increases, and both exhibit regionally specific phenotypes. Due to less discriminating diagnostic methods, aneuploid phenotypes in Drosophila are not as extensively and specifically described as are these various phenotypes in humans. However, in several cases in Drosophila, enzyme levels have been used to discriminate amongst duplications. Enzymes which have been shown to vary with gene dosage include the sex-linked genes for glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Stewart and Merriam, 1972), and the autosomal genes for lactic dehydrogenase (Stewart and Merriam, 1972) and xanthine dehydrogenase (Grell, 1962).

Gene dosage effect in cases of trisomy in humans has been under investigation since the Lejeune, Gautier and Turpin paper in 1959. The following year an abnormally small acrocentric chromosome, the Philadelphia chromosome, was found in cells of patients

with chronic myelogenous leukemia (Nowell and Hungerford, 1960; Baikie et al., 1960). The Philadelphia chromosome was presumed to represent a partially deleted G group chromosome. Since chromosome 21 is one of the very small chromosomes in the G group, and since levels of alkaline phosphatase activity were often found to be elevated in the leukocytes of patients with Down's syndrome (Alter et al., 1963; King, Gillis and Baikie, 1962; Lennox, White and Campbell, 1962; Trubowitz, Kirman and Masek, 1962) and reduced in the leukocytes of patients with chronic myelogenous leukemia (Valentine and Beck, 1951), the suggestion was made that the genetic locus for alkaline phosphatase might be in the segment of G group acrocentric chromosome which was present in triplicate in Down's patients and only once in patients with the Philadelphia chromosome. However, measurements of alkaline phosphatase activity in fibroblast cultures from skin biopsies of trisomy-21 patients failed to produce statistically significant differences in specific activity of the enzyme (DeMars, 1964; Cox, 1965; Nadler, Inouye and Hsia, 1967). Subsequently it was discovered that the Philadelphia chromosome was the other G group chromosome, chromosome 22.

Both elevated and diminished levels of other enzyme activities were also found in blood samples from patients with Down's syndrome. Table 1 provides a list of these enzymes, the site where activity was measured, and the authors.

Table I

A. Enzymes with a significantly elevated level in blood samples from trisomy-21 individuals.

ENZYME: Galactose-1-phosphate uridyl transferase
SITE: Whole blood and leukocytes
AUTHORS: Brandt et al., 1963
Hsia et al., 1964
Mellman et al., 1964
Ng, Bergren and Donnell, 1964
Rosner et al., 1965
Schuppisser, Joss, and Richterlich, 1967.

ENZYME: Glucose-6-phosphate dehydrogenase
SITE: Erythrocytes and leukocytes
AUTHORS: Mellman et al., 1964
Rosner et al., 1965
Shih et al., 1965
Herring et al., 1967.

ENZYME: Acid phosphatase
SITE: Leukocytes
AUTHORS: Mellman et al., 1964
Rosner et al., 1965.

ENZYME: Galactokinase

SITE: Whole blood

AUTHORS: Krone et al., 1964

Donnell et al., 1965.

ENZYME: Phosphoglucomutase

SITE: Erythrocytes

AUTHORS: Priscu and Sichitiu, 1975.

ENZYME: Phosphohexokinase

SITE: Erythrocytes

AUTHORS: Baikie et al., 1965

Naiman, Oski and Mellman, 1965

Bartels, Kruse and Tolksdorf, 1968

Pantelakis et al., 1970.

ENZYME: 5-Nucleotidase

SITE: Leukocytes

AUTHORS: Rosner et al., 1965.

ENZYME: Cholinesterase

SITE: Red cells

AUTHORS: Beutler et al., 1964.

B. Enzymes with a significantly lowered level in blood samples from trisomy-21 individuals.

ENZYME: Monoamine oxidase
SITE: Platelets
AUTHORS: Benson and Southgate, 1971.

ENZYME: Acid phosphatase
SITE: Cultivated skin fibroblasts
AUTHORS: Tamarkina et al., 1974.

ENZYME: Lactate dehydrogenase
SITE: Cultivated skin fibroblasts
AUTHORS: Tamarkina et al., 1974.

ENZYME: Glutamine-oxaloacetate transaminase
SITE: Cultivated skin fibroblasts
AUTHORS: Tamarkina et al., 1974.

The questions which arose from these reports remained: were these elevated levels of specific enzymes due to a gene dosage effect, and could any of these enzymes be used as an indication or marker for chromosome 21? It seemed that increased activity of a certain enzyme in a trisomic individual could not be interpreted in terms of gene location and triple-gene-dose (Stalder et al., 1965). There was too much overlap between normal controls and Down's patients.

In fact, elevated levels of alkaline phosphatase might reflect a generalized disturbance of the leukocytes, which are morphologically atypical in Down's syndrome, rather than a gene dosage effect. This arguement was supported by the observations that the Down's erythrocytes exhibited other generalized abnormalities which were unlikely to relate directly to a triple gene dose effect (Baikie et al., 1965). As well, increased acid phosphatase activity and increased alkaline phosphatase activity were found in other chromosomal aberrations besides trisomy-21 (Stalder et al., 1965; Weber, Mittwoch and Delhanty, 1965), leading to the conclusion that the increased activity of red cell enzymes could only be interpreted as a reflection

of generalized and nonspecific disturbance in cell metabolism (Bender, Ritter and Wolf, 1967). Attempts to correlate blood group phenotype and Down's syndrome have also shown no significant association (Evans et al., 1966; Goodman and Thomas, 1966).

Enzyme levels need not correlate with a particular chromosome dosage. Several investigators have theorized on potential causes for error when attempting to relate enzyme levels and gene dosage effects. For one, it has been suggested that at least some enzymes are multimers, constructed from several different polypeptide chains, and therefore under the control of more than one structural gene locus (Sparkes and Baughan, 1969). When this is the case, a triple dose of the genes for one structural element in the completed enzyme might not appear as a simple gene dose effect.

The effect of a change in gene numbers might also be modified by the regulatory genes controlling production of the structural genes. Although regulatory genes have not as yet been documented in humans, inhibition of enzyme activity resulting from an extra dose of regulator gene has been demonstrated in

microbial systems (Sadler and Novick, 1965). There is the possibility that the extra G group chromosome might contain the regulatory genes for certain enzymes or groups of enzymes (Mellman et al., 1964; Rosner et al., 1965).

Enzyme activities which are substrate inducible might not be effected by gene dosage; similarly, enzymes under the control of inhibitors or activators of enzyme activity, or controlled by enhancers of enzyme synthesis, might not reflect the trisomic condition (Benson, 1967). Where the presence of a specific protein is diagnostically lower in trisomy-21 individuals, either a diminished rate of synthesis or an accelerated breakdown could be responsible (Benson and Southgate, 1971). Either pathway could be affected by overproduction of an enzyme coded on chromosome 21.

There are other aspects which also may interfere with the results of quantitative enzyme assays and the conclusions which may validly be drawn from these assays with regard to gene dosage effect. Modified enzyme activity can be connected with cell age in the trisomic individual (Hook and Engel, 1964; Raab et al., 1966). There may be effects from multiple alleles or

familial modifying factors, which would show up in population studies but only be correctly interpreted through family studies (Herring et al., 1967; Phillips et al., 1967). Even the patient's age can be a modifying factor on certain enzyme levels (Alter et al., 1963).

III. Proteins coded on chromosome 21.

There are proteins which have been definitely mapped to human chromosome 21. Mouse-human somatic cell hybrids have been used to provide evidence that genes for the soluble form of superoxide dismutase (SOD-1) and for the antiviral protein (AVP) induced by human interferon are both on chromosome 21 (Tan, Tishfield and Ruddle, 1973; Sichitiu et al., 1974). Somatic cell hybrids have also provided evidence that glycinamide ribonucleotide synthetase (GARS) can also be assigned to chromosome 21 (Moore et al., 1977). Both 18S and 28S ribosomal RNA genes have been located in the short arm of chromosome 21, as well as in the short arms of chromosomes 13, 14, 15 and 22 (Henderson, Warburton and Atwood, 1972).

Superoxide dismutase-1 is an enzyme which catalyses the dismutation of superoxide radicals to oxygen and hydrogen peroxide (McCord, Keele and Fridovich, 1971). The superoxide radical is produced in the oxidation reactions of flavines, quinones, flavoproteins, and iron-sulfur proteins, and is generated during phagocytosis of bacteria by human granulocytes (Kitani, Hori and Kokubun, 1977). SOD-1 is a cytoplasmic enzyme containing copper and zinc and present in all human cells (Beckman, Lundgren and Tarnvik, 1973; Salin and McCord, 1974). An increase of SOD-1 activity in trisomy 21 has been found in erythrocytes (Sinet et al., 1974; Frants et al., 1975) and in blood platelets (Sinet et al., 1975a). The ratio of the average values of trisomy-21 SOD-1 to diploid SOD-1 is 1.56 in both blood platelets and erythrocytes. This could be due to a direct gene-dosage effect (Sinet et al., 1975a).

There is another type of superoxide dismutase, SOD-2; a mitochondrial enzyme containing manganese (Beckman, Lundgren and Tarnvik, 1973; Salin and McCord, 1974). Although SOD-2 has been mapped to chromosome 6, its activity has been found to be significantly decreased in platelets from Down's individuals (Sinet

et al., 1975a). Whatever the mechanism causing this decrease may be, the salient point is that SOD-2 could also be used as an indicator which would differentiate the trisomy 21 protein complement from that of a normal diploid individual.

Another enzyme which shows increased activity in erythrocytes from Down's patients is glutathione peroxidase, or GPX. This enzyme occurs in the pathway with superoxide dismutase-1, acting to catalyse the reduction of the hydrogen peroxide to water (Cohen and Hochstein, 1963). Levels of this enzyme show a 1.55 ratio when populations of trisomy 21 and normal subjects are compared (Sinet et al., 1975b). This is very close to the ratio observed in the SOD-1 measurements, and may imply that the genes for the expression of both of these proteins are on chromosome 21 (Sinet et al., 1975b).

The expression of the interferon induced antiviral state is regulated by the antiviral protein (AVP). Measurements of gene dosage in normal and trisomic skin fibroblast cultures were used to confirm the assignment of the AVP gene to chromosome 21 (Tan et al., 1974). AVP is presumed to be the protein factor that initiates

specific inhibition of virus replication. It may be that there are other genes also concerned with the regulation of the expression of the antiviral state located on chromosome 21, since both SV40 virus-induced cell transformation and an increased incidence of all types of leukemia involve the G-group chromosomes (Tan et al., 1974). Increased levels of proteins of this nature might be detectable in the trisomy-21 protein complement.

IV. Two-dimensional gel electrophoresis.

The first use of electrophoresis to resolve the complex protein mixture found in human serum was by Tiselius (1937), who succeeded in resolving serum globulin into three components which he labeled alpha, beta and gamma. Raymond and Weintraub (1959) next described a method for the use of acrylamide gel as a support matrix. The two organic monomers, acrylamide and N,N'-methylenebisacrylamide, when properly catalysed, formed a polyacrylamide gel which was optically clear and colourless, elastic, chemically stable, and which could be dried and preserved indefinitely. Raymond and Weintraub used this gel to

resolve several of the serum globulin zones into subcomponents, and found that it improved the resolution of hemoglobin and serum albumin patterns as well.

Improved resolution of human serum proteins was achieved by Ornstein (1964) and Davis (1964), who developed a technique which used controlled variation of the acrylamide gel pore size plus a preliminary electrophoretic step to concentrate the sample ions into a narrow starting zone. They called the technique disc electrophoresis, since the method utilized discontinuities in the electrophoretic matrix and the separated zones of protein had a discoid shape. Their method resolved from twenty to thirty fractions from human serum, in contrast to the five or six seen on other supporting media (Peacock, Bunting and Queen, 1965).

Ornstein and Davis identified albumin, a pre-albumin, from 4 to 8 post-albumins, transferrin, free hemoglobin A, the beta 1-lipoprotein, the alpha 2 glycoprotein, the 7-S gamma globulins, and a variable number of faster and slower haptoglobins. As well, there were 5 or 10 unidentified protein discs. They

attributed most of the variation in numbers of proteins to genetic variation in haptoglobin proteins among individuals. A survey of approximately 200 normal human sera revealed other pattern variations, indicating that the use of acrylamide gel yielded at least twelve discrete groups of electrophoretic patterns (Peacock, Bunting and Queen, 1965).

Shapiro, Vinuela and Maizel (1967) modified the polyacrylamide gel system by using sodium dodecyl sulfate (SDS) to break proteins into their individual polypeptide chains. Laemmli (1970) then worked with Maizel to combine the high resolution power of disc electrophoresis with SDS. This system, known as Laemmli gel electrophoresis, gave more and clearer banding patterns than any previous electrophoretic technique. The anionic detergent SDS coated each polypeptide chain of a protein so that total charge was directly related to molecular weight. Thus, the distance of migration of a protein relative to the distance of migration of the bromophenol blue dye front was found to be a linear function of the logarithm of the molecular weight of that protein, within 10% (Weber and Osborn, 1969).

Additional resolution was then achieved by running the serum sample in two dimensions. A technique for separating serum proteins according to their iso-electric focusing point was used as a first dimension, followed by electrophoresis in SDS polyacrylamide gel. Technical variations on this theme led to greater resolution. By 1968, Dale and Latner were able to resolve as many as 60 serum protein spots, and to identify transferrin, albumin, the haptoglobins and two diffuse arcs assumed to be immunoglobulin G and immunoglobulin A. Margolis and Kenrick (1969) used tubes of polyacrylamide to separate plasma proteins according to their mobility, and then transferred each tube to the surface of a polyacrylamide slab gel, where the proteins were separated according to size. They were able to resolve and identify transferrin, albumin, immunoglobulins M, G and A, a beta-lipoprotein, a macroglobulin, fibrinogen, ceruloplasmin, haemopexin, and a series of horizontal bars which they identified as haptoglobin-haemoglobin complex. Further modification led to suggestions for use of the procedure for studies of pathological sera in diseases affecting the immunoglobulins (Emes, Latner and Martin, 1975), for the analysis of lipoprotein patterns in the lipoproteinaemias (Emes et al., 1976), and for investigations into haptoglobin-haemoglobin interactions (Emes, Latner and Martin, 1975).

Introduction

Studies on the biochemistry of trisomy-21 have revealed a number of accelerated steps in the glycolytic pathway, both increased and diminished levels of certain amino acids in serum, and changes in tryptophan metabolism (Lejeune, 1977). It seemed likely that one or several of these might involve protein changes which could be detected using polyacrylamide gel electrophoresis, a relatively simple and rapid means for obtaining a phenotype of a serum or amniotic fluid sample.

The O'Farrell technique was first presented in 1975. Modifications of this procedure were published in late 1977. A preprint of that article was used as the format for the gels run in this investigation. This study represents the first time the O'Farrell technique has been applied to human serum proteins. It is also the first time that differences in the protein complements of diploids and trisomics were sought using two-dimensional gels.

O'Farrell's high-resolution, two-dimensional electrophoresis technique provided a system which could

resolve complex groups of proteins to a degree which was a vast improvement over the previous systems. When unlabeled proteins were used, as in this investigation, the sensitivity of staining was such that the smallest amount of protein detectable as a compact spot was on the order of a few hundreths of a microgram. For a heavily loaded gel, a spot constituting as little as 0.1% of the total protein in the sample could be detected by staining with Coomassie Blue. About 300 spots are visible in the human plasma proteins pattern, representing thirty identified polypeptides (Anderson and Anderson, 1977).

With O'Farrell gels, a serum sample is separated in two directions, first by isoelectric focusing on an ampholine gradient in a combination of 9M urea and 2% Nonidet P-40, and then by molecular sieving in an SDS-containing poylacrylamide gradient gel. The advantage of this method is that it deals almost exclusively with individual gene products, a fact which led Anderson and Anderson (1977) to use the O'Farrell technique in a program aimed at cataloging human gene products. Characterization of some known genetic polymorphisms has already begun; the O'Farrell system is seen as a powerful tool for human genetic

analysis. Two facts, that there are measurable gene dosage effects from trisomy-21 in humans, and that the O'Farrell technique can reveal individual gene products, led us to investigate the potential of this method in differentiating sera obtained from diploid and trisomy 21 individuals.

We anticipated that areas where the trisomy-21 differed from the diploid would be most clearly seen in the serum proteins from confirmed adults. Once this area was delineated, we hoped to use the technique for screening amniotic fluid samples from mothers at risk for bearing a trisomic child. The possibility of using O'Farrell's technique to screen for proteins in blood or even urine samples from pregnant mothers was another area of potential diagnostic usefulness.

Materials and Methods

I. Samples

Whole blood from diploid individuals was obtained from volunteers in the biosciences department at Simon Fraser University. The samples were collected over the course of one afternoon, at the Simon Fraser Health Services. The clotted samples were spun down, the serum removed, divided into aliquots and placed at -4 C until required. Periodically, a fresh serum sample was run against a frozen aliquot taken from the same individual. Over a period of several months, no protein breakdown in the frozen samples could be detected in the gels.

Whole blood was obtained from individuals with a confirmed trisomy-21 karyotype. In the course of routine blood sampling, ordered by the physician, a 5ml tube of whole blood was set aside for this study. The clotted sample was spun down, the serum removed and kept refrigerated until it could be picked up, usually the same afternoon. Serum was transferred to the laboratory on ice, where it was respun, divided into aliquots and frozen at -4 C until required.

Samples were quickly thawed under hot tap water. Each 100 microliter aliquot of serum was added to 200 microliters of sample buffer, described below, and mixed. Then 75 microliters of the mixture was drawn up and loaded onto the appropriate focusing gel. This quantity was used because it gave the greatest resolution of proteins present in small amounts with the least interference from smearing of the large albumin fraction.

II. Laemmli gel electrophoresis

Laemmli gels were our first choice from the range of types of polyacrylamide gels available. The concentration of both the acrylamide monomer and the bisacrylamide could be varied to produce gels of different pore size. Keeping the bisacrylamide constant and using acrylamide concentrations of 5%, 7.5%, 10% and 15%, as well as 10-16% gradient gels, allowed us to determine empirically the concentration which would give the best resolution of human serum proteins. Sample loading concentration was also determined empirically, to arrive at a quantity which revealed the greatest number of faint bands without undue smearing of the huge albumin fraction. An

advantage of Laemmli's technique was incorporation of the running buffer mixture directly into the gel mixture, so that the gels needed no soaking or other priming prior to electrophoresis. Using TEMED and ammonium persulfate provided a reliable and convenient catalyst. iso-butyl alcohol at the gel surface gave a smooth, straight interface, as well as a rapid means of protecting the gel solution from contact with atmospheric oxygen, an inhibitor of polymerization. The sodium dodecyl sulfate gave a means of determining the range of subunits and their molecular weights in the serum sample for subunits from weight 15,500 to 165,000 (Shapiro, Vinuela and Maizel, 1967). SDS minimized the native charge differences so that all proteins migrated as anions. Both the SDS and beta-mercaptoethanol acted to disrupt hydrogen, hydrophobic and disulfide linkages, helping to solubilize otherwise insoluble protein components.

This technique was not, however, sensitive enough to pick up consistent differences between diploid and trisomy-21 serum samples. We then decided to use the O'Farrell technique, introduced in 1975 and modified in 1977. In 1975 the technique had never been used for separating human proteins.

III. The first dimension.

Chemicals, stocks and solutions used in the first and second dimension are listed in the Appendix. The first dimension apparatus consisted of a series of glass tubes with an internal diameter of 3.0mm. The tubes were cut to a length of 13 cm. and the sharp edges flamed. A small mark was made 5mm down from the top end, for ease in pouring gels to a uniform length. Before use, the tubes were cleaned well by soaking in a 1% chromic acid solution. Tubes were also stored in the chromic acid solution when not in use. When preparing tubes for use in a run, each was carefully rinsed in tap water, then in distilled water, and then immersed to soak in a saturated solution of potassium hydroxide in ethanol for at least ten minutes. The tubes were then thoroughly rinsed in running tap water, rinsed twice with distilled water, and air dried, using a length of rubber tubing attached to an air outlet to blow dry the interior. To seal the lower end of each tube, an approximately 2 x 5cm strip of Parafilm was cut, the middle stretched over the opening, and the ends carefully wrapped around and around the tube. Tubes were then placed vertically in a twelve-tube stand manufactured by Buchler Instruments.

A loading syringe, constructed from a 20ml plastic syringe with a piece of thin plastic tubing attached to the end, was then filled with the mixture. To avoid bubbles when pouring the gels, the liquid was brought right to the end of the plastic tubing, the tubing was inserted to the bottom of the glass tube and withdrawn slowly as the gel filled the tube. All tubes were filled as uniformly as possible to the 5mm mark, overlayered with water, and allowed to sit for one to two hours. All liquid above each gel was then carefully removed with a syringe and 25 microliters of lysis buffer layered onto each gel surface. This was overlayered with water, to prevent the urea from crystallizing out. The gels were allowed to equilibrate with the lysis buffer for one hour, while the upper and lower reservoir buffers were prepared.

After the equilibration hour had passed, the Parafilm was removed from the gel tubes, using a razor blade to scrape the sides of the tubes clean. Each tube was then inserted into a rubber stopper in a standard tube gel electrophoresis chamber designed to hold twelve tubes. A small amount of upper reservoir buffer was added to the upper reservoir and

allowed to stand for several minutes, to check for leaks. The lower reservoir was then completely filled with lower reservoir buffer and the upper reservoir, with gels in place, attached. Each lower gel surface was checked for air bubbles; a bent Pasteur pipette with lower reservoir buffer was used to eliminate these. The liquid was aspirated from each gel surface, and replaced with 25 microliters of fresh lysis buffer, overlayered to the top of the glass tube with upper reservoir buffer. The upper reservoir was then carefully filled with buffer and covered.

The upper base reservoir was connected to the anode and the lower acid reservoir was connected to the cathode. The gels were prerun as follows:

15 minutes at 200 volts

30 minutes at 300 volts

30 minutes at 400 volts

After the prerun, the power supply was disconnected, the upper reservoir buffer siphoned out, and the liquid above each gel surface aspirated.

The samples were then prepared, applied to the appropriate gel surface and overlayered with 10 microliters of overlay buffer. The tube which was later to determine the pH gradient was simply aspirated to the gel surface and topped up with upper reservoir buffer. All tubes were then topped with upper reservoir buffer and the upper reservoir filled with fresh buffer. The power supply was reconnected and the focusing continued overnight as follows:

18 hours at 300 volts

1 hour at 800 volts

Following the run, the gels were removed from the tubes, using a 20ml syringe with a short piece of tubing glued to the end. The tubing was secured over one end of the glass tube and the gel slowly exruded by air pressure from the syringe. Each gel was extruded into a labelled, screw-capped 15ml glass tube containing 5ml of SDS sample buffer.

The gels were equilibrated in the SDS sample buffer for one hour at room temperature and then stored at -4 C. Tube gels which were to be run immediately in the second dimension were put into a fresh 5ml aliquot of SDS sample buffer after one hour, allowed to

equilibrate for a further hour and then added directly to the second dimension.

The iso-electric focusing gel run without sample was used to calibrate the pH gradient. The gel was extruded onto Parafilm which had been cut as a piece slightly longer than the length of the tube and creased down the middle. The gel was aligned with the crease, measured and cut into 1cm pieces. Each piece was placed in a labeled 10ml beaker containing 5ml of double distilled water, pH 8.1. The sections were allowed to equilibrate for two or more hours and the pH determined. This step assured that the pH gradient was relatively constant and reproducible from one first dimension run to the next.

When a frozen gel was selected for use, it was thawed quickly under running tap water and 5ml of fresh SDS sample buffer was added. The gel was equilibrated at room temperature for one hour before it was applied to the second dimension.

IV. The second dimension.

The apparatus for the second-dimension slab gel was constructed as described by Studier (1973), with modifications as noted by O'Farrell (1975). The glass plates were assembled using 15 x 150 x 3mm teflon bars as spacers along the side and bottom edges. Vaseline petrolatum jelly was spread liberally along both sides of each spacer bar to ensure a waterproof seal. The glass plates were then clamped together with 2-inch fold-back clamps along each side and 1-inch screw clamps at each bottom corner. A 10-16% concave exponential gradient gel was poured the morning before a second dimension run, and allowed to equilibrate for at least twenty hours.

To prepare the gel, a light solution and a heavy solution were made up, each in a 125ml sidearm flask. Each solution was made up adding all ingredients but the TEMED and degassed for several minutes. TEMED was then added. All 32ml of the light solution was loaded into the reservoir chamber of a gradient maker; 10ml of the heavy solution was pipetted into the mixing chamber. The gradient maker was attached to a stand at a height sufficient to allow for free gravity pouring

of the solution between the glass plates. A stir-bar was added to the mixing chamber. This chamber was sealed with a rubber stopper, the pressure equilibrated by inserting a 20 gague needle, the needle removed, and the stopcock separating the two chambers opened. The two feed tubes were taped to the back plate and the gradient poured to a mark one inch below the base of the notch. A small amount of isobutanol was layered over the gel surface to create a flat interface, and to facilitate the setting of the polyacrylamide. After one or two hours, liquid was aspirated from the gel surface as much as possible without tilting or disturbing the gel, and a solution of three parts distilled water to one part 1.5 M Tris, pH 8.8 was added. The gradient gel was then left to stand from 20 to 24 hours.

The following morning, the 5% spacer gel was prepared. The buffer mixture covering the gradient gel was removed with a Pasteur pipette, tilting the gel slightly to one edge. The gel surface was rinsed three times with distilled water. The spacer gel was poured on top of the gradient gel by tilting the plates at an angle and allowing the liquid to run down the back plate. The plates were filled to the base of the notch

and set upright. A chilled teflon strip was inserted 2mm below the edge of the notch, moving from left to right to avoid trapping air bubbles. The spacer gel was allowed to set while the first dimension tube gel was thawed and equilibrated.

Shortly before the equilibration time was up, an aliquot of agarose in SDS sample buffer was melted in a boiling water bath. The first dimension gel was then poured from the tube into a crease made down the center of a sheet of Parafilm. All excess liquid was removed from around the gel, to remove any proteins which had diffused from the gel into the buffer since these would run as a smear in the second dimension. The teflon bar was removed, the surface of the spacer gel rinsed twice with distilled water, the plated leaned at a 45 degree angle, and sufficient melted agarose solution pipetted into the groove to form an angled surface between the top of the notched plate and the back plate. The tube gel was immediately eased onto the liquid surface, using a spatula to start one end of the tube at the left edge of the notch and allowing the rest of the tube to slide off steadily, taking care not to stretch or compress the tube or trap bubbles between the tube and the agarose liquid. The agarose was allowed to set for five minutes.

The teflon spacer along the bottom edge was removed and excess petrolatum jelly removed, using one end of the spacer bar. A Layer of petrolatum jelly was spread on the verticle surface of the electrophoresis tank and the gel clamped on, using a bulldog clamp along each side. A 5ml syringe was used to distribute a thin line of melted petrolatum jelly along the crack between the glass plate and the upper reservoir of the electrophoresis tank, to safeguard against leaks. The upper reservoir was then filled with sufficient running buffer to cover the gel, and two or three drops of 0.1% Bromophenol blue added. The lower reservoir was filled with running gel buffer; air bubbles trapped at the lower gel surface were forced out with buffer, using a Pasteur pipette with a u-shaped tip.

Electrophoresis was run at 20 volts until the Bromophenol blue dye front was through the 5% spacer gel. Voltage was then increased to 40 volts and the gel run overnight for approximately 22 hours, or until the dye front was within one centimeter of the bottom edge.

V. Staining and drying.

When the run was completed, the gel was removed from the glass plates. The lateral spacer bars were pulled out, and a spatula used to pry apart the glass plates from one corner. The top plate was removed and the bottom plate, with the gel still adhering, flipped over into a glass dish containing destain solution. After a 20 minute equilibration, to adjust pH, the destain was poured off and the Coomassie Brilliant blue staining solution added. The gel was gently shaken for two hours in this solution, to assure even dye distribution. The stain was poured off, and excess stain rinsed from the gel surface and the glass dish with warm tap water. The gel was destained overnight, with shaking, in several changes of destain solution.

Drying was carried out on a Bio-Rad drying apparatus. Gels were dried two at a time, using four thicknesses of Whatmann #1 filter paper backed with one sheet of Whatmann #25 filter paper. Gels were stored under books, as they tend to curl.

Results and Discussion

I. Gel Parameters.

With a protein mixture, the relative amounts of the component proteins vary considerably. In general, the less protein loaded on a gel the better the resolution. Since albumin makes up a large fraction of the total serum proteins, the amount of serum which could be loaded to give the greatest resolution of minor species without obliterating species running near the albumin component was determined empirically. The effect of overloading the albumin fraction was to shift minor spots close to the major albumin spot rather than engulf them. Thus a comparison of patterns from one gel to another must be made using the relative positions of the spots with regard to marker neighbours in the overall pattern. The acid glycoprotein spot appeared in most gels as the most acidic group resolved, with a pH around 4.5. The basic end of the pH gradient did not extend significantly above pH 7.

Since the method had sufficient resolving power to detect a single charge change in a polypeptide chain, storage conditions were monitored and a periodic

control was run to find if artifactual spots were generated. Specimens were frozen within a few hours of collection and used shortly after quick-thawing, to minimize artifactual charge heterogeneity. Equilibration of the first dimension gel for one hour prior to freezing and for one hour in fresh SDS sample buffer after quick-thawing and prior to loading onto the second dimension resulted in no appreciable loss of protein. Control gels were run without equilibration, directly from the first dimension into the second. In these, there was slightly less diffusion of individual spots, but no apparent losses.

II. Identification of proteins.

Anderson and Anderson (1977) have recently reported the identification of thirty plasma proteins in the two-dimensional pattern. Their identities were established either by running pure preparations or by running immunoprecipitates, made by reacting specific antisera with whole serum. Many of the areas identified in the plasma protein work can be related to similar areas in the serum proteins gels run in this study.

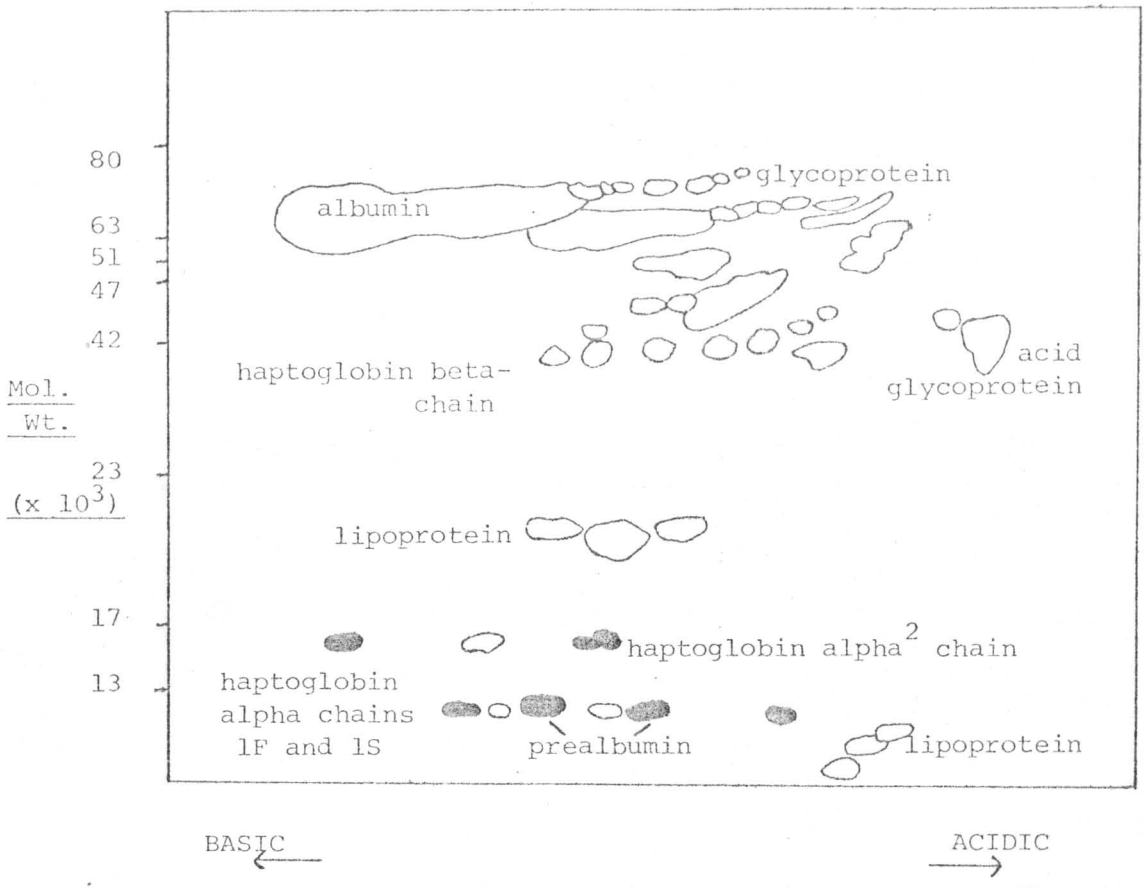
A comparison of serum protein patterns from twelve normal diploid adults and twelve trisomy-21 individuals revealed no consistent area where a difference which might be of diagnostic value occurred. Figures 1 through 14 present photographs of the gels run on two of the trisomy-21 males, three of the trisomy-21 females, five of the normal females and four of the normal males. Above each gel photograph is a simplified diagram of a two-dimensional gel with several of the more obvious proteins labeled. Electrophoretic differences between individuals were seen in areas identified by Anderson and Anderson (1977) as the haptoglobin 2 alpha-chain, and the haptoglobin 1F and 1S alpha-chains, as well as prealbumin. One other unidentified protein spot was seen to vary. The combination of protein spots which appear on any one gel are shaded in the diagram. For quick reference, Table 2, listing all individuals and their status, is also presented.

Table 2

List of examples illustrated indicating presence (+) or absence (-) of spots referred to in text.

(T = trisomy, M = male, F = female, NB = newborn)

Person	Alpha-2 chain				Prealbumins		Alpha-1F & -1S chains		
	1	2	3	4	1	2	1	2	3
TM 1	+	-	+	+	+	+	—	—	—
TM 1	+	+	+	+	+	+	-	+	-
TF 1	+	+	+	+	+	+	-	+	+
TF 2	-	+	+	+	+	-	-	-	+
TF 3	-	-	-	+	+	+	+	-	+
NF 1	+	+	-	+	+	-	-	+	-
NF 2	+	+	+	+	+	+	-	-	-
NF 3	-	-	-	+	+	+	+	-	+
NF 4	+	+	+	+	+	+	-	-	-
NF 5	+	+	+	+	+	-	-	+	-
NM 1	+	+	+	+	+	+	-	-	-
NM 2	+	+	+	+	+	-	-	-	+
NM 3	+	+	+	+	+	+	-	-	-
NM 4	-	-	+	+	-	+	+	-	+
NB 1	-	-	-	+	+	-	-	-	-
NB 2	-	-	-	+	+	-	-	-	-
NB 3	-	-	-	+	+	-	-	-	-
TE	-	-	-	+	+	-	-	-	-



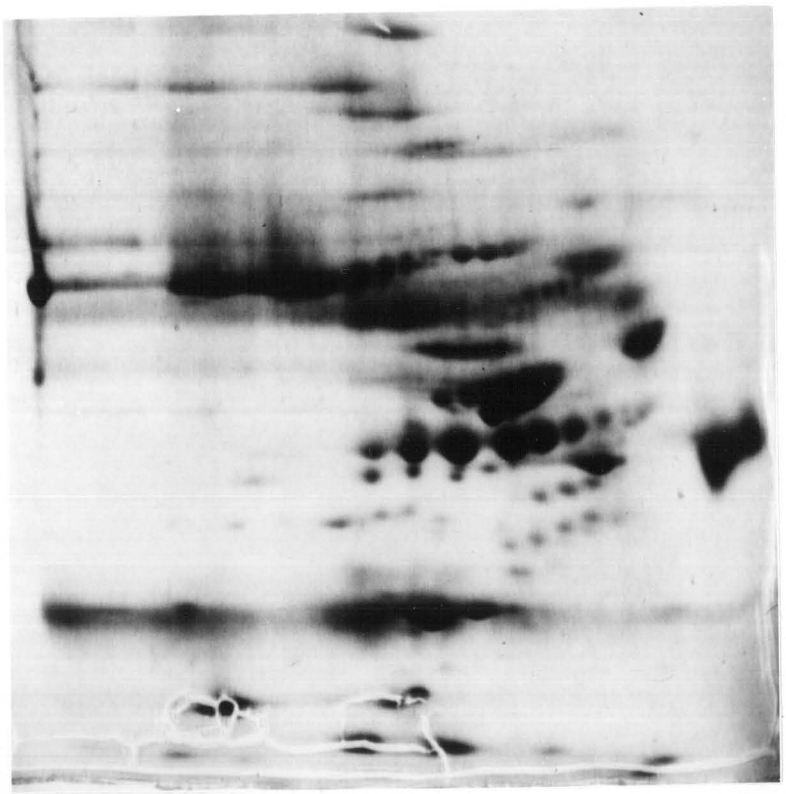
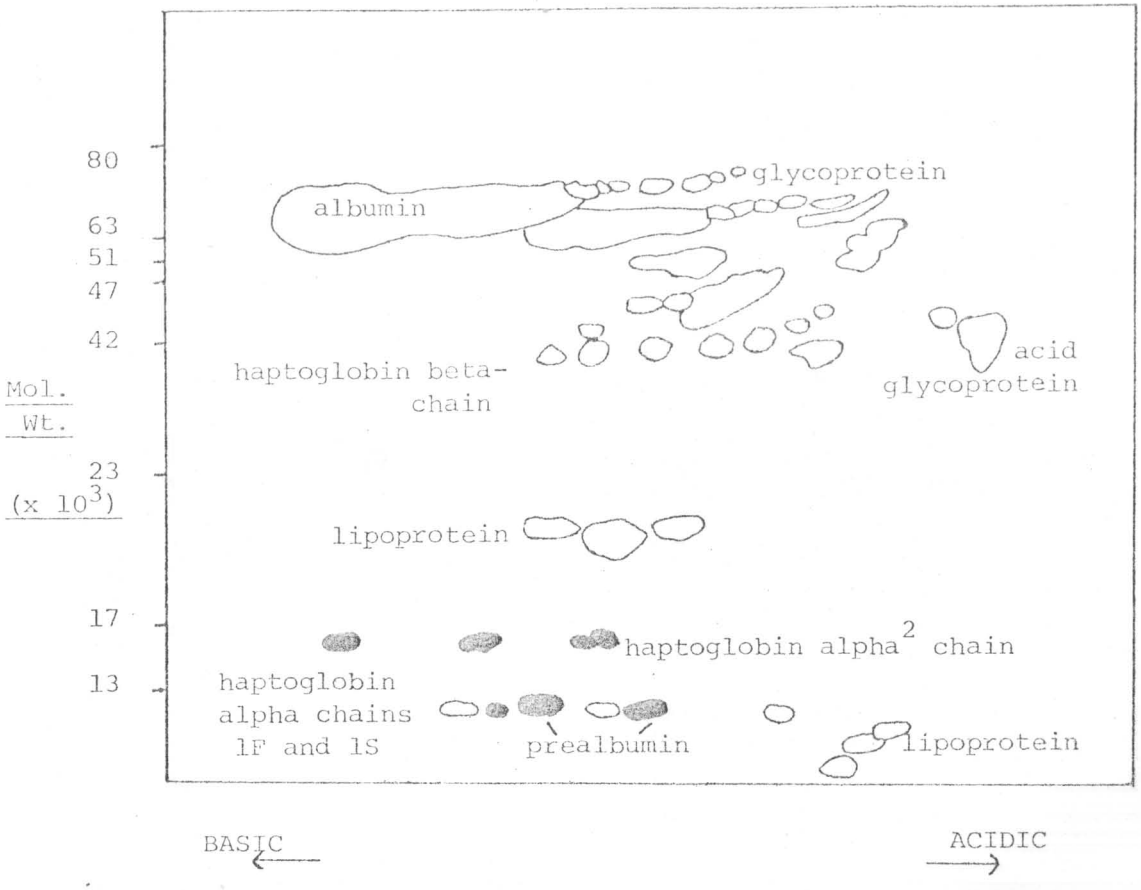


Figure 1

Trisomy
Male 1



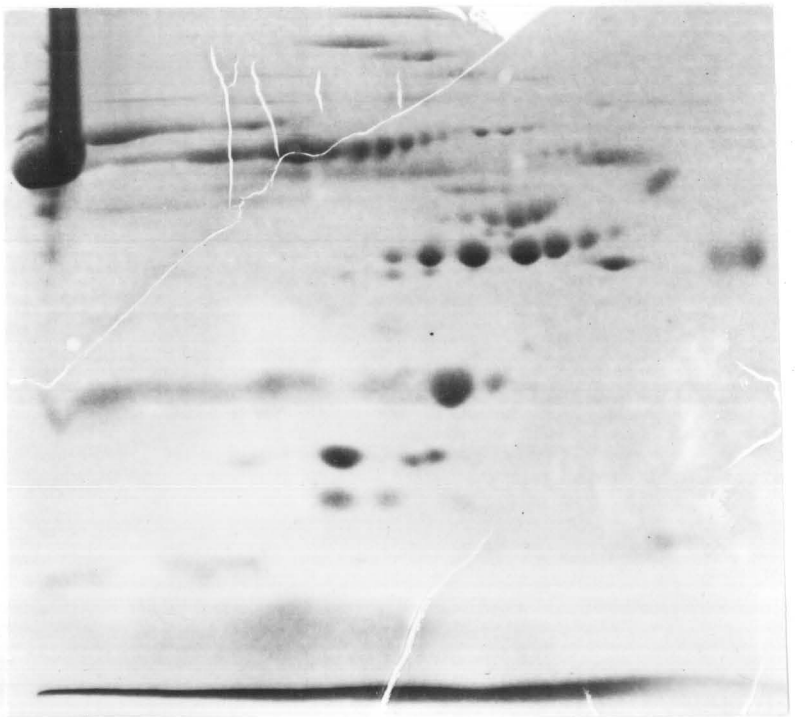


Figure 2

Trisomy
Male 2

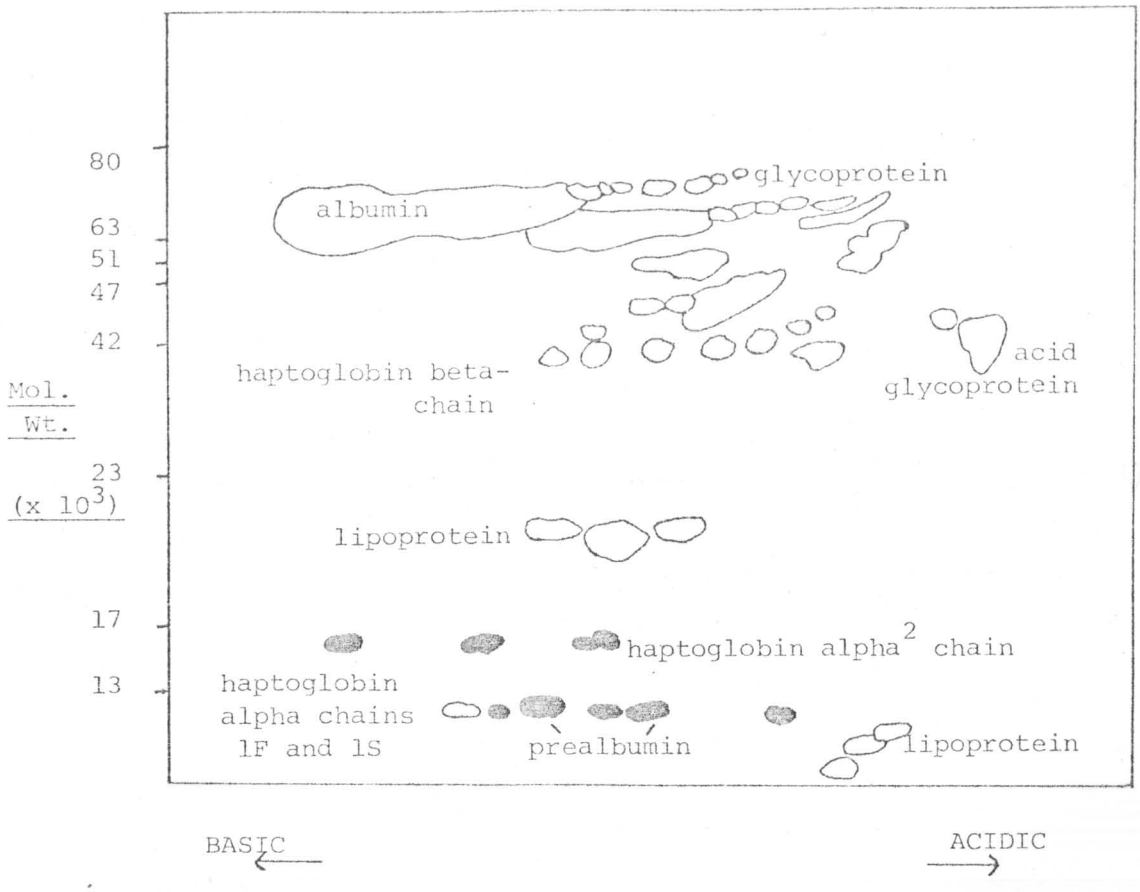
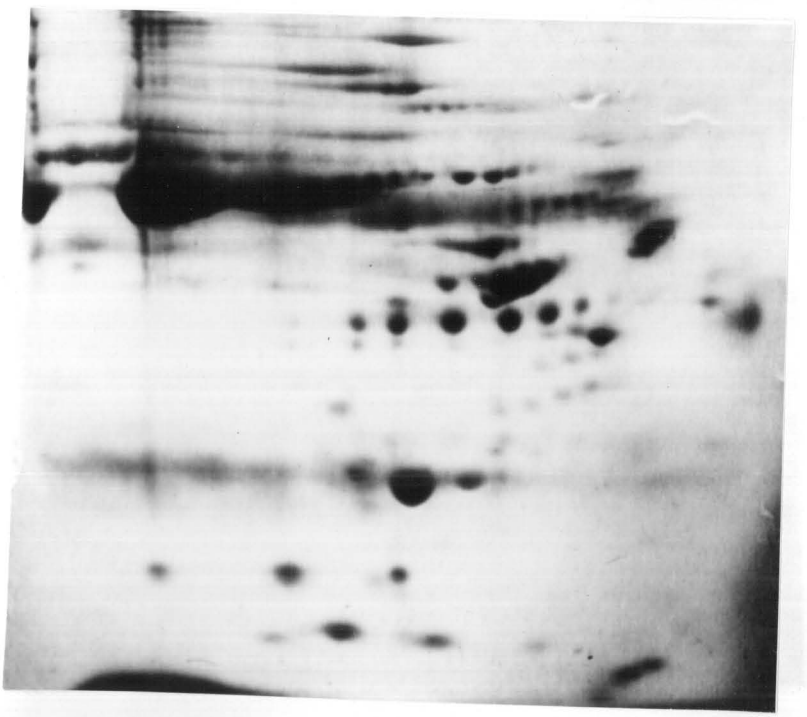


Figure 3

Trisomy
Female 1



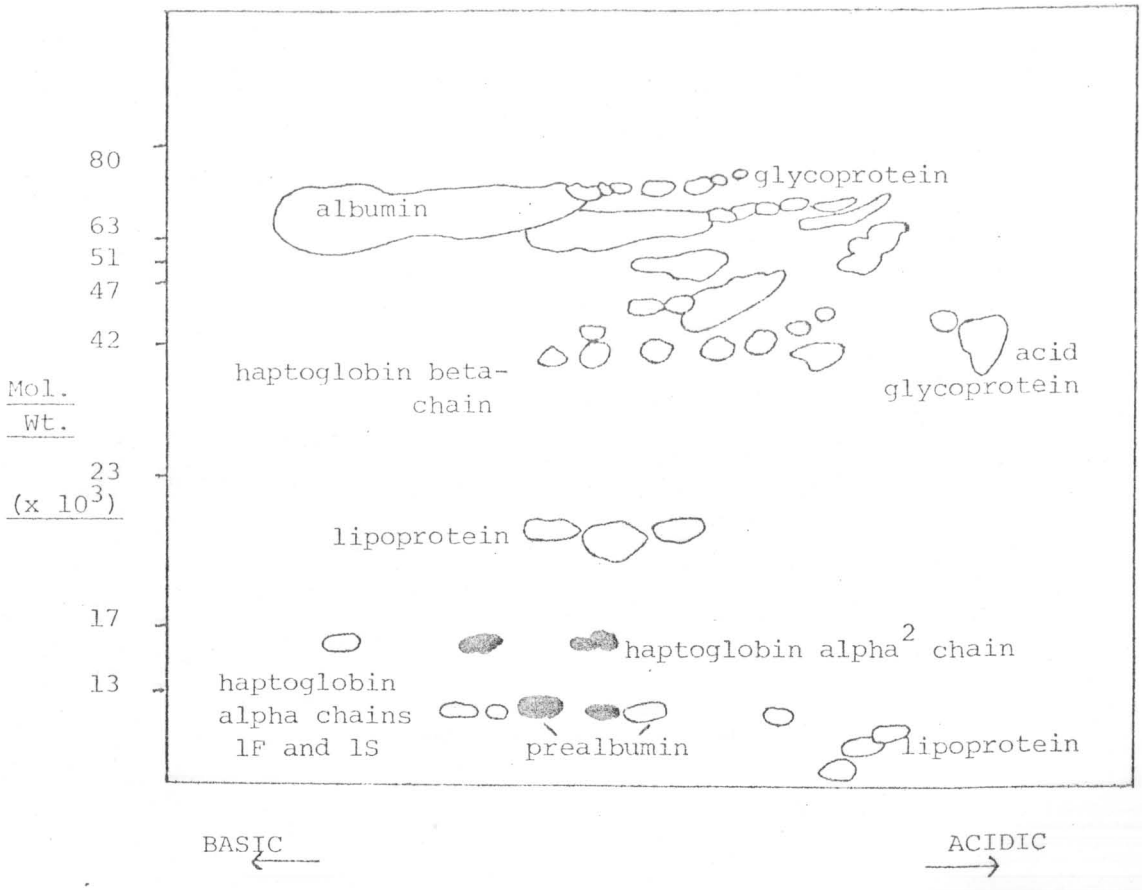
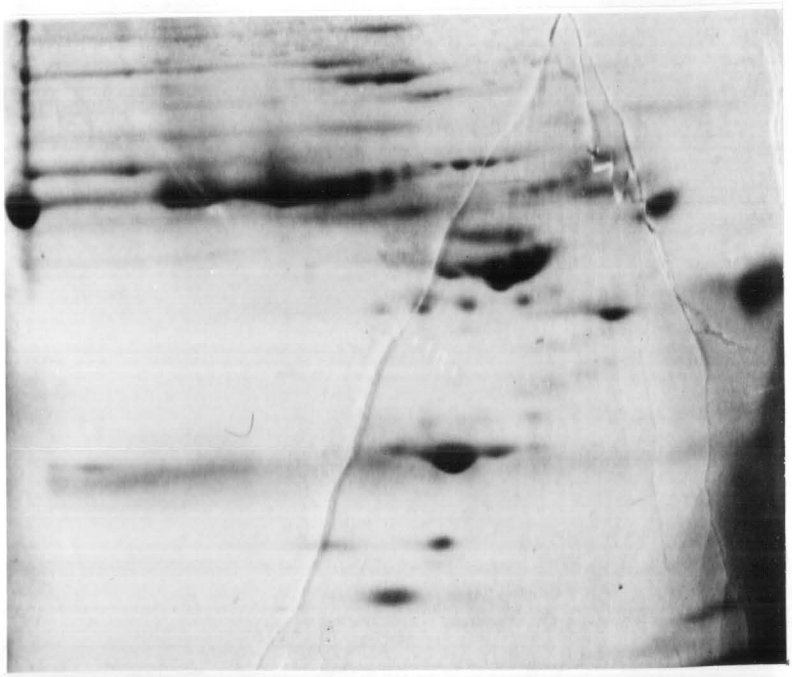


Figure 4

Trisomy
Female 2



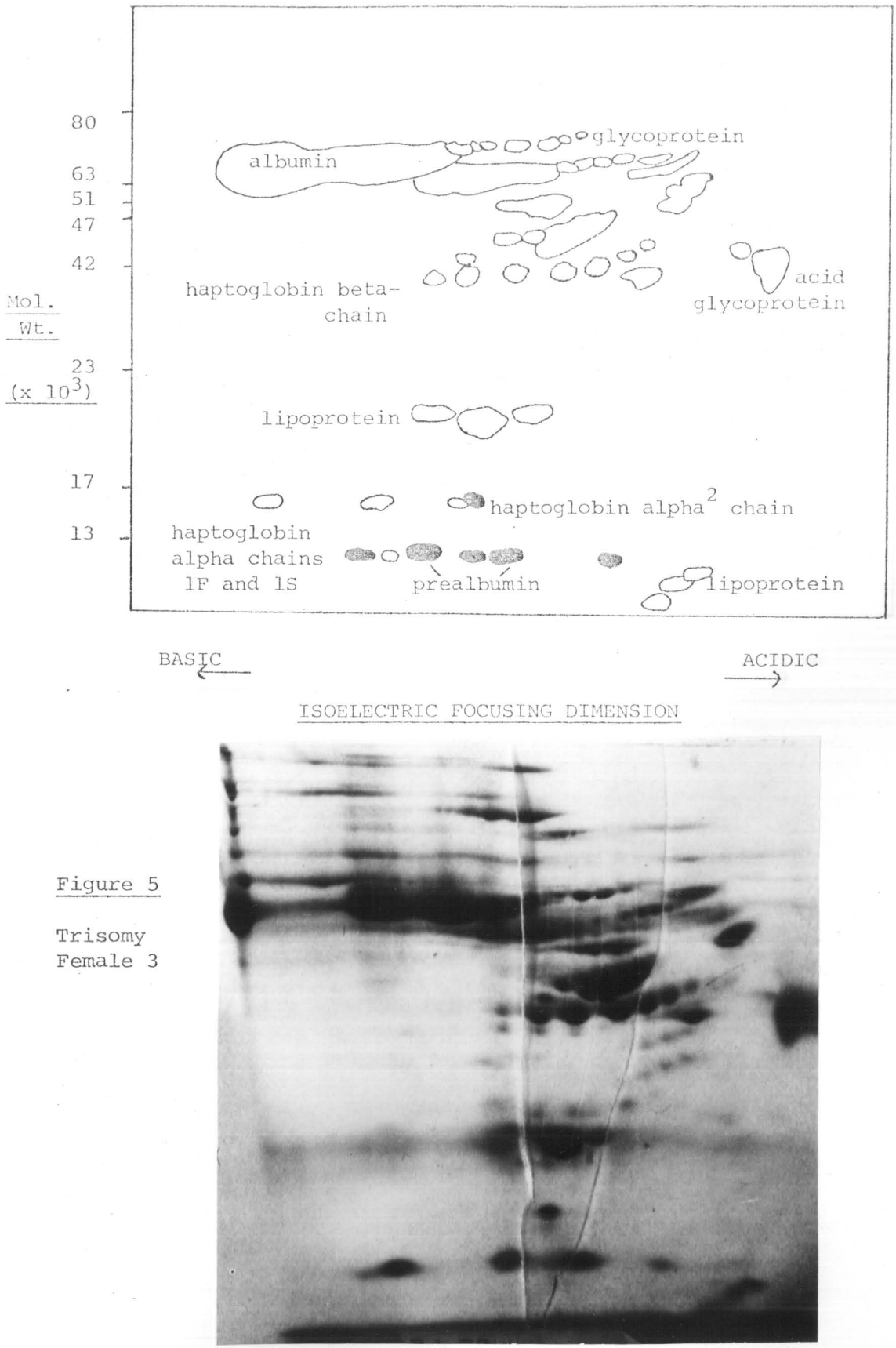


Figure 5

Trisomy
Female 3

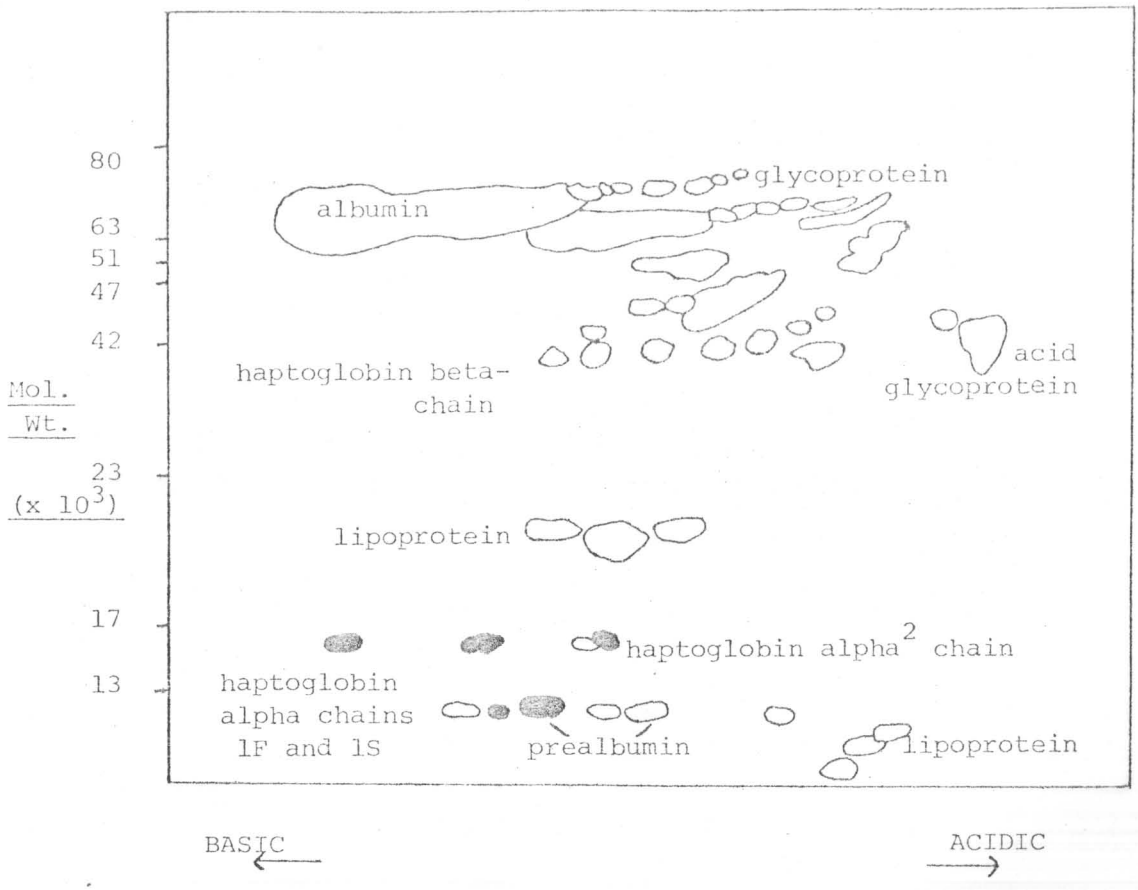
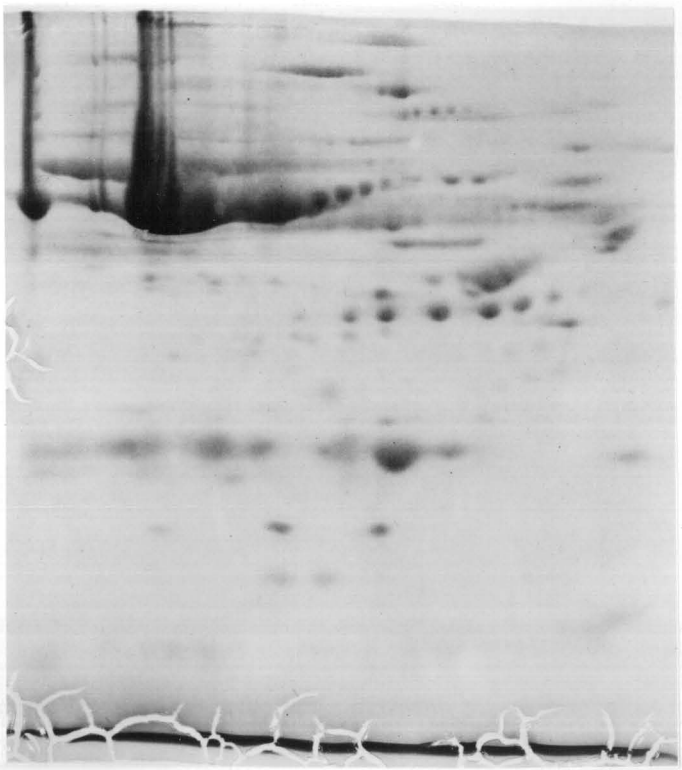


Figure 6

Normal
Female 1



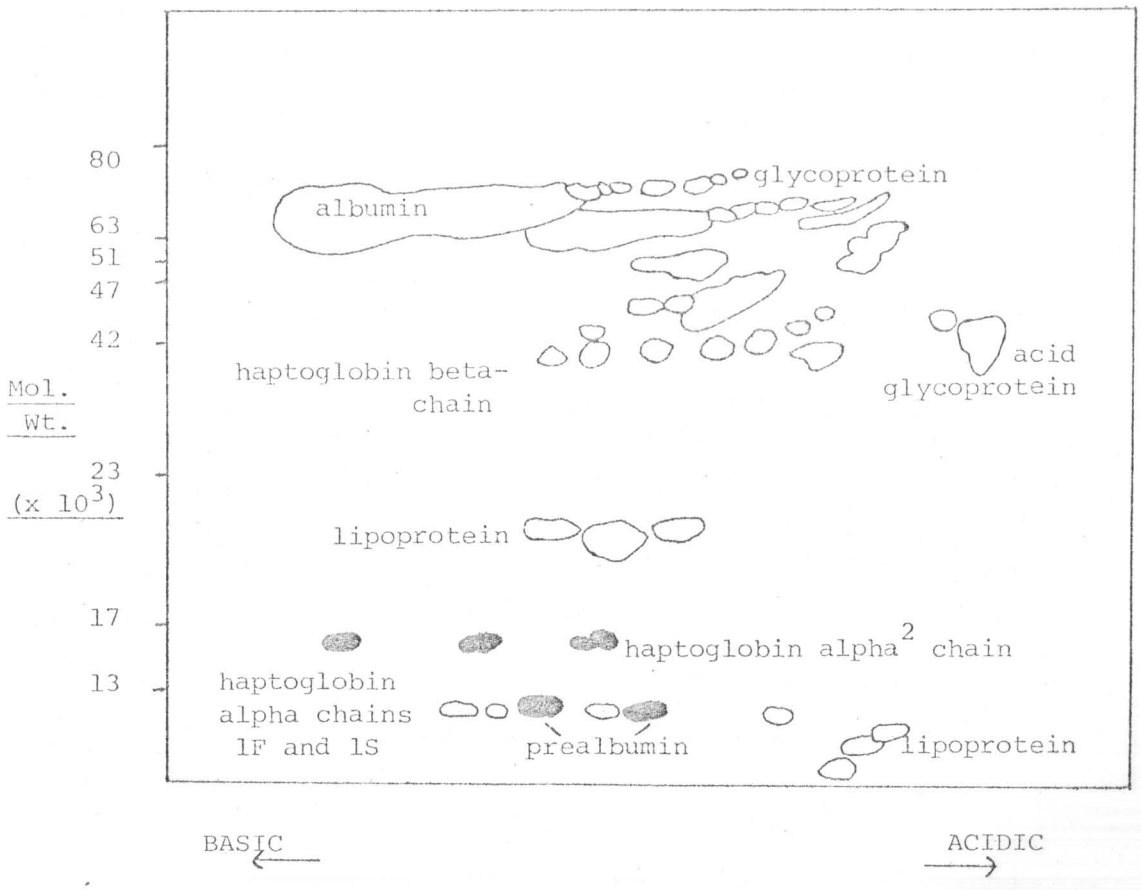


Figure 7

Normal
Female 2

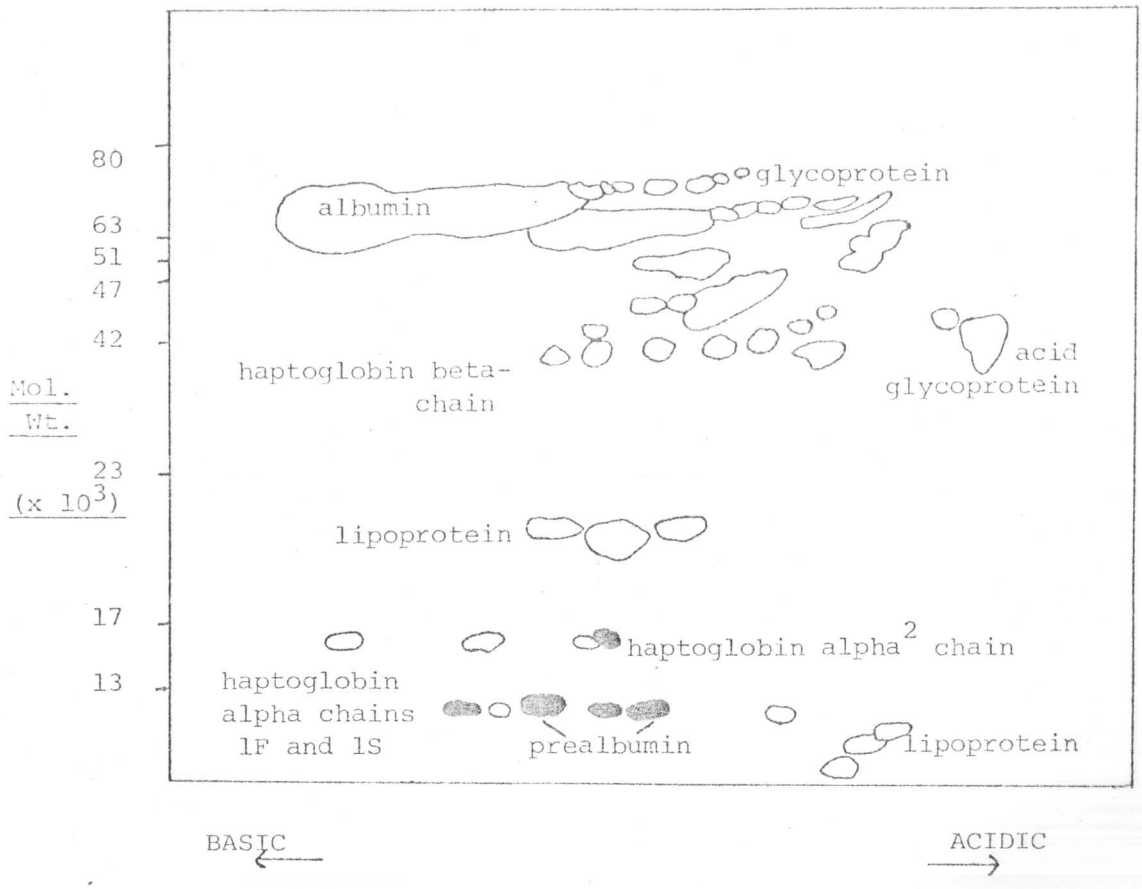
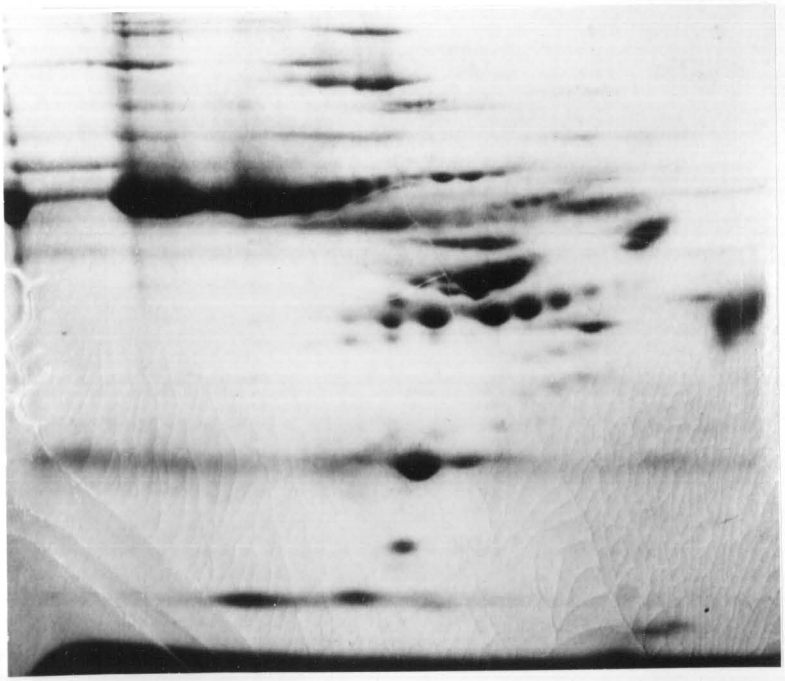


Figure 8

Normal
Female 3



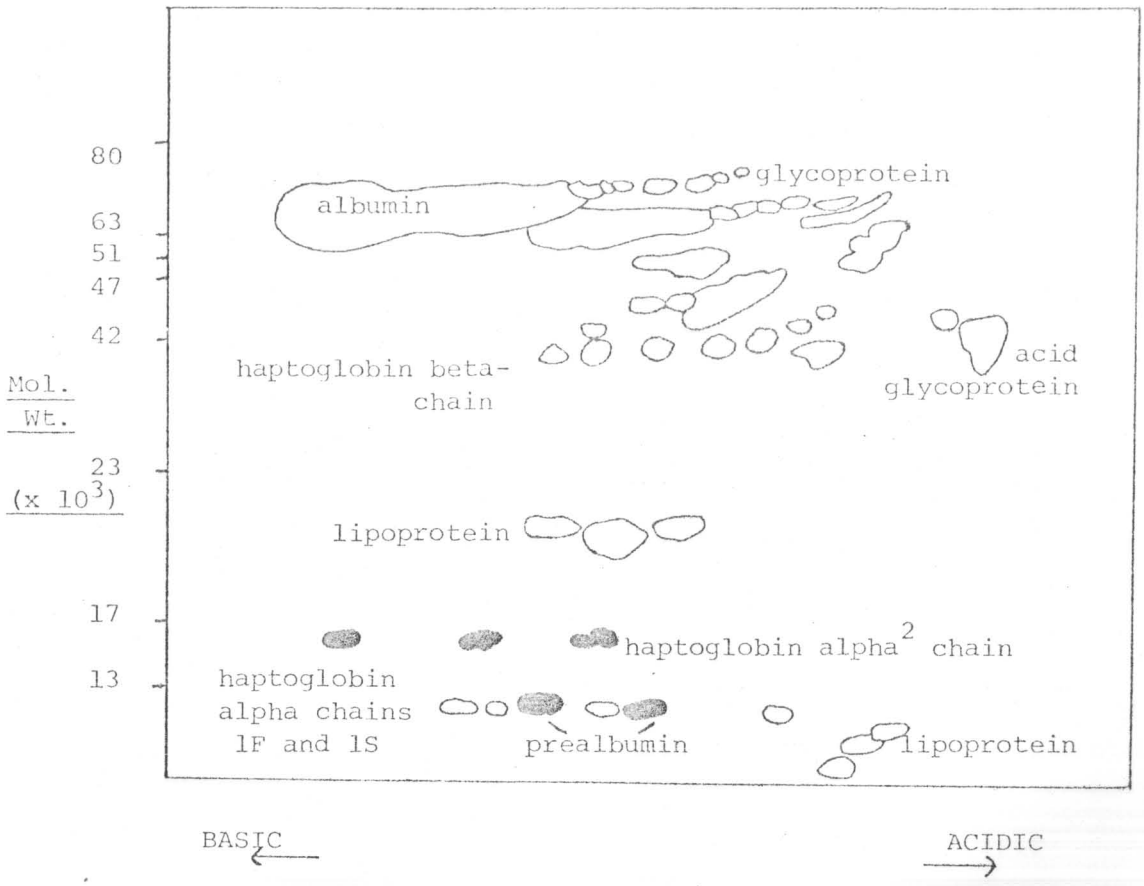
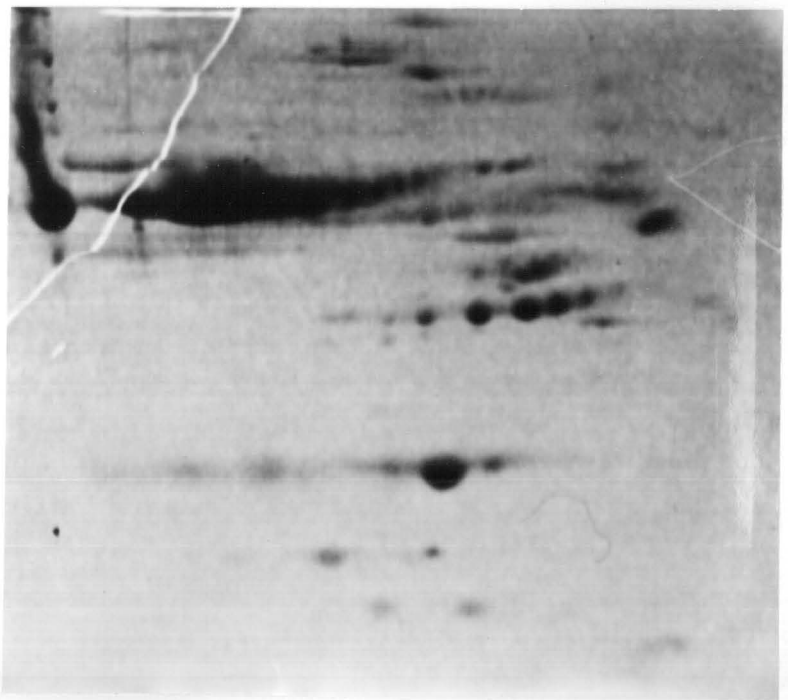
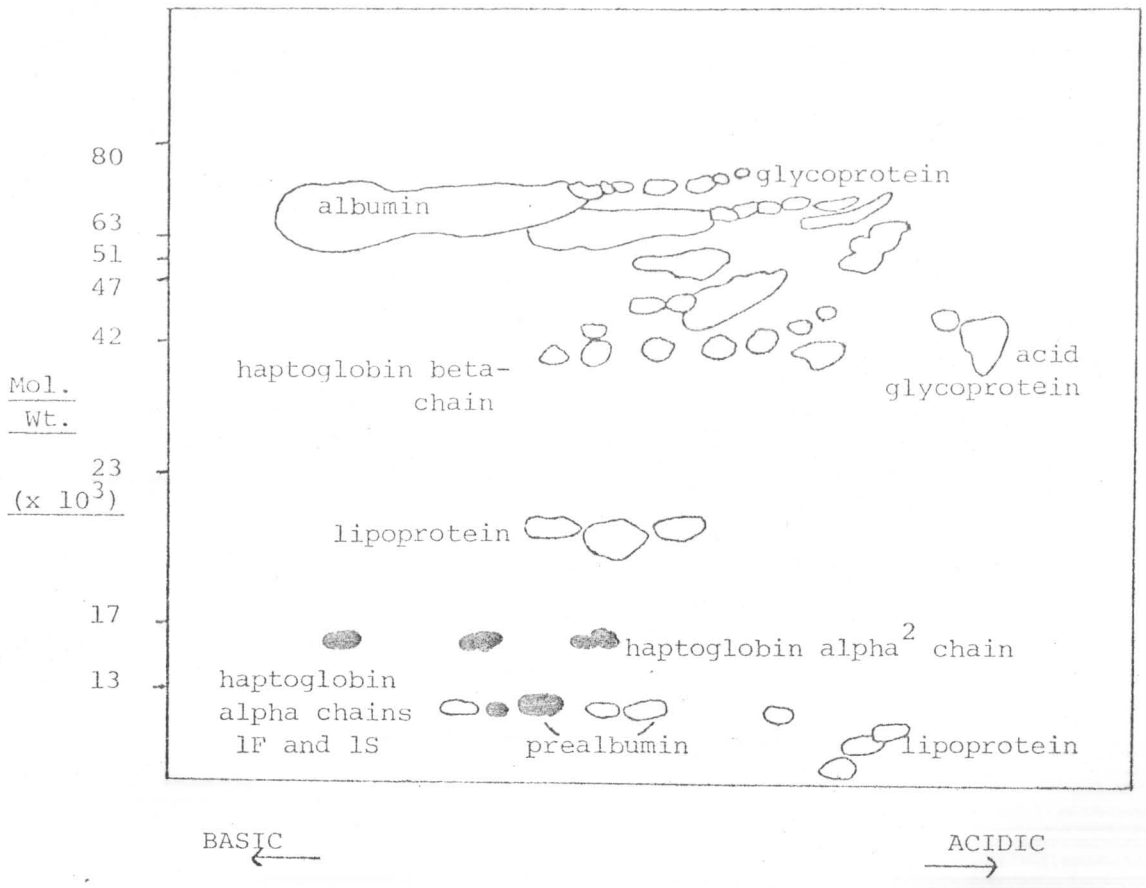


Figure 9

Normal
Female 4





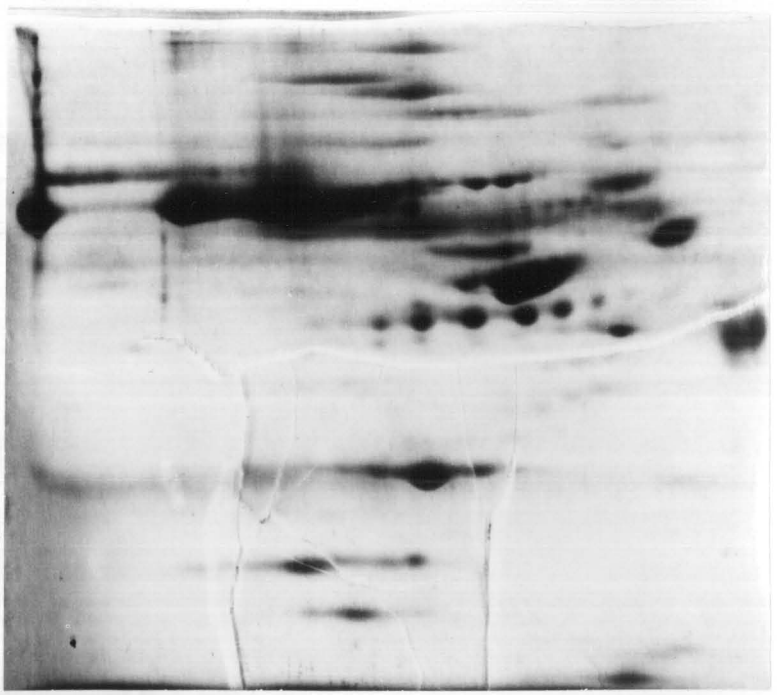


Figure 10

Normal
Female 5

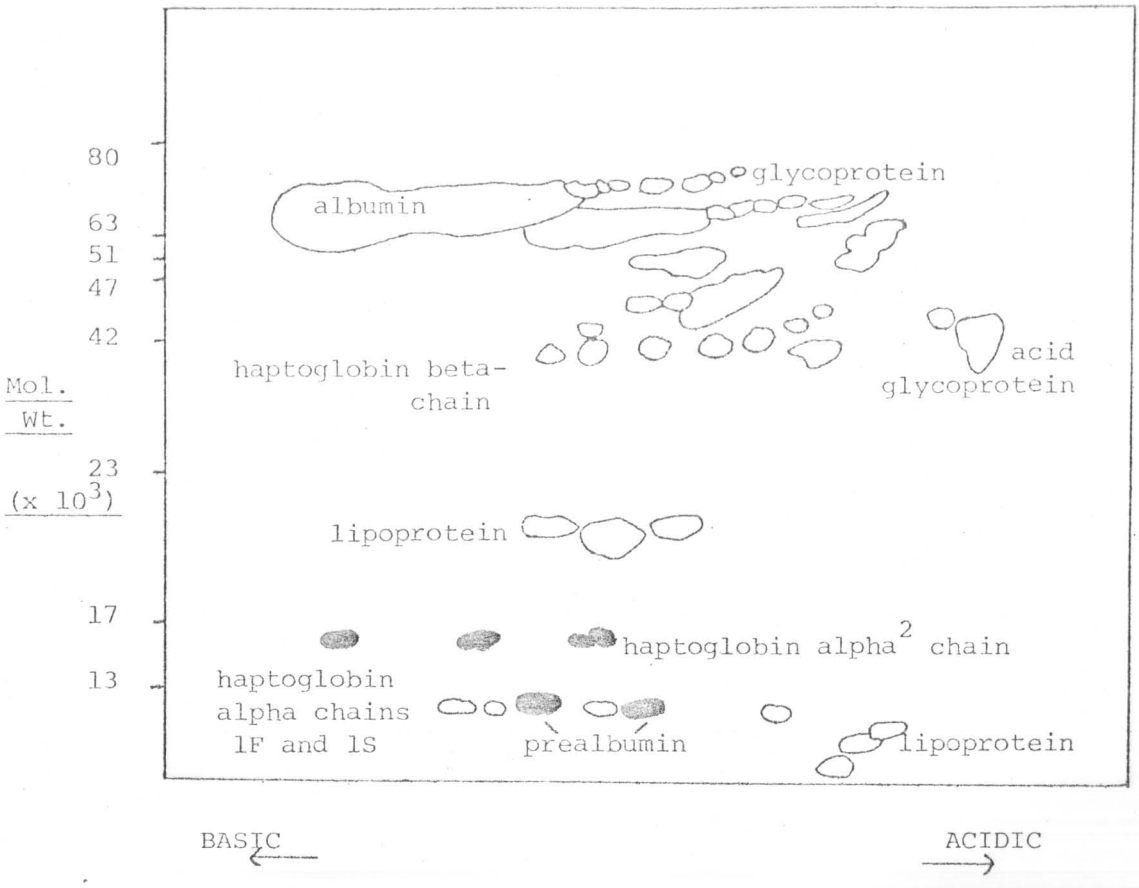
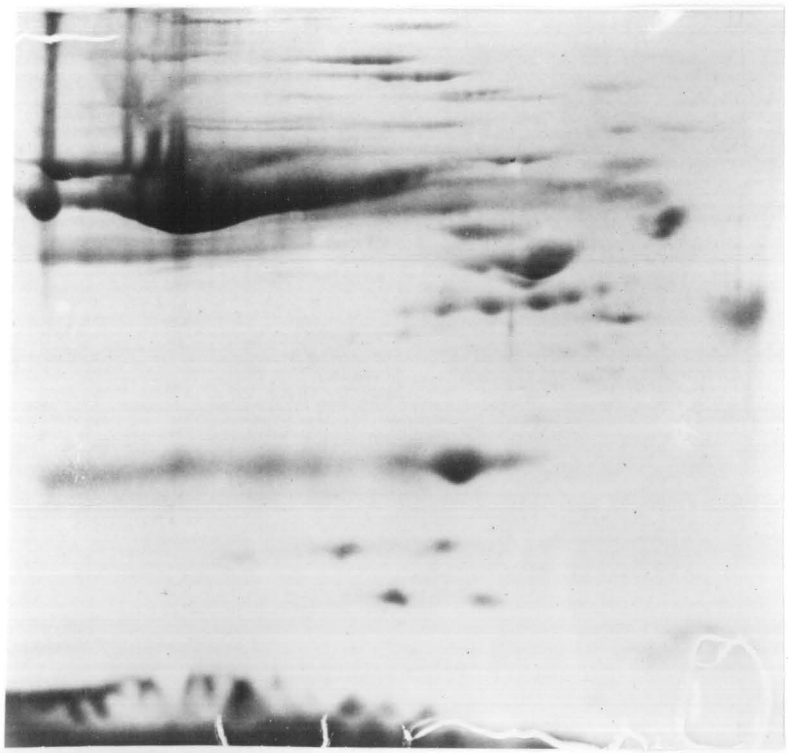


Figure 11

Normal
Male 1



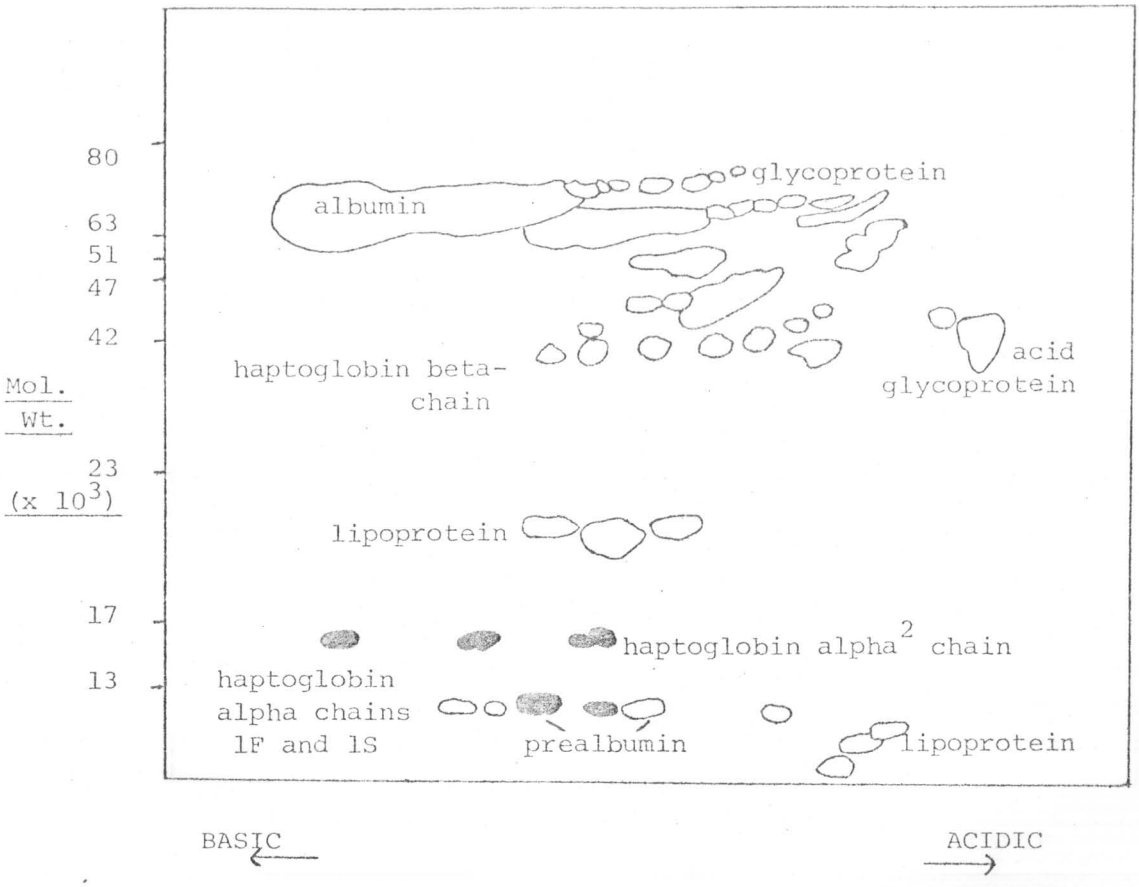


Figure 12

Normal
Male 2

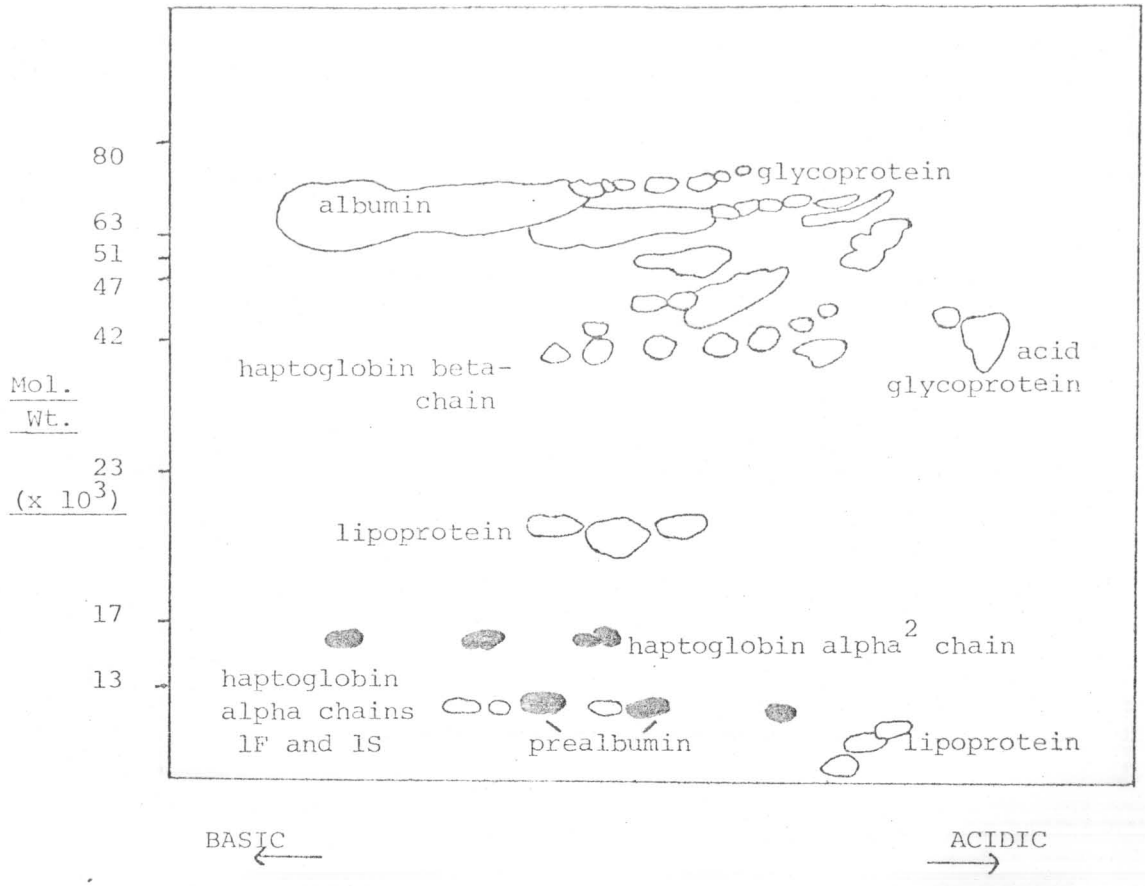
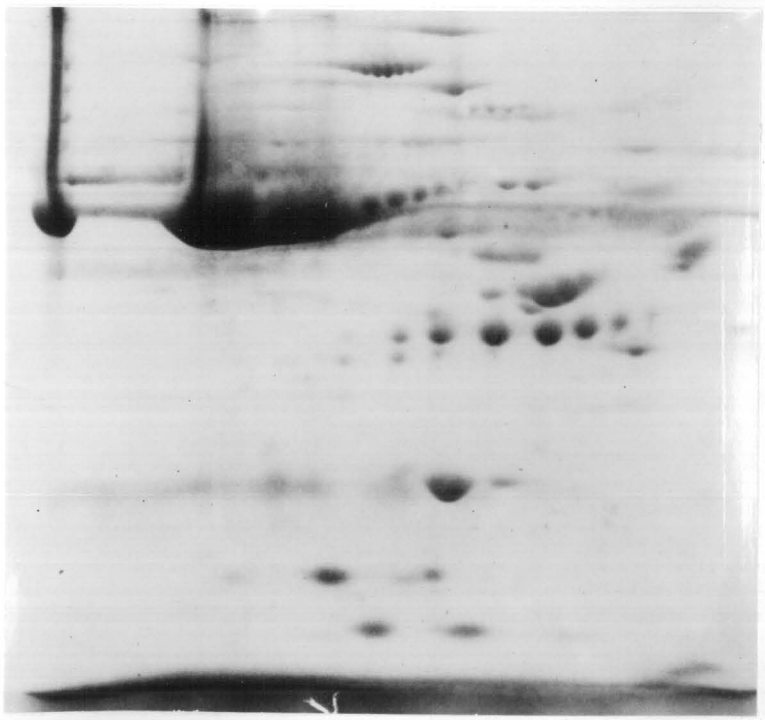


Figure 13

Normal
Male 3



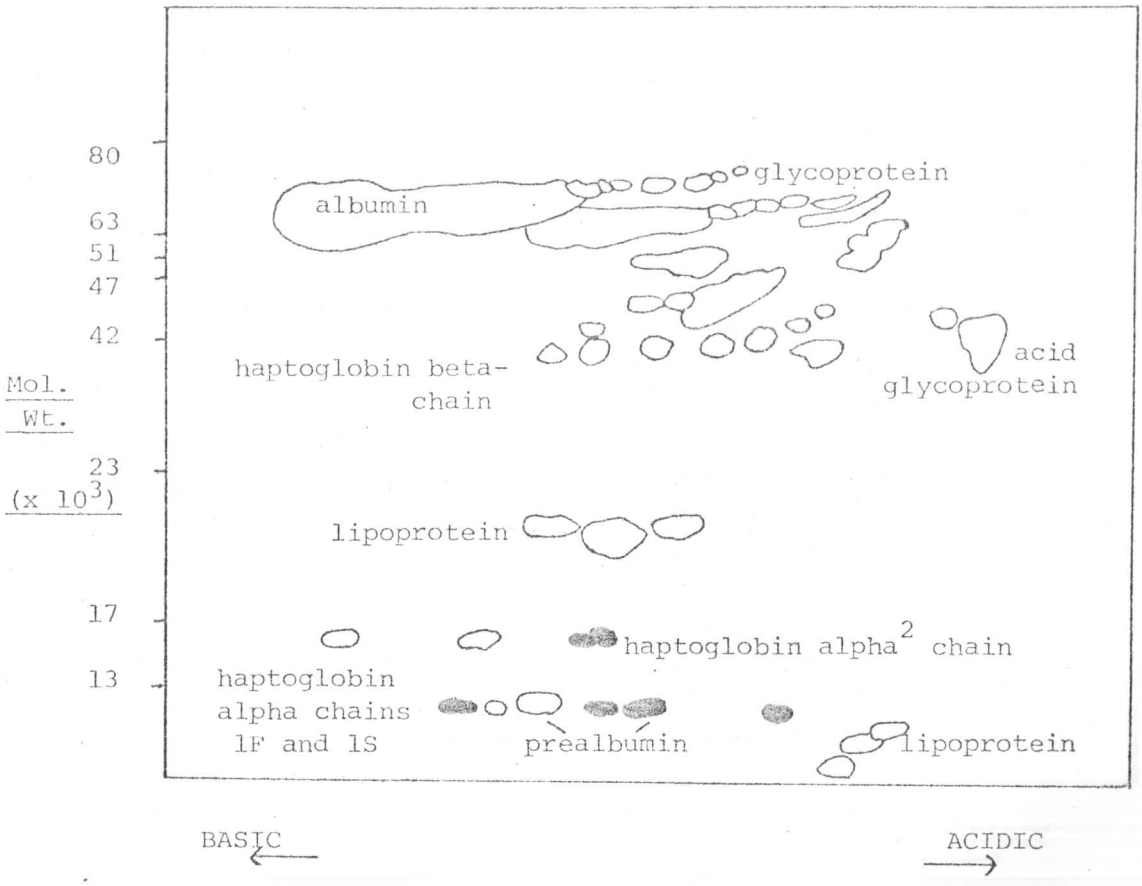
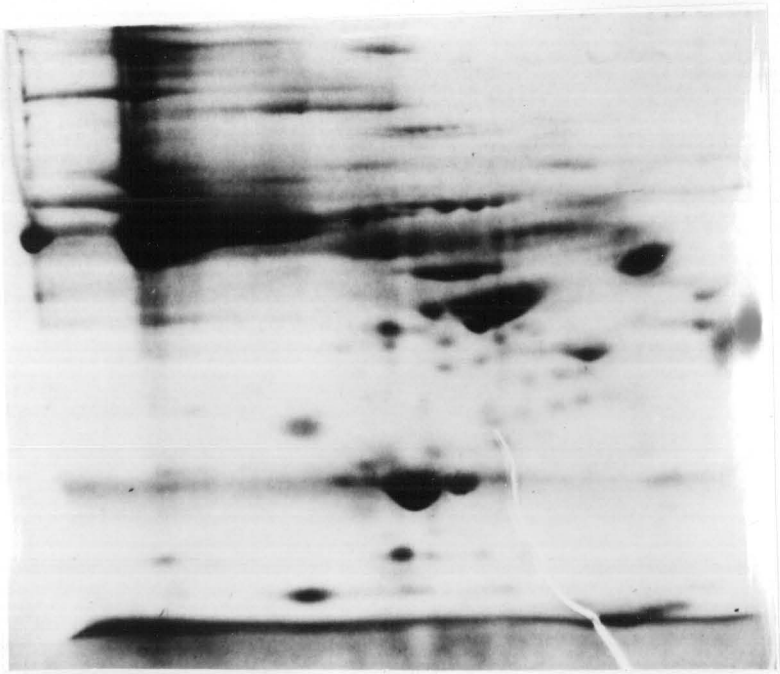


Figure 14

Normal
Male 4



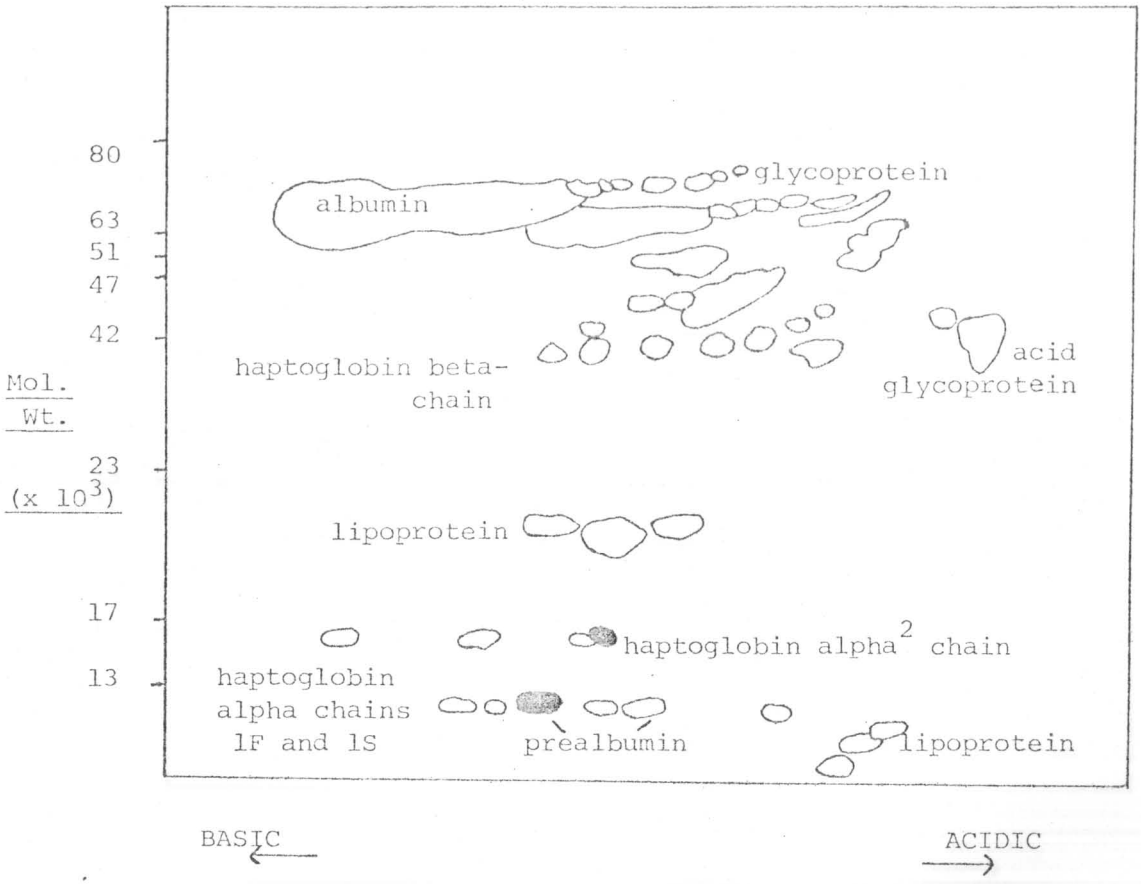
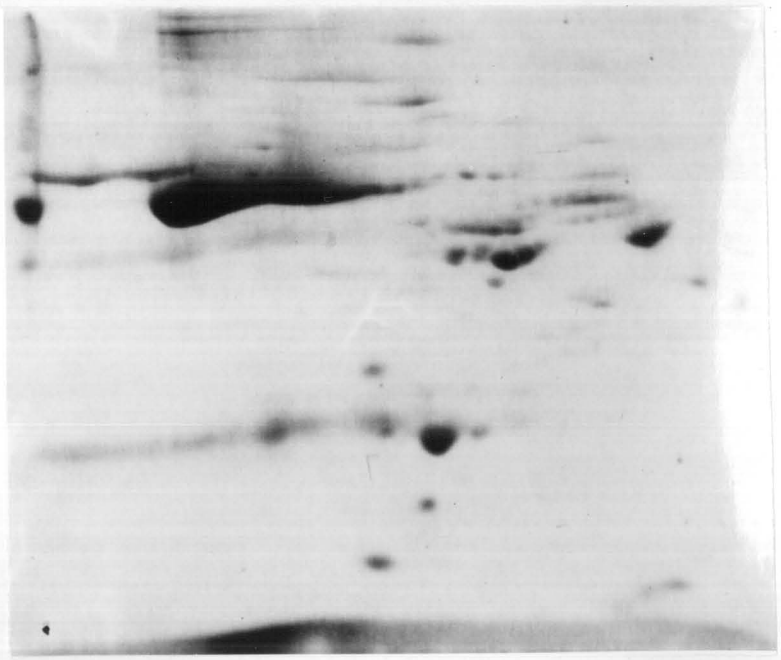


Figure 15

Newborn 1



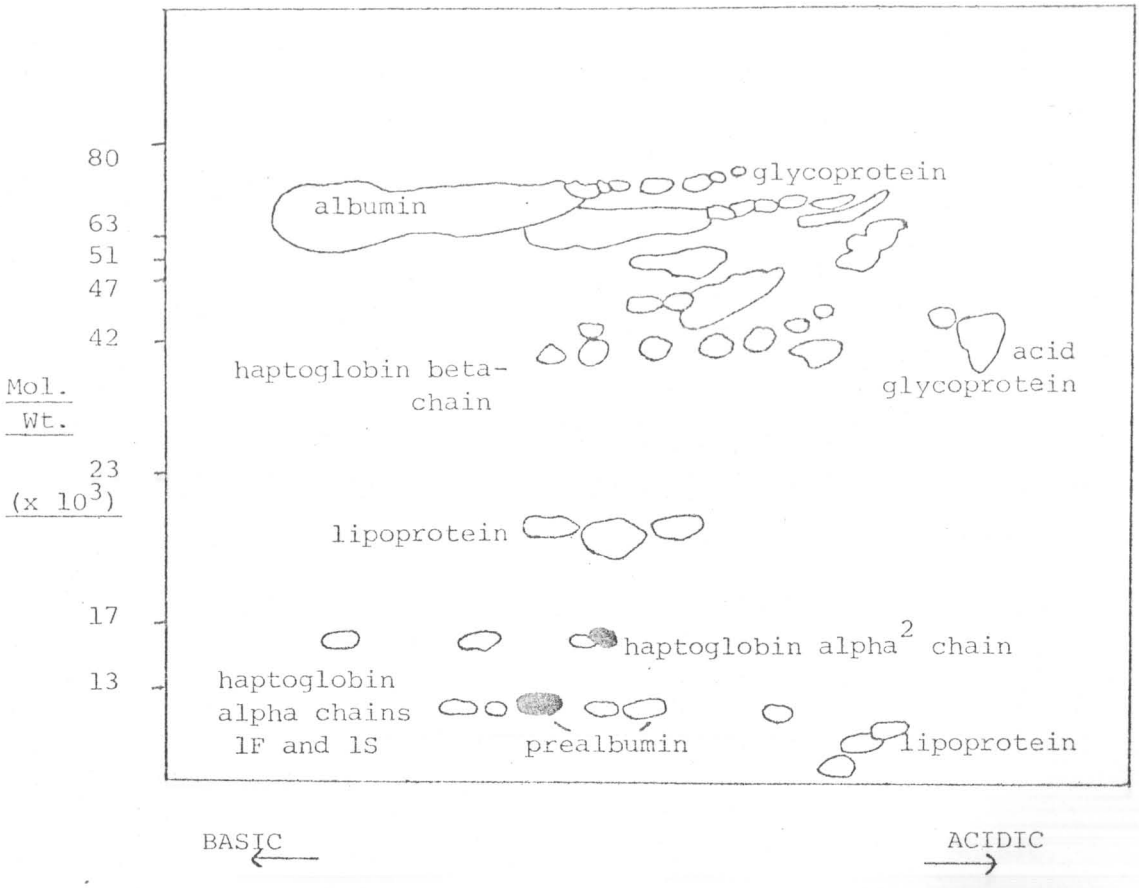
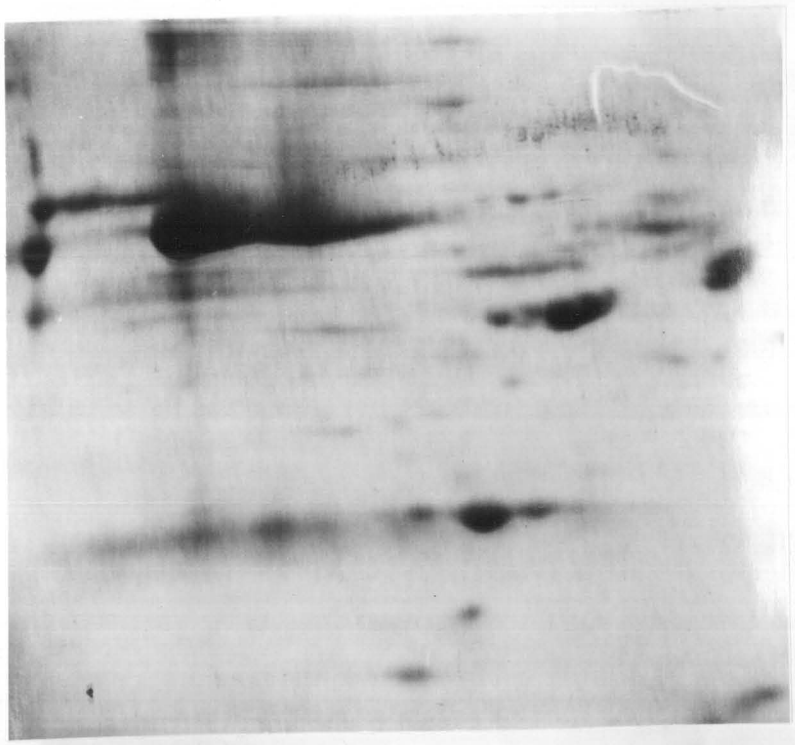


Figure 16

Newborn 2



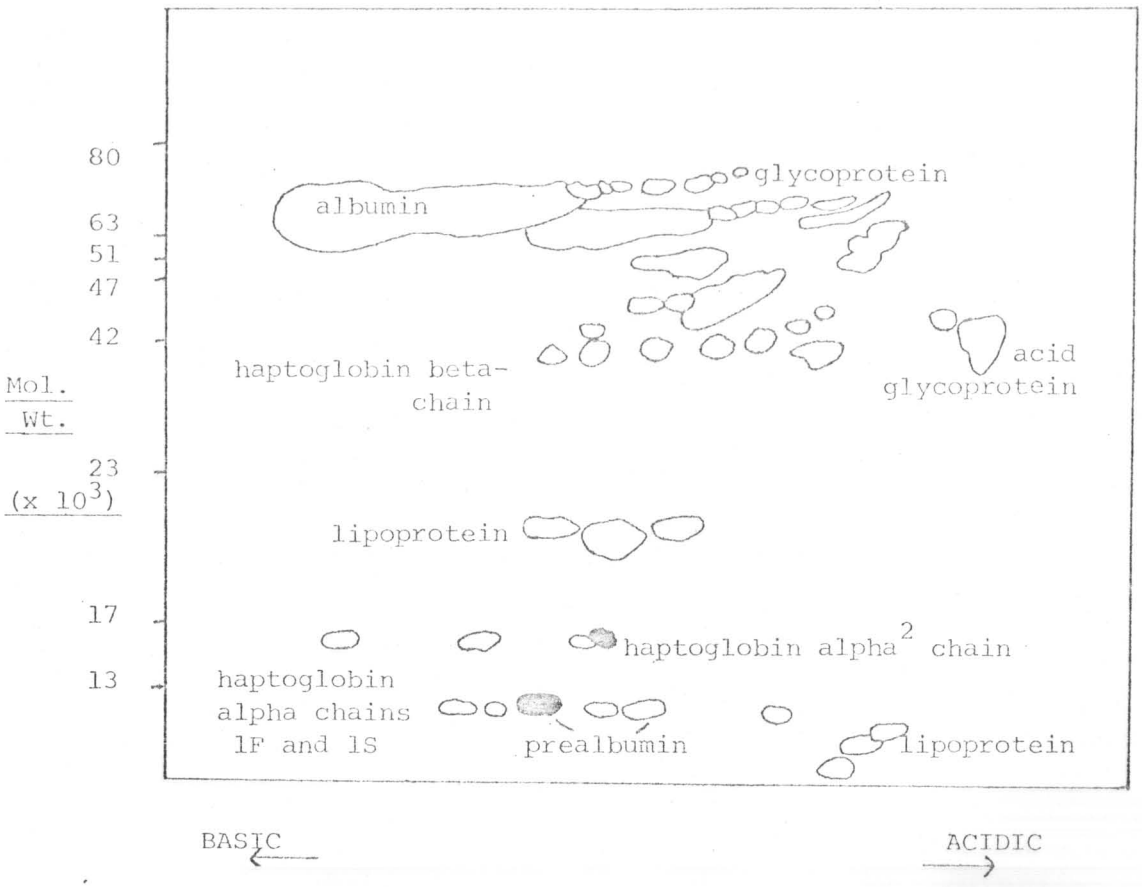
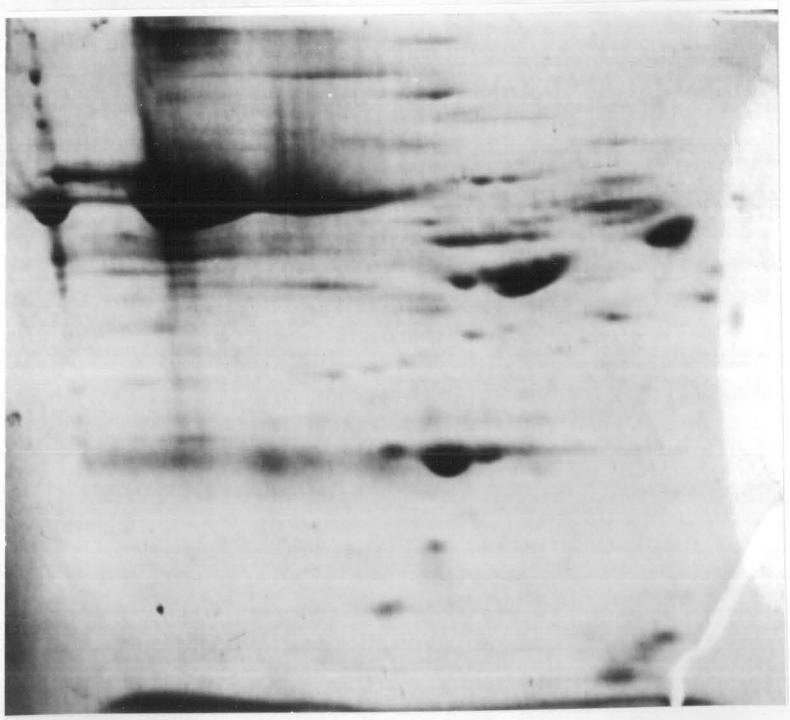


Figure 17

Newborn 3



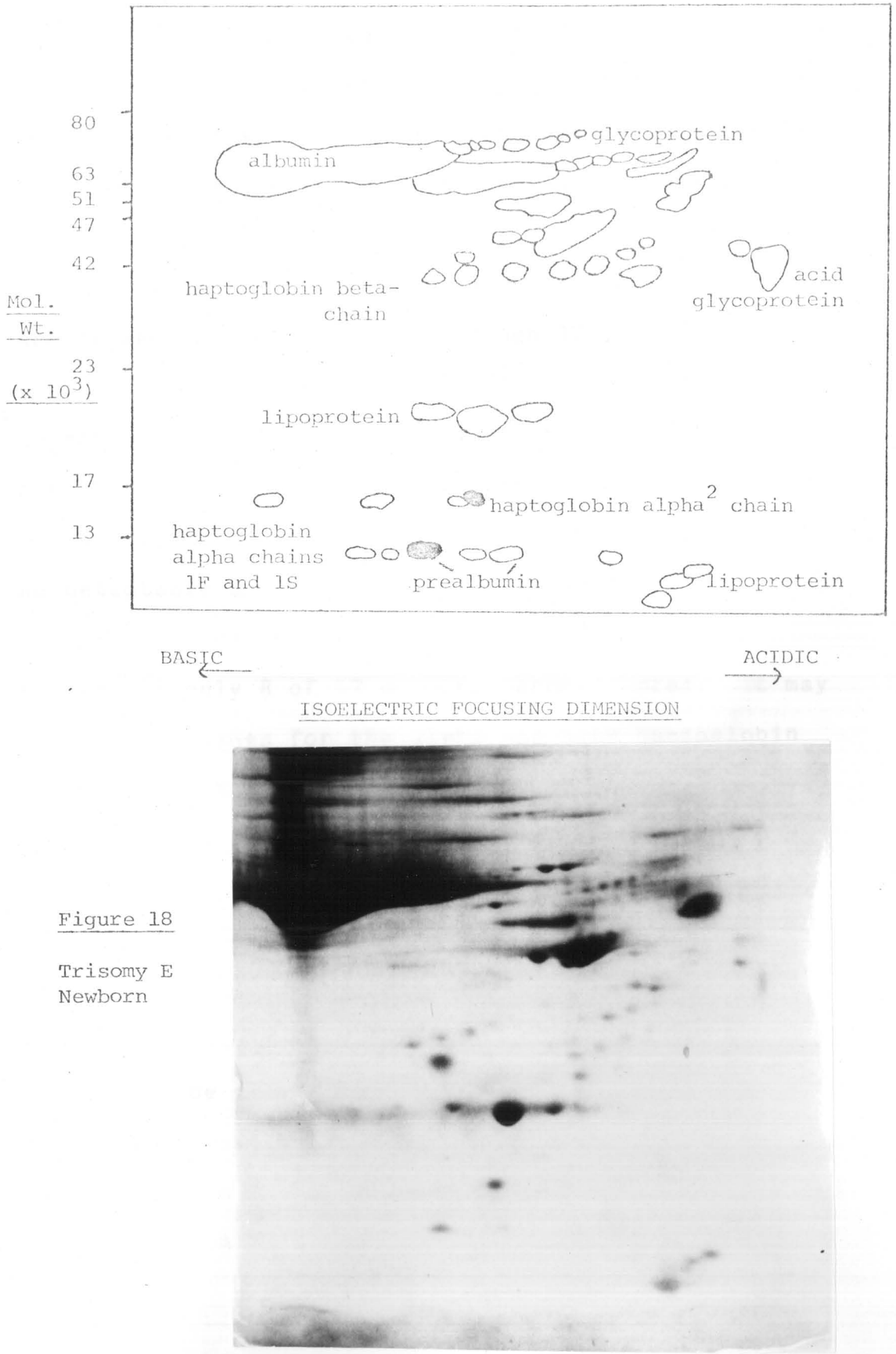


Figure 18

Trisomy E
Newborn

The results of this study yielded 6 different types of haptoglobin alpha-2 chains. All four spots were present in gels from one trisomic male, one trisomic female, three diploid females and three normal males. Spot number one alone was missing in one trisomic female. Spot number two alone was missing in one trisomic male. Spot number three alone was missing in one diploid female. Spots number one and two were missing in one diploid male, while spots number one, two and three were missing in one trisomic and one normal female. This data can be seen in Table 2.

Five types of haptoglobin alpha-1S and alpha-1F chains occurred. Spot number two alone was present in one trisomic male and two diploid females. Spot number three alone was present in one trisomic female and one normal male. Spots one and three occurred in one trisomic and one diploid female and in one diploid male. Spots two and three occurred in one trisomic female. One trisomic male, two diploid females and two diploid males had no haptoglobin alpha-1S or alpha-1F chains.

Prealbumin was always present, either as two distinct spots or as one or the other of the two spots. None of these haptoglobin or prealbumin types, nor any combination of types, is consistent with trisomy-21.

Three of the five normal newborn baby serums run are presented in Figures 15 through 17, as well as one newborn trisomy-E serum sample (Fig. 18). All newborns showed the same single spot in the area identified by Anderson and Anderson as the haptoglobin alpha-2 chain and the same single spot for prealbumin. No detectable amount of haptoglobin alpha-1F or -1S was present. Chung and Shim (1976) found haptoglobin present in only 8 of 93 newborn serum samples. It may be that the genes for the alpha and beta haptoglobin chains are not activated in the majority of newborn infants.

The polymorphism known to exist in the alpha chains of human haptoglobin was seen clearly in these samples. Haptoglobin is a serum protein whose function appears to be one of binding free haemoglobin in plasma and thus preventing it from being excreted out through the kidney tubules, a loss which would also mean a loss of iron. Each haptoglobin molecule contains two sorts

of non-identical polypeptide chains which can be represented two or more times in a molecule, according to molecular size. The two chains are called the alpha- and beta- chains. The beta-chains are evidently identical in all haptoglobin phenotypes (Cleve et al., 1967). A comparison of the multiple components of beta-chains in the gels run in this study revealed no apparent differences in the spot patterns between beta-haptoglobins, although there was occasionally a marked difference in intensity.

The differences between the six common haptoglobin types are due to structural differences in the alpha chains: an Hp 2 allele, and fast and slow migrating alleles of Hp 1. (Connell, Dixon and Smithies, 1962). A comparison of alpha spots revealed an array of different patterns, which are presented in table form. A particular individual may have any combination of two alpha-chains. These account for the six common haptoglobin phenotypes as follows: Hp 1F-1F, Hp 1F-1S, Hp 1S-1S, Hp 1F-2, Hp 1S-2 and Hp 2-2 (Smithies, Connell and Dixon, 1962).

The haptoglobin alpha-1 chains are eighty-three residues long, with a molecular weight of 9,100, while the alpha-2 chain is 142 residues long, with a molecular weight of 16,000. Denaturation with beta-mercaptoethanol and concentrated urea breaks the disulphide bonds holding these alpha- and beta-chains together in the molecule (Smithies, Connell and Dixon, 1966), yielding the patterns of multiple components seen here.

The fourth spot in what Anderson and Anderson have identified as the haptoglobin alpha-2 series appears in every adult. All of the other haptoglobin alpha-2 spots can be seen to vary. This fourth spot also appears in all of the newborns presented, while no other evidence of either the haptoglobin alpha or beta chains occurs in these gels. These facts, along with the observations of Chung and Shim that haptoglobin was seldom present in newborns, lead me to believe that this particular spot may in fact represent some other, as yet unidentified protein. It seems very likely that this spot does not represent any of the haptoglobin alpha-2 polypeptide chains. Whether this is the case awaits further investigation.

There are three spot patterns for the prealbumin fraction seen in these serum samples, with no apparent difference between the trisomic and diploid populations (see Table 1). These results are consistent with the fact that there are a number of serum albumin variants reported in the literature (Weitkamp et al., 1967; Weitkamp et al., 1973).

An unidentified protein was occasionally seen, running far to the right in the SDS dimension at approximately the same molecular weight as haptoglobin 1 and prealbumin (see individual patterns). This spot also showed no relation to the diploid or trisomic status of the individual.

The fact that the absolute position of any particular spot varies from gel to gel has so far made it impossible to automate the pattern analysis. An advance of this type would facilitate analysis of the results reported in this investigation.

III. Polymorphism.

Our understanding of human molecular biology has been enhanced both directly and indirectly by studies of polymorphic components of blood cells and serum. In her recent review of this topic, Giblett (1977) limited the range of the term "genetic polymorphism" to instances where a chromosome locus has been shown to have two or more alleles with frequencies in large populations of more than one percent. Blood specimens may be given phenotype designations based on differences in molecular charge and size as revealed by electrophoretic techniques. Many polymorphisms have been studied from the angle of protein variation amongst populations (Horai, 1976; Neel et al., 1977; Nurse, Botha and Jenkins, 1977), as well as from the angle of enzyme variation in tissue distribution, subunit structure, inheritance, geographic distribution and gene frequencies (Harris and Hopkinson, 1976). There are at least twenty-three electrophoretic variants, and therefore possibly at least twenty-three alleles of human serum albumin (Weitkamp et al., 1973). Over 150 haemoglobins have been reported (Childs and Kaloustian, 1968), most of which were discovered electrophoretically. Buettner-Janusch (1970) has

considered another two of these serum protein polymorphisms, the transferrins and the haptoglobins, from the standpoint of evolutionary significance, while Harris and Hopkinson (1972), and Harris (1975) have used human enzyme variation to calculate the overall degree of allelic variation, concluding that at least 30% of genetic loci have two or three alleles with fairly common frequencies.

Harris (1966) conducted the first systematic search for enzyme polymorphisms in the normal population. Using starch gel electrophoresis to look for differences in molecular charge and molecular size of ten arbitrarily chosen enzymes in human blood, he found three striking examples of genetically determined polymorphism. The following year Lewontin (1967) concluded from an investigation of human blood group factors that the proportion of loci for which the English population was polymorphic was about one third. He observed the remarkable similarity between his estimate, Harris's values for enzymes of human blood and estimates of polymorphism and heterozygosity in Drosophila. And in fact, over the last thirty years polymorphism has been documented in a number of animals from pigs to cattle to mice to fowl to canids (Clark, Ryan and Czuppon, 1975).

In principle, a huge number of alleles could be generated from a single gene by separate mutational events. Some of these changes would result in a codon which coded for the same amino acid as the original, and some would be non-viable, but in 70-75% of cases a single base change would result in a single amino acid change in the polypeptide (Harris, 1971). Only a fraction of these changes would be detected by electrophoresis. It is not known by just how much electrophoresis underestimates the amount of variation in any protein system, since no absolute data is available for how much variation there could be in the structural loci for any human population or even for an individual person. As seen in this study, there are clearcut person-to-person differences in the electrophoretic protein and isozyme patterns which occur.

That these polymorphisms exist among individuals in the trisomy-21 population as well as among individuals in the normal population was a not-unexpected result of this investigation. The results of Hsia et al. (1969) concur with these findings, in that they also found no difference in the gene frequency between Down's syndrome and controls of

the electrophoretic distribution of eight genetic polymorphisms, including haptoglobin phenotypes. Polymorphism of superoxide dismutase has been reported in Finland and in northern Sweden (Beckman, 1973; Beckman and Pakarinen, 1973).

Possibly the cell cultures now used routinely for karyotyping an amniotic fluid sample from a woman at risk for bearing a trisomic child would be a more fruitful source of protein than the serum or the amniotic fluid (Harris, 1978, personal communication). Although this investigation did not lead to a diagnostically useful screen for Down's syndrome, it may lead to use of the O'Farrell technique for identifying other metabolic defects. The possibility of finding clues as to the nature of the fetus in blood or urine samples from pregnant mothers remains. The fact that the haptoglobin and albumin polymorphisms documented in normal adults from various parts of the world also exist in a select population of trisomy-21 individuals is very clearly revealled using the O'Farrell technique. It is also interesting to note that diploid and trisomic serum samples contain a large number of very similar protein components. This was the first study to apply the O'Farrell technique to an

investigation of human populations. There is still a strong possibility that this method could prove diagnostically useful, especially if methods of quantifying proteins can be established.

Appendix

I. Chemicals.

The following chemicals and solutions were obtained and prepared as indicated. Materials were obtained from the suppliers named during the entire course of the experiment. Only the batch of Ampholines indicated was used.

1. Acetic acid, glacial, Allied Chemical Canada, Ltd.
2. Acrylamide, Sigma Chemical Co.
3. Agarose Type I: Low EEO, Sigma Chemical Company.
4. Ammonium persulfate, McArthur Chemical Co. Ltd.
6. Ampholines, supplied as 40% w/v by LKB aminkemi.
7. Coomassie Brilliant Blue R, Sigma Chemical Co.
8. Glycerol, Fisher Scientific Company
9. Methanol, S.F.U. chemistry stores.
10. Glycine 99.5% ammonia free, Matheson Coleman & Bell Manufacturing Chemists.
11. Lauryl sulfate (SDS), Sigma Chemical Company.
12. N,N'-methylene-bis-acrylamide, Sigma Chemical Co.
13. Nonidet P-40, lent by P. Candido, University of British Columbia, Biochemistry Department.

14. Sodium hydroxide pellets,Reagent A.C.S., Allied Chemical Canada, Ltd.
15. TEMED (N,N,N',N'-tetramethylethylenediamine), Eastman chemical purposes. Stored at 4 C.
16. Trizma Base, Reagent grade, Sigma Chemical Co.
17. Urea, Mallinckrodt analytical reagent.

II. Stocks and Solutions.

Bis-acrylamide stock solution. Made up in 100ml portions, filtered, stored at 4 C.

acrylamide	28.38gm
bis-acrylamide	1.62gm
water	to 100ml

10% Nonidet P-40. Made up in 10ml portions, stored at 4 C.

Nonidet P-40	1ml
water	9ml

10% ammonium persulfate. Made up in 1ml portions, shortly before using. Room temperature.

ammonium persulfate	100mg
water	1ml

To make 15ml of gel mixture, sufficient to pour 12 gels, the following amounts were used:

Urea	8.25gm
Acrylamide stock solution	2.0ml
10% NP-40	3.0ml
pH 5-7 Ampholines	.6ml
pH 3-10 Ampholines	.15ml
double distilled water	2.93ml

All ingredients were added to a 125ml vacuum flask and then swirled gently until the urea was dissolved in a 37 C water bath. The flask was vacuated for about one minute, to remove dissolved gases. The catalyzing agents were then added:

10% ammonium persulfate	.015ml
TEMED	.008ml

Upper Reservoir Buffer

sodium hydroxide	1.6gm
distilled water	2.0liter

The above ingredients were added to a 4 liter vacuum flask, a magnetic stirrer was added and the solution was degassed, with stirring, for one hour.

Lower Reservoir Buffer

1 M phosphoric acid	10ml
distilled water	990ml

SDS Sample Buffer

10% sodium dodecyl sulfate	23ml
beta-mercaptoethanol	5ml
glycerol	10ml
0.0625 M Tris-HCL pH 6.8	62ml

Stored at 4 C.

Lysis buffer

Urea	1.71gm
beta-mercaptoethanol	.15ml
pH 5-7 Ampholines	.048ml
pH 3-10 Ampholines	.012ml
distilled water	2.79ml

Overlay buffer

For 10ml, frozen in 1ml aliquots.

Urea	5.4gm
pH 5-7 Ampholines	.08ml
pH 3-10 Ampholines	.02ml
distilled water	9.9ml

Second Dimension gel Solutions

Light solution

Lower gel buffer	8.0ml
Bis-acrylamide	10.6ml
distilled water	13.4ml
ammonium persulfate	.05ml
TEMED	.016ml

Heavy solution

Lower gel buffer	4.0ml
Bis-acrylamide	8.6ml
75% glycerol	3.4ml
ammonium persulfate	.02ml
TEMED	.008ml

Lower gel buffer

Made up to 200ml, stored at 4 C.

Trizma Base	36.3gm
distilled water	to 100ml

Adjust pH to 8.8 with concentrated HCl

Add 10% SDS	8ml
distilled water	to 200ml

5% Spacer gel

0.5 M Tris-Cl pH 6.8	0.75ml
Bis-acrylamide	1.0ml
10% SDS	.06ml
distilled water	4.13ml
ammonium persulfate	.025ml
TEMED	.010ml

Agarose gel

Agarose	1.0gm
SDS Sample buffer	100ml

Heated to dissolve agarose, divided into 5ml aliquots, stored at 4 C.

Second dimension reservoir buffer

glycine	288gm
Trizma base	60gm
distilled water	to 2000ml

Concentrate stored at 4 C. For a run, use

Concentrate	200ml
10% SDS	10ml
distilled water	to 1000ml

Staining Solution

Coomassie brilliant blue R	.5gm
destain solution	500ml

Destaining Solution

Methanol	200ml
Acetic acid	100ml
distilled water	800ml

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