

# CLINIC FOR SPECIAL CHILDREN NEWSLETTER

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\* LANCASTER COUNTY, PENNSYLVANIA \*

Summer 2000

## **AUCTION #10 - LANCASTER COUNTY - SEPTEMBER 16, 2000** **AUCTION - BLAIR COUNTY - SEPTEMBER 2, 2000** **SHIPPENSBURG AUCTION - JULY 15**

### **BUILDING FOR THE FUTURE**

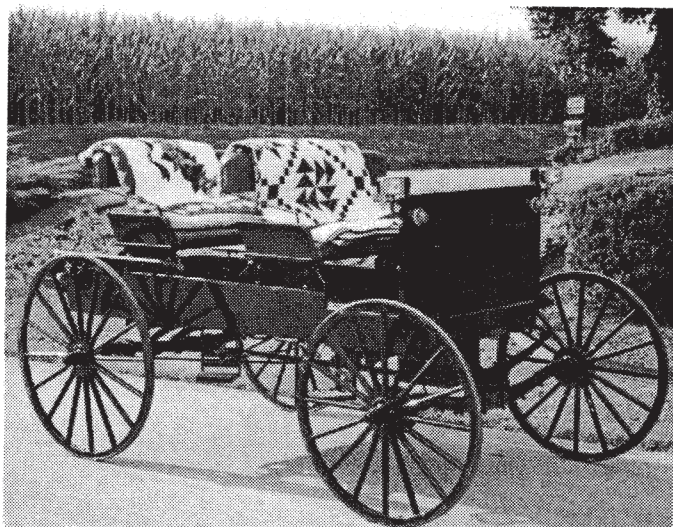
*After 10 years, the clinic is building again. Our work is expanding and so must our space. As soon as plans are finalized and approved, we will be "raising" an addition to the clinic to make room ... for another doctor who will join us next summer ... for more families with children who seek our care ... and for more space for the Special Hearts Circle to gather. We ask for your support this year to help us build for the future ... with additional staff, ... to add work space and needed equipment to provide services children with genetic diseases will need in the next ten years.*

### **TENTH ANNUAL AUCTION**

The 10th Clinic for Special Children Benefit Auction in Lancaster County will take place Saturday, **September 16th** at the Leola Produce Auction center in Leola. (Directions are on back page.) Our 10th auction promises to be as full and rewarding as last year's featuring handmade quilts and wallhangings, locally crafted furniture in cherry and oak, outdoor furniture, storage sheds, swing sets, crafts, farm supplies, housewares, and of course, **FOOD** ! Among the featured items this year will be a new full size two seat spring wagon complete with lights and brakes made especially for this auction as a community effort, a pony sized spring wagon and pony. Another special item is a 1/16th scale millenium series electrified model steam engine. Quilts for this year include one pieced from men's handkerchiefs given over the years to a young man who is handicapped, a postage stamp pattern, log cabin, star designs, samplers, applique designs, and a new pattern named appliqued country grapes. Other items include a martin birdhouse, Bernina sewing machine, new bedroom furniture, swing sets. More appears every day. Food is always an important ingredient in a worthwhile auction and it promises to be in abundance this year. The pony will turn the ice cream maker again as will a hit & miss steam engine. For a preview, a few highlights from last year:

1527 registered bidders with their families participated in the sale of 4116 items. 3650 pieces of chicken were barbecued using 45 gallons of vinegar and 13 lbs of pepper over 1800 lbs of charcoal; 3600 subs were made courtesy of Sheetz, Inc. We also sold 10,000 doughnuts, 4600 soft pretzels, 300 pizzas,

760 pork sandwiches, 3000 servings of french fries, 200 gallons of ice cream, 245 gallons of freshly made lemonade, rhubarb & orange juices, 130 strawberry pies, 300 fresh fruit cups, and over 3800 packages of baked goods went home. The gavels will go into action at 8:30 am, quilts will go up for sale at 11:00 am, large furniture at 11:30am, lawn furniture at 1:00pm. We will pause around noon for Dr. Morton to give a few remarks. If you have items you wish to donate, please call one of the following: (717) 626-4863; (717) 354-5415; (717) 656-9694; or (717) 733-2645.



**Quilts and a Spring Wagon ready for the Auction**

### **4TH AUCTION IN BLAIR COUNTY**

The 4th annual auction to benefit the clinic sponsored by old order Mennonite families in the Blair County region is **September 2**, at the Morrison Cove Produce Auction Center, PA Rt 36 north of Woodbury, or 6 miles south of Roaring Spring, PA. This auction will also feature a two-seat spring wagon, cherry pedestal table, six door oak hutch, oak chairs, benches and other furniture and beautiful hand made quilts. This auction has grown every year and gives many in the central region of Pennsylvania an opportunity to support the clinic. We are very grateful to the families who work hard to sponsor this auction. If you wish to donate items for the sale please call one of the following: (814) 793-3634; (814) 793-3010 or (814) 832-2731.

*Please join us this year for an auction, to help build for the clinic's future and to celebrate the gifts special children bring to all of us. The auction provides a substantial portion of our operating expenses each year, helps keep fees charged to families low, and helps support research for more effective treatment of the complex genetic diseases we see in children, not only from the Plain communities of Pennsylvania, but children in other parts of the world who benefit from what we learn at the clinic. We thank all who contribute their time, their skills, and their bids to give us support.*

## **2ND AUCTION HELD IN SHIPPENSBURG**

The second annual auction sponsored by a local committee of Amish and Mennonite members was held at Leinbach's Auction Center in Shippensburg, PA on July 15th. It was a great success and brings the clinic valuable support and greater awareness of our services to families in that region of Pennsylvania. A new two-seat spring wagon, beautiful quilts, furniture, crafts and food such as chicken barbecue and home made ice cream were featured at the sale. Many thanks to all involved in the auction for your time, effort and contribution to the clinic. It is needed and appreciated.

## **NEW YORK FUND RAISER**

Family and friends of the Colby family in Rochester, NY, are planning the annual pig roast to benefit the clinic. The roast is held in early October each year and we are grateful for this gift of support.

## **MEMORIAL FUNDS**

In the past year the clinic was designated for memorial gifts in memory of:

Paul Morton, Harrisonburg, Virginia;

John Cowen, Ashford, Connecticut;

Rivers Chandler, Greenville, South Carolina.

Funds in memory of Paul Morton, father of Dr. Holmes Morton, were designated to expand the Clinic's library.

Funds received in memory of John Cowen were designated for clinical services to families.

Funds in Rivers' memory, a patient of the Clinic, will be used for research on Glutaric aciduria, Type 1.

We thank their families and friends for these thoughtful gifts.

## **GENETICS TRAINING PARTNERSHIP WITH LGH**

A partnership between Lancaster General Hospital and the Clinic for Special Children was selected as a training team for the Genetics in Primary Care Training Program administered by the Society of Teachers of Family Medicine. One of twenty sites selected nationally and the only team which was not a university based medical center, the Clinic and LGH will develop opportunities for physicians and faculty in family practice residency programs to learn more about genetic diseases and treatments available. Dr. Jeffrey Martin, Associate Director of Family and Community Medicine at Lancaster General will coordinate the team.

## **IMPORTANCE OF HIB VACCINE FOR INFANTS**

During the past year there were at least 8 cases of Hib meningitis reported in Pennsylvania with most of these cases occurring in Amish populations in several different communities. Hib meningitis (*Haemophilus Influenzae* Type b) is a serious, potentially deadly disease. Infants and children under 5 years of age are particularly vulnerable. There was at least one death of an infant due to Hib meningitis this year. Studies by the PA Department of Health and the CDC in two Amish settlements found there is a high rate of carriage of the Hib organism where vaccination rates are low. **Hib meningitis is a preventable disease.** The Hib vaccine is regarded as one of the safest and most effective vaccines for infants and children and the clinic offers this vaccine as part of its immunization service to the local community. In response to the alert for Hib, the clinic sponsored several immunization clinics in Amish homes in different parts of the County to encourage Hib vaccinations. Debbie LaBerge, a nurse practitioner, directs our immunization service at the clinic and is available every Tuesday to answer questions or concerns about vaccinations and schedule appointments for children. The clinic highly recommends that all children under 5 receive the Hib vaccination in addition to the other immunizations.

## **NEW FACES IN THE CLINIC**

We welcomed Miriam Echternach to our staff in February as the new Assistant Office Manager, part time. Mim has been busy getting to know clinic families, surprising some with her ability to speak "Dutch" and updating patient account information for the recent change to a computer format. We are grateful for Mim's help and hard work.

Linda Stoots, joins us one day a week as a volunteer to help with financial accounting. Doris Dunkle also lends her nursing experience to help us organize medications and formula. Our work is made much easier by their contributions of time and effort and we extend our thanks to each of them.

Two students from Franklin & Marshall College in Lancaster worked on research projects at the clinic this year. Alicia Timme, a biology/anthropology major developed her senior thesis based on her research in the lab at the clinic. This summer, Alicia Haupt, a junior at F & M, researched a project related to maple syrup disease. Carol Brenneman, a prospective medical student, also donated time this spring to organize research materials into a useful file system. We hope their work with the clinic inspired them to pursue their interests in the field of medicine.

## **OTHER WORK UNDER THE CLINIC ROOF:**

The following programs/studies continue to operate in space provided in the clinic:

- \* Studies and clinic on the genetic basis of adult on-set diabetes directed by Dr. Alan Shuldiner, University of Maryland Department of Medicine, Baltimore;
- \* Studies on the genetic basis of osteoporosis, Dr. Elizabeth Streeten, Univ. of Maryland Dept. of Medicine, Baltimore;
- \* Studies on genetic eye diseases in the Amish population, directed by Dr. Dwight Stambolian, University of Pennsylvania.
- \* Special Hearts Circle meets Wednesday-Thursday-Friday.



## MEDICAL PROGRESS NOTES

Holmes Morton M.D.

### BILE SALT DISEASE

***Poor growth, itching, and vitamin K responsive bruising and bleeding in infants may be caused by inherited disorders of bile salt transport.***

Morton DH, Salen G, Batta AK, Shefer S, Tint GS, Belchis D, Shneider B, Tint GS, Belchis D, Shneider B, Puffenberger E, Bull L, Knisely AS: *Abnormal hepatic sinusoidal bile acid transport in an Amish kindred is not linked to mutation in FIC1 and improved by ursodiol.* Gastroenterology 2000, 119:188-195.

Two disorders of bile salt transport are now recognized in the Amish population in Lancaster County. Both disorders interrupt the normal circulation of bile salts. Bile salts are made in the liver from cholesterol then are carried across liver cell membranes into bile by specific transport proteins. After a meal the bile empties from the gallbladder into the small intestine, mixes with food, and makes possible the efficient absorption of dietary fats as well as the fat soluble vitamins A, D, E, and K. The bile salts are reclaimed from the intestine and return to the liver in the portal blood and are actively transported from the portal vein sinuses into the liver cells then back into the bile ducts and gallbladder. *Byler disease*, also called *familial intrahepatic cholestasis or FIC1*, is related to an abnormal bile transport protein in the membranes between the liver cells and bile ducts. A second newly recognized disorder of bile salt transport prevents the reclamation of bile acids from the portal blood. Both disorders are usually diagnosed in infants with poor weight gain who may also have bruising and bleeding caused by vitamin K deficiency.

Byler disease was described in 1969 as *familial intrahepatic cholestasis* and is more common in the Amish population of Lawrence County than in Lancaster County. In Byler disease the blocked transport causes the accumulation of bile salts within liver cells and deterioration of the liver between five and ten years of age. Liver transplant remains the only effective treatment. In contrast, *Blank disease* causes high concentrations of bile acids in blood and intestinal losses of bile acids, fats and vitamins but responds to treatment with a replacement bile salt called ursodiol (Actigall, Urso). *Blank disease* does not cause progressive injury of the liver in early childhood but may be associated with gallstones and scarring of the liver in young adults.

### VITAMIN K DEFICIENCY AND INHERITED LIVER DISEASES: Mistaken diagnosis of child abuse

Last December an Amish infant died at four months of age from a hemorrhage into the brain. In the emergency room where the comatose infant was first taken, a hemorrhage in the brain was suspected, bruises were seen on the infant's face, trunk, legs and multiple hemorrhages were found in the retina of one eye. A diagnosis of *shaken baby syndrome* was suggested by an emergency room physician and was subsequently offered with confidence by a neurosurgeon at the

hospital where she underwent surgery and subsequently died. The case was reported by the neurosurgeon and a pathologist as a probable homicide to the regional district attorney and child protection agency.

The legal investigation went forward rapidly and was based upon an assumption of guilt. The district attorney, police, and child protection workers assumed that one of the parents was guilty of either violently shaking or striking the head of the infant. Within hours after the infant was taken to the emergency room, while the parents were still at the hospital with their dying infant, state police took the six brothers from the family home and from the homes of Amish neighbors and placed them in *protective custody* in non-Amish homes.

What parent can imagine what it must have been like for these parents first to watch this infant become ill and seek help at a hospital and then have the physicians they asked for help falsely accuse them of abuse, neglect, and murder? Today, knowing that this infant died of bleeding caused by vitamin K deficiency, it is easy to feel sorry, ashamed and horrified by the manner in which this innocent family was treated by a medical-legal system that is intended to protect children from violent and negligent parents. This and other cases wherein parents are wrongly accused of neglect and abuse of children because of unrecognized underlying medical conditions remind us why our legal system is supposed to be based upon *assumption of innocence until proven guilty*. Physicians in this case were also misled by an *assumption of guilt*. Laboratory information that led to a diagnosis of vitamin K deficiency was present in the medical record before the infant died but was ignored. Neither x-rays nor the findings at autopsy supported the diagnosis of shaken baby syndrome or head trauma, yet medical-legal efforts to establish guilt went forward and kept this family separated for months. An *assumption of innocence* should have required physicians and the police to carefully consider all the evidence in the case.

Within days of the infant's death, the family requested help from the clinic to determine the cause of the baby's illness and death. Based on lab information present in her chart at the hospital and confirmed in subsequent labs performed at the clinic and elsewhere, I established a diagnosis of vitamin K deficiency with bile salt transport disorder a contributing cause. Despite these medical reports the investigation of child abuse and separation of the family continued for several months. After a medical board review in Harrisburg, officials ended the investigation and the children were allowed to return home. Neither the district attorney, nor the hospital where the infant died, nor the neurosurgeon, nor the pathologist who erroneously reported death by head trauma has publicly expressed regret about the mistaken diagnosis of child abuse or the suffering of this family.

We wish to acknowledge and express appreciation to many who helped the family in this tragic case. Charles Hehmeyer, an attorney from Philadelphia volunteered his time to help the family. Mr. Hehmeyer and I spent more than a hundred hours offering proof of the innocence of these parents. Many others also helped: Dr. Lucy B. Rorke, Pathologist-in-Chief and neuropathologist at Children's Hospital of Philadelphia and a

nationally recognized authority on shaken baby syndrome reviewed the case and found the retinal and brain hemorrhages to be typical of vitamin K deficiency and found no evidence of shaken baby syndrome or blunt trauma to the head. Dr. Gerald Salen, whose research led to the discovery of the bile salt transporter syndrome called *Blank syndrome*, showed abnormal bile levels in the blood of the infant and helped establish that the bile salt transporter defect was a contributing cause of vitamin K deficiency. Dr. David G. Nathan, who coauthors the major textbook of pediatric hematology and is Smith Professor of Medicine and Pediatrics at Harvard Medical School, reviewed the laboratory data about the bleeding disorder and agreed that all of the lab data fit the diagnosis of vitamin K deficiency. Representative Merle Phillips supported the family and continues to hold hearings in Harrisburg related to the case. Maria Coole and Ed Klimuska kept this injustice in the public eye by writing forcefully and well about a complex medical and ethical case.

The most important and lasting support for this family through weeks of hardship came from their extended family, neighbors, and the Amish community. Hearing the old farmhouse needed repairs and its poor condition might be offered as evidence of neglect, building supplies were donated and van loads of family and friends from Lancaster County and from as far away as Ohio completely rebuilt the house. This restored home will stand as testimony to a true and meaningful *assumption of innocence* long after the medical, legal, and political arguments about the case are forgotten.

**Medical lessons:** Many pediatricians assume that retinal hemorrhages are diagnostic of shaken baby syndrome or non-accidental trauma. Retinal hemorrhages may be seen in association with intracranial hemorrhages that occur in vitamin K deficiency, hemophilia, primary liver failure or hemorrhages caused by vascular malformations. Intracranial bleeding associated with vitamin K deficiency is particularly characteristic of late hemorrhagic disease of the newborn which is almost always associated with an underlying abnormal liver function. In Sweden 1987-1989 there were 332,686 births, 80% of these infants received oral vitamin K 2 mg and 20% got 1 mg IM. There were 17 vitamin K deficiency related hemorrhages. Of these 17 infants with hemorrhages, 8 had intracranial bleeding and 15/17 infants were subsequently found to have an underlying liver disease. (Ekelund E *Acta Paediatr Scand* (1991) 80: 966-968.) In the series of 32 infants with brain hemorrhages associated with vitamin K deficiency reviewed by Chaou WT et al in 1984, 91% had subarachnoid hemorrhage and 32% had subarachnoid and subdural hemorrhages, 31% had intracerebral hemorrhages, 13% intraventricular hemorrhages and 9% had posterior fossa hemorrhages. (Volpe JJ *Neurology of the Newborn* (1995) 3rd edition, 393-4). In *Holt's Diseases of Infancy and Childhood*, 11th Edition, D. Appleton-Century Co, 108-111, the authors note hemorrhages are almost always of venous origin and retinal hemorrhages are commonly seen.

Retinal hemorrhages are also associated with tears of veins that bridge the subdural space in children with glutaric aciduria and have led to the mistaken diagnosis of child abuse in this disorder as well. (Baric I, Zschocke J, Christensen E, Duran M, Goodman SI, Leonard JV, Muller, Morton DH et al., Hoffman G,

(1998) *Diagnosis and management of glutaric aciduria type 1*. *Journal of Inherited Metabolic Diseases* 21 326-340., Morton DH Letter to Editor *Contemporary Pediatrics* 14(1),158, 1998.)

*Although some authors have suggested that retinal hemorrhage in children under two or three years is pathognomonic of child abuse, retinal hemorrhages can definitely occur in infants from non-traumatic causes including severe hypertension, vasculitis, meningitis, endocarditis, generalized sepsis, coagulopathy and blood dyscrasia. Fundus appearance in such cases may be virtually indistinguishable from that seen in shaken baby syndrome.* (Levine A *Ophthalmology Clinics of North America* 1990; 3: 249-264.)

Several genetic disorders are found in the Amish/Mennonite populations that are associated with abnormal bile salt circulation or primary liver disease and cause abnormal blood clotting, bruising and an increased risk of acute intracranial hemorrhage. These disorders include: 1) several cholestatic diseases of the liver - Blank syndrome, Byler syndrome, Allagilles syndrome, alpha-1-antitrypsin deficiency, cystic fibrosis, and biliary atresia; 2) classical galactosemia; 3) glutaric aciduria type-1; 4) vascular malformation syndromes - anterior-venous malformation of the brain, Moya Moya disease (which usually presents as stroke-like injuries); and 5) hemophilias. Classical hemophilia with factor VIII deficiency or Christmas disease factor IX deficiency, are both x-linked and have a prevalence in males of 1/5000 infants in all ethnic groups. Although not associated with bruising and hemorrhages, but with fractures, the molecular basis of an autosomal dominant form of *osteogenesis imperfecta* has recently been identified in the Amish population. This disorder most often presents in adults with early onset osteoporosis but some children have frequent fractures and could easily be mistaken for victims of abuse.

The important lesson is that bleeding in an infant, Amish or non-Amish, has a long differential diagnosis. Physicians must always make an *assumption of innocence* and understand that although unfortunately common in our violent society, child abuse is not the only cause of retinal hemorrhages and intracranial bleeding. All possibilities must be fully examined.

## CHICKEN BREAST DISEASE

One of the most commonly seen disorders at the Clinic is a lethal disease of muscle known within the Amish community as *chicken breast disease*. It has been said that there is always at least one child living with this disease within the community at any one time, and in the 11 years of the clinic's involvement in caring for children with this disease we know that has been true. It has always been lethal, the children have died at around two years of age. We remember each one and their families and have continued to keep our promise to work on understanding this disease. There is new hope.

**We now know the cause of chicken breast disease is a mutation in the gene for troponin T1 - a protein**



required for normal contraction and relaxation of large skeletal muscles. Drs. Jennifer Johnson, Les Biesecker and colleagues at the National Institutes of Health who have been working with us on this disease have identified the gene mutation that causes chicken breast disease. Their findings will be published in the American Journal of Human Genetics in October.

(Johnson JJ, Kelley RI, Crawford TO, Morton DH, Agarwala R, Kock T, Schaffer AA, Francomano CA, Biesecker LG. *A novel nemaline myopathy in the Amish caused by a mutation in troponin T1*, (American J Hum. Genet. in Oct. 2000).

**Troponin and the normal structure and function of muscle:** Skeletal muscle is made of collections of muscle fibers. Muscle fibers contain thick and thin filaments that can be seen with an ordinary microscope. When a nerve impulse causes a muscle to contract the rounded projections of the thick filament attach to the thin filament and pull the thin fibers inward. Contraction requires energy (ATP) to pull the thin fibers along the thick fibers, and calcium which is required for attachment of the thick filament heads to the thin filament. Relaxation of the muscle fibers requires that calcium be removed from the filaments and moved back into the storage area called the *sarcoplasmic reticulum*. As concentrations of calcium around the filaments decrease, troponin T1 moves to block the thin filament binding sites, muscle tension decreases, and the thin filaments pull away from the thick filaments back into the relaxed position.

In chicken breast disease, skeletal muscle troponin is missing from the thin filament because of a gene mutation and normal muscle fiber relaxation does not occur. The large muscle fibers of the shoulders, back, hips and upper legs, which are predominantly made up of slow type-1 muscle fibers, are affected. The type 1 fibers of these muscles degenerate and become increasingly weak and stiff. Troponin is also found in heart muscle and in fine fast muscles of the hands and eyes, but these troponins for other muscle types arise from different genes and are not affected in children with chicken breast disease.

**Features of the neurological examination & natural history of the disorder that allow diagnosis of the syndrome:** At birth affected infants have a normal appearing chest but have generalized jitteriness and ankle clonus. The jitteriness and clonus are similar in frequency and amplitude to that seen in normal infants but are abnormally sustained. Jittery movements that involve the large muscle groups of the legs, trunk, and upper arms, are provoked by spontaneous movements or by startle. The tremors have a frequency of approximately 5-10 cycles per second. Mothers usually report feeling the jittery movements throughout the last half of pregnancy. Such movements have also been reported during pregnancies with siblings of affected infants who subsequently do not have clinical disease, but may be carriers of the disorder.

In the first weeks after birth, tendon reflexes at the jaw, biceps, knees and ankles are increased and usually provoke generalized sustained jitters. Sustained ankle clonus is readily obtained by upward flexion of the foot and stretch of the

Achilles tendon. Large muscle groups also have an unusual feel not unlike muscles in *rigor mortis*. The fine muscles of the hands and forearm, eye muscles, and heart muscle are not affected by the disorder.

Affected newborns usually have restricted movements of the hip, shoulders, and jaw. The deformity of the chest wall, which gives the disorder the common name of "chicken breast disease," develops late in the course of the disease, usually between 4-12 months of age. Progressive involvement of the intercostal muscles restricts chest wall movement and finally appears to pull the ribs down and away from the sternum causing a striking chest wall deformity that may worsen markedly over a few days. Usually respiratory efforts become weaker over weeks and months. Aspiration and lung infections cause sudden deterioration.

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A search of the medical literature from 1963, when troponin was first discovered, through July of 2000 shows that much research has been done to understand this protein. More than 6500 papers have been published about the structure and function of troponin. The work of Jennifer Johnson, Les Biesecker and others has made this information relevant to the care of infants with chicken breast disease. The next challenge is to translate this new insight of the cause of a genetic disease into an effective treatment of the disease. In September physicians from the Clinic, National Institutes of Health, Johns Hopkins, and other medical centers interested in the treatment of disorders of muscle will meet at the Clinic to discuss new approaches to the management of troponin 1 deficiency. The physicians and scientists will also meet with families to answer questions about the significance of this discovery to the care of affected children. It is important to note this particular gene mutation could potentially be repaired by the method of gene repair referred to as *chimeraplasty*.

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## **NEW ABI PRISM 310 GENETIC ANALYZER**

Erik G. Puffenberger, PhD

The clinic currently offers carrier testing and newborn screening for 15 different genetic disorders. These DNA-based tests require knowledge of the causative gene for the disorder as well as the specific mutation within that gene. Once this information is known, a clinical test can be developed to detect the mutation. The information used to develop the current set of carrier and diagnostic tests has come from collaborations with research laboratories around the country. While this arrangement has provided important information, the priorities of a research laboratory are often at odds with ours and usually do not include clinical applications which are vital to the clinic. Thus, there is a need to pursue research on diseases relevant to clinic patients in a timely and accurate manner. In order to improve and expand our important clinical services, we are initiating genetic research projects in the clinic laboratory. This will allow us to concentrate efforts on those disorders where important research discoveries are needed to improve diagnosis and management. In addition, this will provide us with the tools and reagents to perform non-clinical

basic research on these disorders (e.g. population genetic studies). Research projects involve gene mapping, gene identification, mutation detection, and development of diagnostic services. To accomplish these aims, the clinic recently purchased an ABI Prism 310 Genetic Analyzer. The 310 Genetic Analyzer is an automated system for sequencing, sizing, and quantitating nucleic acids (i.e. DNA).

The new equipment allows us to sequence genes in order to identify mutations and polymorphisms. It is often difficult to solicit research laboratories to perform such analyses on patient samples. Since mutation identification is usually not supported by federal grants, most research laboratories will not perform these procedures. In addition, CLIA regulations prohibit the dissemination of clinical information from a research laboratory. In the future, the need to identify specific mutations in patients will increase, particularly if chimeraplasty becomes feasible on a large scale. Thus, to meet this need, we should be capable of performing our own mutation analyses as a clinical laboratory service for those disorders relevant to clinic patients.

Sequencing a gene from a patient provides us with the string of DNA bases (i.e. A, G, C, and T) which comprises the gene. The sequence from the patient is then compared to a consensus sequence for that gene which is stored in a computer database called GenBank. Once a sequence variant is found in the patient, we then determine whether the variant is a neutral change (i.e. polymorphism) or a mutation. We then use this information to develop a clinical test to detect the presence of

the variant in any DNA sample we choose to test. The clinic currently performs 15 different carrier tests as outlined in the following table. The first 14 of these mutations were identified in other laboratories. We collaborated with many research groups to identify the specific gene mutations in clinic patients. The latest mutation to be added to the list, the c.35G→A mutation for 3-B-hydroxysteroid dehydrogenase deficiency, was identified in the clinic laboratory using the new DNA sequencer. DNA was obtained from two Amish patients known to have this disorder. The entire gene was sequenced in these two individuals and their sequence was compared to the normal consensus sequence. Two variant DNA bases were identified in the patient samples. One change was a normal variant while the other, a G to A transition at nucleotide position 35 of the gene, altered the amino acid structure of the enzyme. This change was predicted to have a deleterious effect on the enzyme and thus cause disease. We studied 3-B-hydroxysteroid dehydrogenase deficiency first because it was difficult and costly to diagnose; however, the identification of the mutation has led now to an inexpensive and accurate test to diagnose this condition.

In anticipation of the chimeraplasty clinical trials, we also have initiated mutation analyses of the bilirubin-UDP-glucuronosyltransferase-1 gene (UGT1A1) on human chromosome 2q37. Mutations in this gene cause Crigler-Najjar syndrome. All Amish and Mennonite patients with this disorder carry the same mutation (see table). However, the clinic follows several Crigler-Najjar patients who do not harbor this particular mutation. To date, we have analyzed the UGT1A1 gene in two of these non-Plain patients and have identified the unique mutations which they carry.

#### Diagnostic DNA analyses performed at the clinic:

DISEASE	GENE	MUTATION	POPULATION
Aldosterone deficiency	CYP11B2	5 bp deletion	Amish
Byler disease	FIC1	c.923G→T	Amish
Congenital nephrotic syndrome	NPHS1	c.1481delC	Mennonite
Crigler-Najjar syndrome	UGT1A1	c.222C→A	Amish and Mennonite
Glutaric aciduria	GCDH	c.1298C→T	Amish
Glycogen storage disease, type 6	PYGL	IVS13+1G→A	Mennonite
Hirschsprung disease	EDNRB	c.828G→T	Mennonite
Maple syrup urine disease	BCKDHA	c.1312T→A	Mennonite
Medium-chain acyl-CoA dehydrogenase deficiency	ACADM	c.985A→G	Mennonite
Mevalonate kinase deficiency	MVK	c.803T→A	Mennonite
Nemaline rod myopathy	TNNT1	c.505G→T	Amish
Osteogenesis imperfecta	COL1A2	c.2098G→T	Amish
Pyruvate kinase deficiency	PKLR	c.1436G→A	Amish
Spinal muscular atrophy, type 1	SMN1	large deletion	Mennonite
3-B-hydroxysteroid dehydrogenase deficiency	HSD3B2	c.35G→A	Amish



The clinic was honored a second time with a nomination by Carleton S. Fiorina, CEO of Hewlett-Packard Company, for inclusion in the Smithsonian Institution's Permanent Research Collection at the National Museum of American History. The awards are sponsored by Computerworld and The Smithsonian to recognize innovative uses of technology. The clinic was selected for its innovations in the field of medicine. We thank Hewlett-Packard and Agilent Technologies for their recognition and for their continued support of the clinic.

**Lectures 2000 by Holmes Morton, M.D. :**

- \* Franklin and Marshall College, Department of Biology. Lancaster, Pennsylvania. March, 2000. *From gene to disease: The neurobiology of maple syrup disease.*
- \* University Hospital Rotterdam, The Netherlands April 2000. *Management of Crigler-Najjar Syndrome.*
- \* Academic Medical Center of Amsterdam, April 2000 Biochemical Genetics. *The neurobiology of maple syrup disease: Essential amino acid deficiencies and the brain.*
- \* Blanchard Memorial Lecture, Society of Teachers of Family Medicine Annual Spring Conference, Orlando FL, May 2000. *Genes, Diseases, & the Practice of Medicine in Year 2000.*
- \* Graduation Speaker & Honorary Degree Recipient Elizabethtown College. May 2000, *Difficult learning.*
- \* MSUD Symposium, Danvers, MA, June, 2000
- \* Visiting Scientist & Lecturer: National Youth Science Camp, West Virginia. July 2000. *The neurobiology of an inherited disorder of biochemistry.*
- \* Mid-Atlantic Connection for PKU and Allied Disorders Conference, Lancaster, PA, August 2000

Time To Muse....

**Butterflies**

*Butterflies in meadows -  
Beautiful, fragile, ephemeral -  
To see them tossed about by breezes  
And dancing from flower to flower  
I would think they would go nowhere.*

*I am told  
Butterflies endure the storms of summer  
and winter's cold  
and that, finally, they go far to the south -  
To Mexico I am told.*

*Butterflies in meadows remind me of children -  
Tossed and dancing.*

*I am told  
that children too endure the storms of summer and winter  
and that, finally, they go far.*

*Beautiful enduring travelers.*

by D.Holmes Morton, M.D.



**AMOS & SADIE'S FARM ~~~~~  
~~~~~A PATHWAY TO SAFETY**

Many children say the picture cards are the best part of the game. One teacher used it in her daily lesson routine, dividing the classroom into teams and taking a few questions each day. We hope the farm safety game is fun, challenging for all ages and will encourage lessons about safety around the farm.

*Amos & Sadie's Farm - A Pathway to Safety* leads children on a journey around a typical farm - through the fields, meadow, past a pond, manure pit, through a barn with hay holes and ladders, past equipment, across a road, past a machine shed, past all the animals to learn safety lessons along the way. It was invented by a collaboration between the Clinic for Special Children, Lancaster County Safe Kids Coalition and nurses from Penn State. Children's Miracle Network, a charitable foundation, provided funds to produce 300 copies of the game manufactured by YZ Printing in Elizabethtown, PA. Some of the initial work at the Clinic that led to the game was funded by a gift from Merck & Co.

The game's questions were compiled from stories in the Diary and Die Botschaft over several years, from admissions statistics at area hospitals, suggestions from Amish and Mennonite school children, and advice from Plain families involved in farming. This is the only game of its kind that reflects cultural traditions and farming methods and equipment on Amish and Mennonite farms. The game board was drawn by a young Amish woman and the picture cards were from photographs contributed by Jim Stansbury for the Safe Kids Coalition. Dr. Kathleen Fisher, a professor of nursing at Penn State, coordinated the project. The game is available in limited quantities at Gordonville Bookstore, Clay Bookstore, and Ken's Joys in Ephrata. Funds collected from the sale of the game will help produce more games if the demand is there or to fund other farm safety educational programs.

THE CLINIC FOR SPECIAL CHILDREN, INC.  
P.O. BOX 128  
STRASBURG, PENNSYLVANIA 17579



**Tenth Annual Benefit Auction**  
to support  
**THE CLINIC FOR SPECIAL CHILDREN**  
*Quilts Handmade Toys Furniture Crafts Baked Goods Chicken Barbeque*  
*Donations Appreciated*



**September 16, 2000**

*Bring your checkbook, no credit cards accepted*

Time: 8:30 am Location: Leola Produce Auction, Brethren Church Road, Leola, PA  
Directions: From PA Turnpike, exit 21 Rt. #222 south, exit to Rt. #772 south east, left on Peace Rd., 2nd right.  
From Lancaster: Rt. #23 east, turn left (north) on Brethren Church Rd. past Leola.  
From Rt. 30 east: right to Rt. #772 (Newport Rd.) north west to Rt. #23, right on #23 (New Holland Pike), left  
on Brethren Church Rd. Auction is approximately 1 mile.

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*The Clinic for Special Children is a non-profit diagnostic and primary pediatric medical service for children with inherited metabolic disorders in Lancaster County, Pennsylvania. The clinic serves Old Order Amish, Mennonite and other families who suffer from a high incidence of genetic diseases such as glutaric aciduria and maple syrup urine disease. Clinic services include infant testing programs for early diagnosis, primary medical care to prevent devastating effects of metabolic diseases, clinical research to improve treatment, and services to support the needs of parents. The Clinic is funded through a combination of fees for services, benefit auction proceeds and private contributions.*

*The Clinic is tax exempt under IRS 501 (c)(3), ID # 23-2555373.  
P.O. Box 128 Strasburg, PA 17579 (717) 687-9407*

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The Clinic for Special Children is a registered charitable organization. In accordance with Pennsylvania law, we are required to advise you that a copy of our official registration and financial information may be obtained from the PA Dept. of State by calling toll free 1-800-732-0999. Registration does not imply endorsement.