Special Article

Experience of the Manitoba Perinatal Screening Program, 1965-85

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The Manitoba Perinatal Screening Program is guided by a committee of medical specialists with skills in the diagnosis and management of disorders of metabolism in the newborn. The program is voluntary and is centralized at Cadham Provincial Laboratory, in Winnipeg. A filter card blood specimen is collected from newborns on discharge from hospital, and a filter card urine sample is collected and mailed to the laboratory by the mother when the infant is about 2 weeks of age. The overall compliance rates for the blood and urine specimens are approximately 100% and 84% respectively. The blood specimen is screened for phenylalanine and other amino acids, thyroxine, galactose, galactose-1-phosphate and biotinidase. The urine specimen is screened for amino acids, including cystine, as well as methylmalonic acid and homocystine. Between 1965 and 1985, 83 cases of metabolic disorders were detected, including 23 cases of primary hypothyroidism, 14 of classic phenylketonuria, 5 of galactosemia variants, 3 of galactosemia, 2 of maple syrup urine disease and 1 of hereditary tyrosinemia. The direct cost per infant screened is $5.50, and the cost:benefit ratio is approximately 7.5:1. Maternal serum α-fetoprotein screening is being made available as the necessary supporting clinical facilities become available. On the basis of this experience, the author outlines the components that are important for an effective screening program.

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Sous la direction d'un comité de médecins spécialistes, le Manitoba Perinatal Screening Program s'attache au diagnostic et au traitement des troubles métaboliques du nouveau-né. Son centre nerveux est le Laboratoire provincial Cadham à Winnipeg. Ce dépistage n'est pas obligatoire. Chez tout nouveau-né on fait deux prélèvements sur carte-filtre: un échantillon de sang à son départ de la maternité et un échantillon d'urine recueilli par la mère, qui l'adresse par la poste, quand l'enfant est âgé de quelque 2 semaines. Le taux de fidélité à fournir ces échantillons est d'environ 100% pour le premier et 84% pour le second. Dans le sang on recherche la phénylalanine et autres acides aminés, la thyroxine, le galactose, le galactose-1-phosphate et la biotinidase; dans l'urine on recherche la cystine et autres acides aminés, l'acide méthylmalonique et l'homocystine. Entre 1965 et 1985 on a dépisté 83 cas de maladies métaboliques, y compris 23 d'hypothyroïdie primitive, 14 de phénylcétonurie classique, 5 des variantes de galactosémie, 3 de galactosémie, 2 de leucinose et 1 de tyrosinémie. Le coût direct par nourrisson est de 5,50 $, le rapport de rentabilité d'environ 7,5:1. On offre aux gestantes le dosage de l'alpha-foetoprotéine sérique au fur et à mesure de la mise en place des services cliniques correspondants. À la lumière de ce qui précède, l'auteur indique les composantes nécessaires à l'efficacité d'un tel programme de dépistage.

The Manitoba Perinatal Screening Program was started on a voluntary basis in 1964–65 with the introduction of the Guthrie test for neonatal hyperphenylalaninemia on a pilot basis at the provincial laboratory. This initiative was taken in response to the interest and encouragement of

the pediatrician community. Since then all perinatal metabolic screening testing in Manitoba has been centralized in the Metabolic Diseases and Chemistry Section of Cadham Provincial Laboratory, in Winnipeg.

The objective of perinatal screening is to detect selected disorders that affect the developing fetus or newborn for which effective therapeutic measures are available and can be applied in time to allow for the normal mental or physical maturation of the infant.

Perinatal screening includes testing that is directed either at the mother in the prenatal period or at the newborn. Although there are many disorders that may affect the normal development of the fetus or newborn and that can be detected in the perinatal period, screening tests must be selective and must meet certain well-defined and accepted criteria. The chief criteria adopted by the Manitoba program include the following.

- Screening must be accessible to the population at risk, with account taken of the geographic distribution of the population and the available health facilities.
- The specimen must be easy to collect, stable and easy to transport.
- The test must be inexpensive and easy to perform.
- The sensitivity and specificity of the test must be such as to allow for no false-negative results and an acceptable proportion of false-positive results.
- The diseases screened for must have a high enough frequency and associated rates of illness or death to make testing economically practical.
- The program must have immediate access to an identified panel of specialists with backup technical resources who will be able to promptly investigate suspected cases and treat and manage confirmed cases.
- There must be effective and inexpensive methods of treatment or prevention that will be acceptable and of benefit to the patient.

In general, the diseases screened for are genetically determined disorders of metabolism, morphogenesis or endocrine function or some combination of these. All the tests are studied and put through a pilot evaluation period of at least 1 year before being adopted. The proportion of repeat specimens requested for confirmatory testing that gave a negative result on retesting (false-positive result) was 1% in 1986.

Perinatal Screening Committee

The Manitoba program is guided by a committee with special interest and experience in pediatric disorders of metabolism, endocrinology, genetics, biochemistry, and maternal and child health. Referrals for clinical or ancillary laboratory investigation are made to appropriate members of the committee who are also associated with the University of Manitoba's Department of Paediatrics and Child Health and with the Children's Hospital of Winnipeg. The committee operates within the framework of well-defined terms of reference. Briefly, these are as follows.

- To make regular (quarterly) reviews of the Manitoba program and of other national and international programs.
- To review and document all current data on findings of the Manitoba program.
- To evaluate new programs, review current literature and make recommendations to the Director of Cadham Provincial Laboratory.
- To consider the costs and funding of screening programs.
- To coordinate existing laboratory services.
- To establish contacts with similar groups in other provinces, states or countries.
- To develop and maintain a satisfactory relationship with consumers of the program and health professionals, particularly community physicians.

Screening methods and protocols

In 1969 the Guthrie test for hyperphenylalaninemia was replaced by a procedure involving descending paper chromatography, performed according to a modification of the method of Efron and colleagues.[1,2] The specimen continued to be dried blood collected on Schleicher & Schuell #903 paper (Schleicher & Schuell, Inc., Keene, New Hampshire). An overlay stain for histidine[3] was subsequently incorporated.

The Manitoba program was expanded in 1975 to include urine screening. A urine collection kit comprising a Schleicher & Schuell #903 filter card and an information brochure describing the purpose and scope of the program is distributed in a postage-prepaid, self-addressed envelope by the hospitals to mothers at the time of discharge. The mothers are asked to collect a urine sample on the filter card when the baby is approximately 2 weeks of age and mail the card back to the laboratory. In addition to identifying disorders not detectable in blood, such as Hartnup disease, cystinuria and methylmalonic aciduria, the urine specimen serves as a reference for missed initial blood specimens.

A synopsis of the protocol for blood and urine testing is outlined in Figs. 1 and 2. In both cases if all the tests give normal results, no report is generated.

The most recent addition to routine perinatal chemistry testing has been the introduction of maternal serum α-fetoprotein (AFP) screening, in 1985. This test is being made available as the necessary ancillary clinical follow-up facilities are developed. It is expected to be as valuable in the recognition of pregnancies at high risk due to multiple gestation, intrauterine growth retardation and prematurity as in the detection of congenital malformations.[4,5]

Detailed protocols have been devised for laboratory staff, providing clear guidelines as to when a particular finding should lead to a request to the attending physician for another specimen or when the result must cause immediate referral to one of the specialist committee members for clinical consultation and additional investigative procedures. All routine requests for repeat specimens are made by telephone, and a form letter outlining the reason for the request follows. Failure to comply within a given time results in automatic referral to the Maternal and Child Health Division of the Manitoba Department of Health, which pursues the request through its network of public health

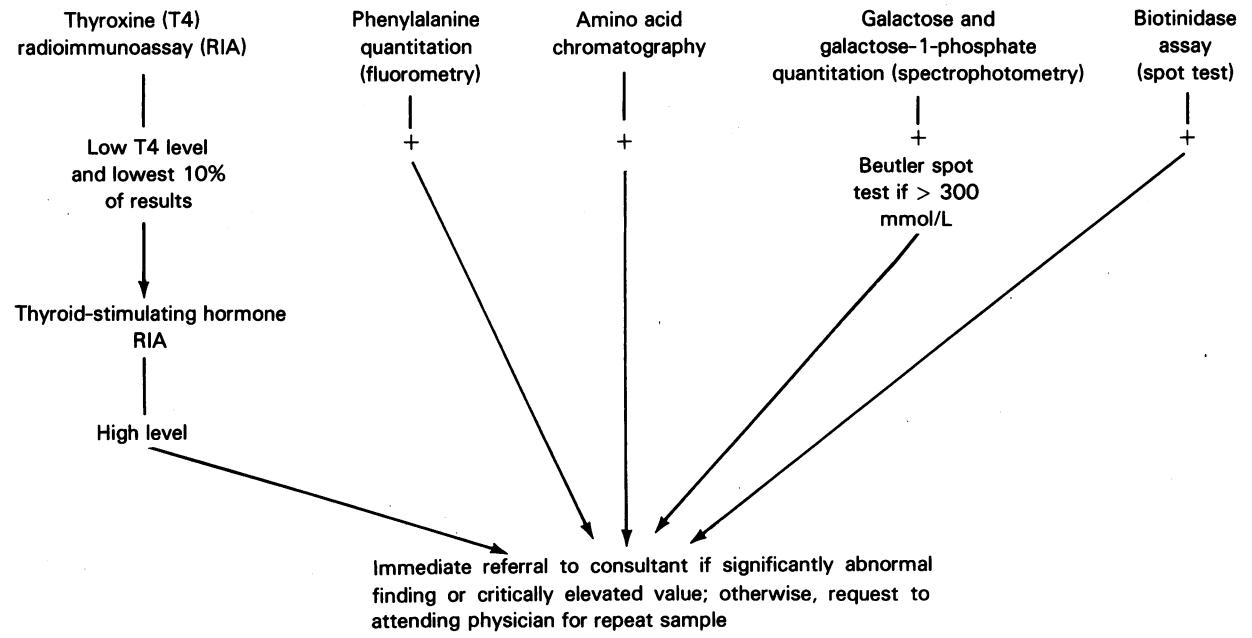

Fig. 1 — Simplified protocol for testing blood specimens in Manitoba Perinatal Screening Program.

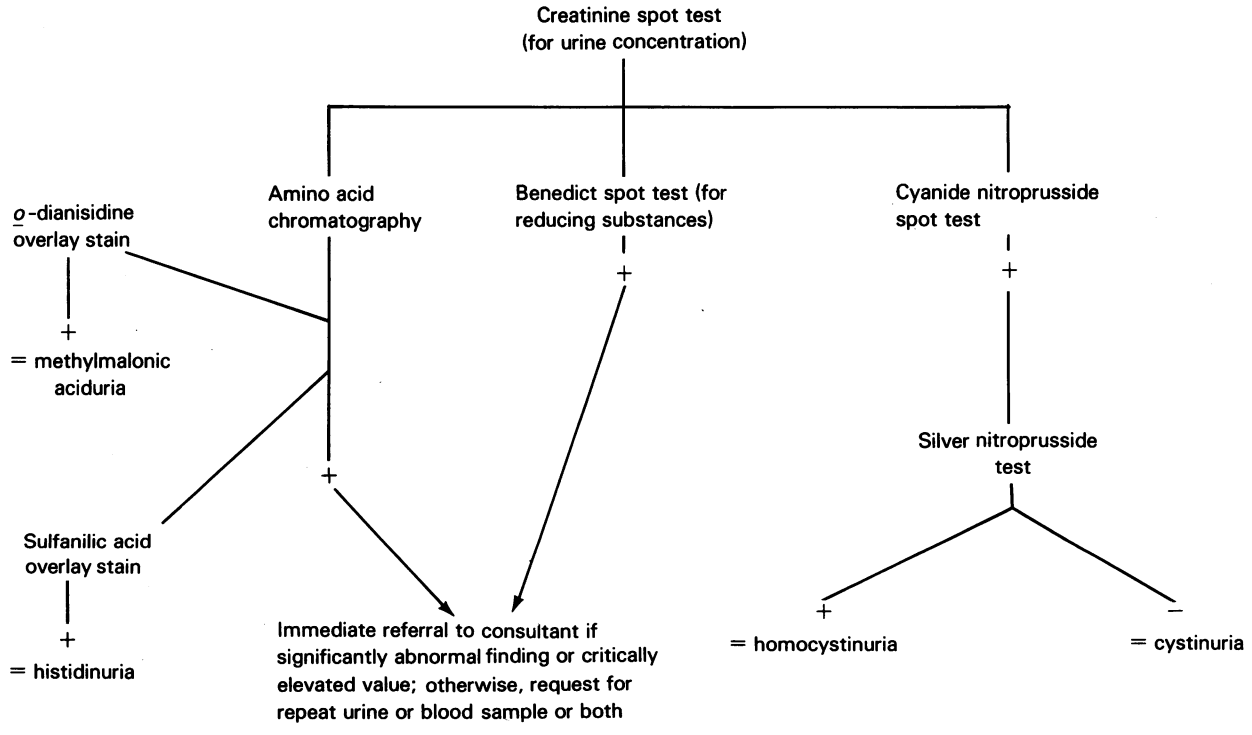

Fig. 2 — Simplified protocol for testing urine specimens.

nurses. Follow-up form letters are sent out when repeat specimens show that the abnormality has cleared or if more specimens are needed. The clerical component of the program, which formerly consisted of manual files, is undergoing automation.

Quality assurance is provided in two ways. Analytical precision is monitored for each test and specimen by the daily incorporation of control materials appropriate for each procedure. Accuracy is monitored regularly by participation in external proficiency test programs, such as those available from the College of American Pathologists, Centers for Disease Control, Atlanta (recently discontinued), and an international metabolic screening survey based in Heidelberg, Germany.

Table I — Compliance rates for the Manitoba Perinatal Screening Program

Type of screening	Result
Blood (1965–85)	
No. (and %) of infants screened	352 501 (97.0)*
No. (and %) of repeat tests	11 837 (3.4)†
Urine (1975–85)	
No. (and %) of infants screened	124 556 (81.5)‡

*The rate has increased to 100% in the last 10 years owing to acceptance of the program, public health education and hospital policy.
†Includes tests done because of improper collection of the initial specimen (2.4%) or the need for confirmation of the initial result (1.0%).
‡The rate increased to 84% in 1985.

Program findings

The results of the program for 1965–85 are presented in Tables I and II (derived from data provided through the courtesy of Dr. James C. Haworth, Department of Paediatrics, University of Manitoba).

Cost-effectiveness

In 1985 blood screening was carried out in 17 712 newborns and urine screening in 14 871 newborns, for compliance rates of 100% and 84% respectively. The direct cost of the tests (the costs of the technologists' time and of the reagents) per infant screened was $5.50. The indirect cost, including all screening program costs but not the salary of the coordinator, was $17, for a total cost of $22.50. Committee members, except for the consulting chemist, are not paid through the program. Clinical costs resulting from the program are borne by the respective hospitals. Comparison of our program costs with costs averted indicates a consistently high cost:benefit ratio.

Since 1977, 23 cases of primary hypothyroidism and 8 cases of phenylketonuria (PKU) alone were detected through screening. If we assume that 40% of these children (12) would have required lifelong institutional care and if we assume an average life span of 50 years and an average annual cost of custodial care of $40 000 per child, the costs averted would be $24 million. Given an average yearly total cost of the screening program

Table II — Results of the Manitoba Perinatal Screening Program, 1965–85

Metabolic disorder	Screening period	No. of confirmed cases*	No. of infants screened	Frequency rate	Frequency rate reported elsewhere
Phenylketonuria (PKU)	1965–85	16 (2)	352 501	1:22 031	1:14 254[6]
Histidinemia	1969–85	17	298 771	1:17 575	1:16 740[7]
Prolinemia	1969–85	3	298 771	1:99 590	
Maple syrup urine disease	1969–85	2	298 771	1:149 386	1:226 760[8]
Hereditary tyrosinemia	1969–85	1	298 771	1:298 771	1:700 000[9]
Glycine encephalopathy	1969–85	1	298 771	1:298 771	1:245 000[10]
Galactosemia	1969–85	6 (3)	298 771	1:49 795	1:60 000[11]
Compound galactosemia/ Duarte variant	1983–85	5	45 378	1:9076	1:3500[11]
Hypothyroidism	1977–85	23†	135 812	1:5905	1:7000[12]
Thyroid binding globulin deficiency	1977–85	2	135 812	1:67 906	
Cystinuria	1977–85	7	127 397	1:18 200	1:7000[10]
Hartnup disease	1977–85	5	127 397	1:25 479	1:26 000[10]
Total		88			

*Numbers in parenthesis signify cases not detected through the screening tests. In 1967 PKU in a sibling of a child known to have PKU was missed by the Guthrie test in a specimen taken at 3 days of age; fluorometric assay of a sample taken at 6 days of age gave a positive result. One case of PKU was missed by chromatography in 1970. One case of galactosemia was missed because screening was not done, one was in a sibling of a child known to have the disorder whose mother was put on a diet during pregnancy, and one was missed by chromatography before a quantitative assay was instituted. No other clinical cases have come to light in which screening had given negative results.
†Includes three cases of iodine trapping defect, two cases of ectopic thyroid and two cases of familial dyshormonogenesis.

of $400 000, the cost for 8 years would be $3.2 million. The cost:benefit ratio is thus 7.5:1. These calculations do not take into account the loss of human potential and earning power in undetected cases.

Recent improvements

Half of the disorders detected were diagnosed in 1981–85. This is attributable in part to the introduction of technical improvements and additional screening tests over the years. The change to chromatography in 1969 allowed for the detection of a wider range of aminoacidopathies. Two cases of maple syrup urine disease have been detected since this technique was implemented. An overlay stain for histidine and spot tests for cystine and homocystine in the urine sample also added to the scope of detection. However, the most significant additions to the program were the introduction of screening for congenital hypothyroidism, in 1977, and of automated quantitative screening for galactose and galactose-1-phosphate with a Multistat III microcentrifugal analyser (Instrumentation Laboratory, Lexington, Massachusetts), in 1983. The latter has provided a sensitive method for identifying the various types of galactosemia. Between 1983 and 1985 one case of classic galactosemia (uridyl transferase deficiency) and five cases of compound galactosemia/Duarte variant have been diagnosed.

Children with metabolic or endocrine disorders detected through the program are followed up regularly at specific clinics at the Children's Hospital of Winnipeg. Those identified to date who are receiving treatment appear to be developing intellectually and physically within normal limits.

Future developments

New screening procedures for disorders that meet selective criteria are constantly under review by the Perinatal Screening Committee and under methodologic investigation by Cadham Provincial Laboratory. The latest test to be investigated for its technical feasibility and introduced for a pilot period of laboratory and clinical appraisal is a biotinidase assay.[13] Biotinidase deficiency results in an inability to recycle the vitamin biotin. Patients with this disorder may suffer hearing loss, developmental delay and metabolic decompensation leading to coma and death during infancy or early childhood. If the disorder is detected at birth, inexpensive biotin supplementation of the diet can prevent neurologic damage and may be life-saving. It is important for cost-effectiveness reasons to establish the incidence of this disorder in Manitoba. It has been reported elsewhere in North America in approximately 1 in every 40 000 infants screened.[14]

Another assay awaiting final approval (it has met all the technical and feasibility criteria for universal screening) is that for detecting an elevated level of 17-α-hydroxyprogesterone, which is associated with the congenital adrenal hyperplasia syndrome. It is estimated that the true incidence rate of this disorder in most populations may be as high as 1 per 10 000 births.[15] Aside from the genital virilization in girls, this disorder is associated with an acute salt-losing crisis that occurs soon after birth in a significant proportion of boys and of girls thought to be boys; the crisis is often misdiagnosed and has a high death rate. Replacement steroid treatment in the neonatal period can result in normal development and life expectancy.

Other assays under preliminary consideration for possible implementation are screening tests for cystic fibrosis and for Duchenne-type muscular dystrophy.

A concern facing screening programs in the last several years has been the progressively earlier age at which samples are obtained and newborns discharged from hospital. Whereas formerly samples were obtained and infants discharged about the fourth or fifth day of life, after several days of protein feeds, programs of discharge at 48 or 24 hours — or even sooner — are gaining in popularity, especially in times of financial restraint. The recommended policy in Manitoba has been to obtain samples as close as possible to the time of discharge from hospital, regardless of the infant's age. The sensitivity of some screening tests, especially tests for elevated levels of amino acids, when done within hours of birth has not been satisfactorily established. Accordingly, some provincial and state programs require repeat sampling when initial samples are taken within 24 hours after birth, and others may prescribe delayed sampling. Repeat sampling is inefficient, time consuming, expensive and often incomplete, and delayed sampling results in a serious drop in the compliance rate.

In 1975 the blood amino acid chromatographic assay was supplemented with a quantitative fluorometric assay for phenylalanine, modified from that described by McCaman and Robins.[16] It is thought that this sensitive and specific additional assay should minimize the necessity for repeat sampling in infants in whom samples obtained shortly after birth give negative results. It is also expected that the early sampling experience gained by other, larger programs will clarify the relation of time to the sensitivity of the assays and the need for repeat sampling. The state of California reports that no missed cases have been documented when testing was done in the first 24 hours of life. They do not recommend rescreening infants who were tested early. They screen with automated fluorometry, using a cutoff point of 4.3 mg/dl (260 μmol/L) (George C. Cunningham, chief, Genetic Diseases Branch, Department of Health Services: personal communication, 1985).

Other common difficulties encountered are a delay in transporting the specimen to the laboratory, unsatisfactory collection of the specimen and

the necessity for repeat collection because of a previous abnormal test result. To address these difficulties Cadham Provincial Laboratory has prepared an audiovisual presentation on specimen collection and transportation for circulation to the province's hospital nurseries.

Essential elements of a screening program

In working toward an effective metabolic screening program we have found that the following components are necessary.

- Program centralization or regionalization is essential to provide the necessary expertise, experience and economics of operation. In Manitoba, with a population of just over 1 million, all testing is done at Cadham Provincial Laboratory.
- As important as accurate and efficient laboratory screening tests are, the overall effectiveness of the program will fall short of the mark if it is not supported by a designated group of consultants with expertise in the disorders encountered. These people must be readily available to the screening laboratory to interpret laboratory and clinical findings in consultation with the baby's attending physician so that an expeditious and appropriate plan of follow-up action can be instituted. In Manitoba the referring specialists are also members of the program's consultative committee and provide advice on the overall direction of the program.
- Program coordinators are required to liaise with the laboratory, the attending physicians and the appropriate medical specialists and reference facilities. In Manitoba one person is responsible for the newborn screening program, another for the maternal serum AFP program. These people are essential to the efficient coordination of the many interrelated facets of the programs.
- A documented referral protocol for laboratory staff, detailing the appropriate action to be taken for particular test results, is essential to efficient performance of the program.
- Technical methods need constant upgrading to ensure incorporation of the latest improvements. The screening laboratory must have well-qualified, experienced professional and support staff.

The clerical component of the program and the collation and interpretation of the demographic, medical and laboratory data can no longer be adequately done by manually searching hard-copy files. Writing an appropriate computer program is necessary to optimize the daily operation and the ongoing review and evaluation of the program.

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References

1. Efron M, Young D, Moser H et al: A simple chromatographic screening test for the detection of disorders of amino acid metabolism. *N Engl J Med* 1964; 270: 1378–1379
2. Fox JG, Haworth JC, Sekla L: Newborn screening for hereditary metabolic disorders in Manitoba, 1965–1970. *Can Med Assoc J* 1971; 104: 1085–1088
3. Shih VE (ed): *Laboratory Techniques for the Detection of Hereditary Metabolic Disorders*, CRC Pr, Cleveland, Ohio, 1973: 59
4. Sellers MJ: Is routine maternal serum α-fetoprotein testing a waste of time in an area of low incidence of neural tube defects? *J Obstet Gynecol* 1983; 3: 139–143
5. Doran TA, Valentine GH, Wong PY et al: Maternal serum α-fetoprotein screening: report of a Canadian pilot project. *Can Med Assoc J* 1987; 137: 285–293
6. Veale A: Screening for phenylketonuria. In Bickel H, Guthrie R (eds): *Neonatal Screening for Inborn Errors of Metabolism*, Springer-Verlag, Berlin, 1980: 7–18
7. Thalhammer D: Neonatal screening for histidinemia. Ibid: 59–64
8. Naylor E: Newborn screening for maple syrup urine disease. Ibid: 19–28
9. Halvorsen S: Screening for disorders of tyrosine metabolism. Ibid: 45–57
10. Shih VE (ed): *Laboratory Techniques for the Detection of Hereditary Metabolic Disorders*, CRC Pr, Cleveland, Ohio, 1973: 115
11. Gitzelmann R: Newborn screening for inherited disorders of galactose metabolism. In Bickel H, Guthrie R (eds): *Neonatal Screening for Inborn Errors of Metabolism*, Springer-Verlag, Berlin, 1980: 72–74
12. Dussault J, Coulombe P, Laberge C: Neonatal thyroid screening. In Fisher D, Burrow G (eds): *Perinatal Thyroid Physiology and Disease*, Raven, New York, 1975: 228
13. Wolf B, Grier R, Allen R et al: Biotinidase deficiency: the enzymatic defect in late-onset multiple carboxylase deficiency. *Clin Chim Acta* 1983; 131: 273–281
14. Wolf B, Heard G, Jefferson L et al: Clinical findings in four children with biotinidase deficiency detected through a statewide neonatal screening program. *N Engl J Med* 1985; 313: 16–19
15. Hofman LF, Klaniecki JE, Smith EK: Direct solid-phase radioimmunoassay for screening 17 α-hydroxyprogesterone in whole-blood samples from newborns. *Clin Chem* 1985; 31: 1127–1130
16. McCaman M, Robins E: A fluorimetric method for the determination of phenylalanine in serum. *J Lab Clin Med* 1962; 59: 885–890

Nature's heart

For faithful life-long study of science you will find no better example than John Hunter, never satisfied until he had the pericardium of Nature open and her heart throbbing naked in his hand.

— Oliver Wendell Holmes (1809–1894)

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