Screening for Galactosemia in New York State

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CONVENIENT blood tests for galactosemia (*1–5*) now permit wide-scale screening and early identification of babies affected, hopefully before irreparable damage or death ensues. In conjunction with New York State's mandatory screening program for phenylketonuria (PKU) at birth (*6*), we tested a large population of newborns for galactosemia with a fluorescent spot method (*5*) and found the procedure a practical means of identifying babies in whom galactosemia is likely to develop.

Materials and Methods

Whole blood samples on PKU cards from 141,402 infants were also tested for galactosemia (*5*). The infants were born in 1968 and 1969 and screened for PKU by the department's division of laboratories and research (*7*). The infants, born in the two New York State Health Regions of Albany and White Plains (including Long Island and excluding New York City), comprised nearly one-fourth of the live births recorded in the State during the period.

The spot test method, chosen for its ease and adaptability to the schedule of screening newborns for PKU, indicates activity of galactose-1-phosphate uridyl transferase by development of a fluorescent spot on filter paper after incubation of sample with substrate (see table). Blood from normal persons contains enzymes, including the transferase, which catalyze a series of reactions, converting galactose-1-phosphate to ribulose-5-phosphate through steps involving reductions of coenzymes from nonfluorescent (TPN) to fluorescent (TPNH) forms. Samples from persons with galactosemia have little or no transferase and fail to catalyze the conversions; thus their test spots do not fluoresce (*5, 8*).

The tests were carried out by incubating the dried blood samples on filter paper disks of $\frac{3}{16}$-inch diameter in the depressions of Disposo trays (*A*) containing 0.2 ml. of substrate for 3 hours or longer at 37° C. Aliquots of the incubation mixtures were spotted on sheets of No. 1 Whatman filter paper (*B*) after 3 hours and, if nonfluorescent, again 18–20 hours later. The spots were examined under long-ray ultraviolet radiation when dry.

Samples with nonfluorescing spots were also tested for glucose-6-phosphate dehydrogenase activity (*9*)—a deficiency in such activity may interfere with the transferase endpoint (*5*)—and for excessive galactose by paper chromatography (*10*). The annual cost of the program was approximately $10,000 for 70,000 samples, including technical assistance, reagents, and postage when carried out in the same laboratory used for PKU screening.

Results

Samples from 97 percent of the newborns had normal transferase activity, that is, fluoresced within 3 hours. All but a few of the remaining samples gave fluorescent spots after

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overnight incubation and were also considered normal. The samples which did not fluoresce after 3 hours were more frequent in the summer and were attributed to enzyme inactivation from excessive heat. Quenching by hydration on humid days may also have contributed to the delayed fluorescence, as such samples were fewer when the spotting sheets were stored in a desiccator.

The 143 samples which failed to fluoresce after 3 hours failed also after overnight incubation. Second samples from 132 of the newborns were received; 110 of these samples fluoresced after 3 hours' incubation and were considered normal. Physicians of the few infants from whom second or third samples were not received, as requested, were called; they indicated the infants were well and chose not to send in the later sample.

Second samples from 22 infants failed to fluoresce after either a 3-hour or an overnight incubation period. Third samples were requested at this point, and if the nonfluorescing spots of the previous samples were especially dark or if the paper chromatogram showed galactose, the physician was called to inform him of the abnormal result.

Nine of the 22 samples were from infants whose third samples were normal; six, from infants from whom third samples were not received and whose physicians indicated they were well; and seven, from infants classified after intensive clinical investigation by attending physicians or specialists as affected (homozygotes) or as carriers.

Three infants were homozygotes: one, who had carrier parents and a carrier sibling, died at 9 days of age with typical clinical and postmortem findings; one with hepatosplenomegaly, kidney damage, and a family history of galactosemia was treated and survived; and one healthy infant who excreted galactose and had carrier parents was recommended for prophylactic treatment. The fourth, a healthy infant with minimal enzyme activity and two carrier parents with different degrees of the transferase deficiency, may or may not be homozygous, pending the outcome of electrophoretic studies to differentiate it from a "double heterozygote" of the galactosemia/Duarte variant type (*11*, *12*). The three classified as heterozygotes had demonstrable enzyme and no clinical signs; single parents of two and both parents of the third were carriers.

Substrate for the detection of galactose-1-phosphate uridyl transferase activity in 300 samples of blood

Reagent	Milliliters
Uridine diphosphoglucose, 0.095 M	2
Galactose-1-phosphate, 0.27 M	4
Triphosphopyridine nucleotide, 0.066 M	6
Digitonin, saturated solution	8
Tris buffer,[1] 0.75 M, pH 8.0	20
Disodium ethylenediamine tetraacetic acid, 0.27 M	.3
Distilled water	19.7
Total volume	60

[1] 2-amino-2-(hydroxymethyl)-1,3-propanediol.

Fluorimetric measurements (*13*) of later samples were helpful in distinguishing between those with minimal and those with no visual fluorescence. The objective measurement identified a strikingly abnormal sample (a black spot) with greater certainty on the day of the initial screening test.

None of the samples was deficient in glucose-6-phosphate dehydrogenase. Galactose was detected in initial samples from three of the infants, two of whom were clinically affected homozygotes, and one was the possible "double heterozygote." The initial sample from the healthy homozygote did not contain galactose, although he excreted it at a later date.

The incubation periods in our tests were longer than those originally described (*5*), due perhaps to our use of a different sample, a dried blood spot which had been mailed. Although liquid samples of 10 λ volume and fresh blood spots both shortened the fluorescence time, such samples were impractical for our schedule.

Discussion

Our estimate of the incidence of galactosemia at birth, based on the number of infants with persistent transferase deficiencies and later classified as homozygotes, was four in 141,402 or one in 35,000, including the uncertain "double heterozygote." The figure compares with Beutler's estimates of homozygous galactosemia at birth of from one in 25,600 to one in 35,000,

based on the screening for transferase deficiencies of smaller (*8*) and heterogeneous populations (*14*) and corrected for the likely incidence of the Duarte variant. "Classic galactosemia," that is, infants with appropriate clinical signs at the time physicians were notified of abnormal screening results (by telephone on the day of the test, if strikingly abnormal), was certain in two of the 141,402 infants, giving an incidence of one in 70,000. This frequency was remarkably similar to an early estimate of the incidence of galactosemia at birth in Great Britain (*15*), projected from clinical reports of the disease.

Our finding of one and possibly two homozygotes without clinical signs, both white, adds to the growing evidence of heterogeneity of gene expression in the "inborn errors of metabolism." Several variants of galactosemia have already been described, and more are likely to be recognized as casefinding increases through screening programs.

Our acceptance of the samples which required 24 hours' incubation for fluorescence as normal may have excluded Duarte variants and some heterozygotes, because the test in the hands of its authors (*5*) and others (*16*) detected the homozygote only. Conversely, the greater incidences of galactosemia estimated by others (*17–21*) may have been due to the inclusion of heterozygotes and Duarte variants because they used more sensitive methods of detecting transferase deficiency.

The test is, indeed, a practical means of identifying infants likely to manifest signs of galactosemia. Coupled with prompt dietary measures, the test may prevent disease or death. Subsequent studies, for example, quantitative enzyme assays, electrophoresis identification of isozyme pattern, and characterization of the parents' phenotypes, are necessary, however, for classification of the disease form and for valid estimates of gene frequency from homozygote incidence.

Summary

In conjunction with New York State's mandatory screening program for phenylketonuria, 141,402 infants born in 1968 and 1969 were also tested for galactosemia.

The tests were carried out by incubating dried blood samples on filter paper disks in the depressions of trays for 3 hours or longer. Each depression contained 0.2 ml. of substrate. Aliquots of the incubation mixtures were spotted on filter paper and, if nonfluorescent, spotted again 18–20 hours later. The spots were examined under long-ray ultraviolet radiation when dry.

The annual cost of the program was approximately $10,000 for 70,000 samples.

Ninety-seven percent of the newborns had normal transferase activity. Of 143 infants whose samples failed to fluoresce, samples from 132 were tested a second time. Of these, 110 were considered normal.

Seven infants had persistent transferase deficiencies, and four of these were homozygotes or "double heterozygotes." The incidence of galactosemia at birth was considered one in 35,000.

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EQUIPMENT REFERENCES

(*A*) Disposo trays, Linbro Chemical Co., New Haven, Conn.

(*B*) Whatman chromatography paper No. 1., W. and R. Balston, Limited, England.

Tearsheet Requests

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Disposing of Clamshells

Disposing of waste clamshells by planting them in Delaware Bay as cultch (material that forms a spawning bed for oysters) is being studied by Delaware State health officials. The Environmental Control Administration's Bureau of Solid Waste Management is assisting the project through a $27,274 demonstration grant awarded to the Delaware State Board of Health in Dover. The State is providing $13,636 as its share of the total $40,910 first year costs.

Shell disposal poses a perplexing problem in all coastal States where mollusks are harvested and processed. When clamshells are dumped on land, they emit odors and attract flies. Clamshell wastes are estimated to amount to as much as 112.5 million pounds a year in the mid-Atlantic region alone.

The 2-year project is under the direction of Richard B. Howell III, of the State health board. The board is collaborating with the University of Delaware's Marine Laboratory to monitor water quality and spat (oyster egg) count. According to a marine biologist at the laboratory, scavengers such as crabs and eels clean the shucked shells within hours after deposit and prevent water pollution.