TABLE I. Infectability of Spleens of Susceptible (Chick) and Non-susceptible (Mouse) Species Bitten by *A. aegypti* Mosquitoes Vectoring *P. gallinaceum*.

Donors		Days to implant	No. positive donor bloods at implant	No. positive recipient bloods after implant	No. negative recipient chicks infected by later bites
Chicks	10	7	6	10	
"	10	6	1	10	
"	10	5	0	7	3
"	10	4	0	7	3
Mice	10	7	0	0	10
"	10	6	0	0	10
"	10	5	0	0	10
"	10	4	0	0	10

6 days in our propagating routines with *P. gallinaceum*. It is extremely unlikely that the 7 infections resulting from 10 implantations from chicks 5 days after their infecting bites, and the similar number after only 4 days, could have been due to transfer of infected erythrocytes; we never find organisms in the blood-stream this early following the bites of so few mosquitoes as were used here. In the case of the splenic implants from mosquito-bitten mice, there is no evidence that they contained viable forms of any sort, since no infections resulted from their presence in clean chicks no matter how long after the bites the implants were made.

Summary. When *mice* were bitten by *A. aegypti* mosquitoes infected with *P. gallinaceum* sporozoites, and portions of their spleens were subsequently implanted intraperitoneally in clean chicks after intervals of 4 to 7 days, the latter did not develop infections in any instance. When similar implantations were made from bitten *chicks*, there resulted an impressive number of infections that were not easily ascribable to transfer of infected erythrocytes from the blood-stream. The evidence strongly suggests that the well-known exoerythrocytic cycle of *P. gallinaceum* in the chick, a species that is fully susceptible to infection with sporozoites of the organism, does not find a counterpart in the mouse, a species that is not demonstrably susceptible to such sporozoite infection.

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Estimation of Frequency of Occurrence of Galactosemia in the Population. (28968)

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Since the discovery of the biochemical defect in the hereditary disease of galactosemia as a deficiency in galactose-1-phosphate uridyl transferase(1), a number of procedures have been developed to identify the homozygous diseased individual(2). These can be applied with certainty to give chemical support to the clinical diagnosis.

In several family studies where parents, siblings and other relatives have been sampled it has become obvious that identification of the heterozygous carrier of the dis-

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ease is possible with almost complete certainty, since the parents and some of the relatives possess levels of transferase intermediate between the diseased and normal individual. More positive confirmation of the trait as an autosomal recessive has thus been provided by quantitative chemical tests for transferase in relatives of the galactosemic.

In distinguishing the heterozygous carrier from the normal individual, varying degrees of success have been achieved depending upon the method of estimating transferase, and somewhat upon the experimentalist. Since there is an overlap in transferase levels of about 5% between the normal and heterozygous or carrier individual(3,4,5,6), it is not possible to classify an individual with absolute certainty as a heterozygote on the basis of chemical analysis by presently available methods. For clinical purposes, however, a family study of transferase levels will indicate the possibility of hereditary galactosemia with almost complete certainty, and family studies have been used in the present report to give added assurance of the classification of isolated heterozygotes in a general population.

A knowledge of the gene frequency would give a useful indication of the expected occurrence of the disease. In assessing the gene frequency by a survey of pediatricians, an estimate has been made(5) that one in 70,000 individuals is born with the disease, or from the Hardy-Weinberg equilibrium, one in 134 is expected to be a carrier. Many cases of galactosemia die suddenly, and often within 2 weeks after birth; therefore accurate clinical diagnosis may not always be possible.

During studies of various galactosemic families(4), several other populations have been sampled and transferase levels have been measured. From these chemical data estimates of the expected frequency of the occurrence of galactosemia have been made. The findings are summarized here.

Procedures. A group of 132 male penal prisoners, 67 random admissions to the local hospitals and 69 unclassified patients at a hospital for the mentally retarded have been sampled in addition to parents of galactosemics. The analyses were performed in accordance with the procedures of Bretthauer *et al*(7) and Nordin *et al*(8). As the enzyme is labile in red cells, care was taken to preserve the samples at ice temperatures for the 2 to 3 hours between veni puncture and analysis.

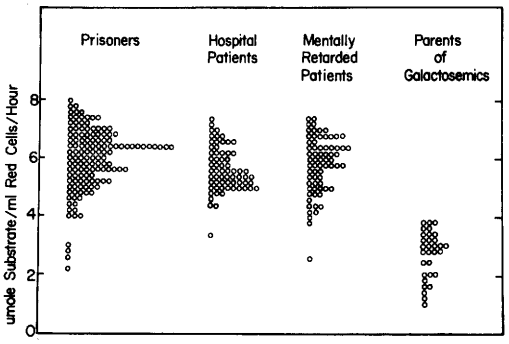

FIG. 1

In accordance with previous findings(4,7) individuals sampled in the present study with a level of transferase below 4.0 units were suspected to be heterozygous. As a further validation family studies were made wherever possible to give added significance to the identification of the heterozygote.

Results and discussion. The transferase levels found in the red cells from subjects in all categories sampled are shown in Fig. 1. Four of the prisoners and one each of the hospital patients and the mentally retarded patients are suspected heterozygotes on the basis of transferase levels equivalent to less than 4 μmoles of substrate per ml of erythrocyte per hour. In the case of each of the suspected heterozygotes it was possible to obtain one or more additional blood specimen. The transferase levels observed, although not always the same, agreed well and in no case did they change the classification (Table I). In 3 cases it was possible to verify the heterozygotes by doing family studies; 2 of the prisoners (C and D) and the mentally retarded patient (H). In each of these 3 examples at least one of the parents, as expected, may be classed chemically as heterozygotes (Table I). Further, 2 of the siblings of the mentally retarded patient (H) are clearly heterozygotes as is the mother but not the father. Heterozygotes are generally

TABLE I. Galactose-1-phosphate-uridyl Transferase Levels in Red Cells of Heterozygotes and Their Families.

Population	Family	Heterozygote	Mother	Father	Relatives
		μmoles of substrate per ml red cells per hr			
Prisoners	A	3.0 (3.0)*	—	—	
	B	2.2 (2.6)	—	—	
	C	2.6 (2.6)	4.3	3.7	
	D	2.8 (2.6)	3.7	3.7	6.3, 4.6, 5.1
Hospital patient	F	3.4 (3.6) (3.6)	—	—	
Mentally retarded patient	H	2.6 (2.2)	2.6	5.3	6.8, 6.5, 2.4, 2.9

* Value obtained on another sample of blood.

asymptomatic; thus it is of interest that the mother was a carrier in the case of the mentally retarded child. It may be that *in utero* toxicity of galactose contributed to the mental retardation. This possibility has already been suggested(5). Since mental retardation is sometimes associated with galactosemia, it is prejudicial to consider the mentally retarded in estimates of gene frequency. However, on the basis of this limited data this group does not appear to have a higher gene frequency than the other populations.

The number of homozygous diseased subjects that may be expected from such chemically identified heterozygotes has been estimated using the Hardy-Weinberg equation (Table II). The data, though limited, indicate that the gene defect may be more prevalent than heretofore suspected (perhaps 1 in 18,000 births) and galactosemia, though rare, will be seen in most hospitals with a large children's department.

During this study, a number of samples have been received from parents of clinical galactosemics and appropriate controls. From one hospital there have been 3 instances in which both parents were clearly classified as heterozygotes, thus confirming the clinical diagnosis (Table III). In one case (I) initially the transferase level was appreciable but this was due to a blood transfusion; however, at one year the red cells showed essentially no transferase. In a second case (J), the child died without sampling. That 3 galactosemics should be recognized by a pediatrics group within a few months gives further support to the possibility that the occurrence of galactosemia may be more frequent than estimates currently available. The possibility exists that galactosemia occurs more frequently in some sections of the United States than in Great Britain, where estimates of frequency were based on surveys of pediatricians(5). In any event it should be possible to extend chemical studies of isolated heterozygotes to include an adequate family sample to confirm the classification of suspected heterozygotes. The galactosemic infant can be best maintained on a galactose-free diet; therefore it is important to recognize the disorder as early as possible. Chemical identification of the heterozygote may now be made to predict the possibility of galactosemia in clinically questionable cases.

TABLE II. Calculation of Occurrence of Galactosemia from Chemical Estimation of the Heterozygote Frequency.

Population	Sample size	No. of heterozygotes	No. of galactosemics
Prisoners	132 (132)	4 (2)	1 in 4,300 (1 in 17,400)*
Hospital patients	67	1	1 in 17,900
Mentally retarded patients	69	1	1 in 19,000

* Estimated from the 2 prisoners where family studies were possible.

TABLE III. Galactose-phosphate-uridyl Transferase Levels in Red Cells of Galactosemics and Their Parents.

Family	Galactosemics	Mother	Father	Controls
	μmoles of substrate per ml of red cells per hr			
I	2.4 (0)*	2.6	2.0	4.8
J	—	1.6	2.6	5.4
K	0	2.8	1.4	5.5

* At one year of age.

Summary. From estimates of galactose-1-phosphate uridyl transferase levels in red

cells it has been estimated that galactosemia in humans may occur as frequently as 1 in 18,000 births. Since galactosemic heterozygotes are generally asymptomatic the identification of a heterozygote of maternal origin among the mentally retarded suggests the possibility that the symptomology may have resulted from inadequate metabolism of galacose by the mother during maternity.

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Effect of Erythropoietin on Cultured Human Leukocytes.* (28969)

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Phytohemagglutinin-stimulated cultures of mature human leukocytes produce a mitotically active cell which appears to be derived from lymphocytes(1,2). Since the lymphocyte has long been suspected of playing a role as stem cell(3,4), a primitive cell originating from lymphocyte precursors might be multipotential. This possibility and evidence that the hemoglobin type can be altered in irradiated mice by transplantation of leukemoid leukocytes(5) prompted us to study the effect of erythropoietin on cultured human leukocytes.

Method. Human peripheral blood leukocytes were cultured in the presence of phytohemagglutinin according to the technique of Moorhead *et al*(6). Cultures were set up in duplicate in each experiment so that one culture could serve as a control for the erythropoietin-treated culture. Erythropoietin was added in concentrations of .01, .05, 0.1, 0.5 and 2.5 units/ml culture medium at 0, 24 and 48 hours in separate experiments. At 66 hours colchicine was added to each culture and at 72 hours cells were harvested, exposed to hypotonic saline solution and fixed in methanol-acetic acid fixative. The cells were then resuspended and total cell counts determined. An aliquot of each culture was spread on a glass slide, air dried, stained with Orcein and studied for the per cent of cells in metaphase.

Results. Average total cell counts and per cent of cells in metaphase are shown in Table I. Erythropoietin appeared to have no significant effect on this cell system.

TABLE I.

Time of adding erythropoietin	Cell × 10^6/culture at 72 hr Mean (Range)		% cells in metaphase at 72 hr Mean (Range)	
	Control	Erythropoietin treated	Control	Erythropoietin treated
0	7.0 (3.3–9.6)	5.0 (2.1–7.0)	3.1 (1.9–5.0)	3.9 (2.0–4.8)
24	5.9 (3.5–8.8)	5.4 (2.0–9.4)	3.6 (1.5–5.8)	3.3 (2.2–4.6)
48	5.8 (4.0–6.9)	5.9 (5.2–6.5)	3.1 (2.0–6.1)	3.7 (1.6–7.4)

* The erythropoietin used in this experiment was sheep plasma erythropoietin concentrate, kindly supplied by the U.S.P.H.S.