know whether petroleum from wells separated by several miles comes from the same or from different sources. A knowledge of the content of these two hydrocarbons in the given wells may aid in solving this problem. With the information now available an analysis for these two compounds can be made by gas-liquid chromatography, using only retention times for identification provided samples of the pure hydrocarbons are available for calibration.

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Cystinuria: Genetic Heterogeneity and Allelism

Abstract. *Studies of four stone-forming cystinuric subjects from three unrelated pedigrees indicated that each was heterozygous for two of the three described mutant genes producing cystinuria (I, II, III). Their genotypes were I–II, II–III, I–III, and I–III, respectively. These doubly heterozygous patients were phenotypically indistinguishable from cystinuric homozygotes of genotype I–I, II–II, or III–III. The data provide the first direct evidence that all of the known mutations responsible for the genetic heterogeneity in cystinuria are allelic.*

Results of definitive studies (*1, 2*) on the genetics of cystinuria from 27 pedigrees indicated that the occurrence of cystinuria followed classical Mendelian laws for autosomal recessive inheritance. Homozygous affected subjects excreted large quantities of cystine, lysine, arginine, and ornithine in their urine. Renal, ureteral, and vesical calculi composed of cystine were formed because of the very limited solubility of cystine. The findings in heterozygous individuals were heterogeneous. In some families urinary dibasic amino acid excretion among heterozygous individuals (parents and children of affected subjects) was normal, whereas in other families, modest to marked excesses of cystine and lysine were excreted by all heterozygotes. Thus Harris suggested that cystinuria was, in fact, more than one disease entity. We have reported that there are three types of cystinuria (*3*). Type I was characterized by the absence of mediated intestinal transport mechanisms for dibasic amino acids in homozygotes and by normal urinary cystine values in heterozygotes. Type II differed most significantly in that heterozygotes excreted markedly increased quantities of cystine. In type III cystinuria, intestinal transport of all dibasic amino acids was retained by homozygotes, and heterozygotes excreted cystine in slight excess. Quantitative determinations of lysine, arginine, and ornithine in these heterozygotes have confirmed the differences noted in cystine excretion. Thus we have demonstrated statistically significant differences among type I, II, and III heterozygotes for each of the four dibasic amino acids measured (*4*).

In the first nine pedigrees studied, our results were in agreement with those of Harris (*1*), Crawhall (*5*), and their co-workers in that all heterozygotes within a single pedigree showed remarkably similar values for the aforementioned urinary amino acids. That is, there was no example in these nine pedigrees of more than a single type of heterozygote within a single family. Recently, however, we have found three unrelated families in which two types of heterozygotes have been demonstrated in a single pedigree. These results form the basis of this report.

Three individuals, G.F. (31-year-old male), J.P. (25-year-old male), and B.B. (35-year-old female), showed recurrent renal or ureteral cystine stone formation. Each excreted in the urine great excesses of cystine, lysine, ornithine, and arginine, the values being in the range noted for cystinuric homozygotes. However investigation of their families revealed (Figs. 1 and 2) that G.F.'s father (F.F.) showed a urinary amino acid pattern typical for a type III heterozygote (Fig. 1) while his mother (M.F.) and son showed a normal urine pattern characteristic of type I heterozygotes. These results were confirmed by analysis of three separate urine samples from each subject, and were strengthened by finding other type I and type III heterozygotes within the pedigree (Fig. 2). These data represent the first unequivocal demonstration of heterogeneity within a single cystinuric family. Similar, but not identical, results were encountered in the other two families (Fig. 2). J.P.'s father (H.P.) was a type II heterozygote (Fig. 1) while his mother (T.P.) was a type I heterozygote. Analyses for the four amino acids in urine samples from each of B.B.'s five children revealed that three were type II heterozygotes and two were type III heterozygotes (Fig. 2). Representative results from this pedigree also appear in Fig. 1. These findings suggested that J.P., B.B., and G.F. were not homozygous for one of the mutations producing cystinuria but, rather, were each heterozygous for two of the three mutant genes, their genotypes being I–II, II–III, and I–III, respectively.

These findings necessitated additional studies (Table 1) of intestinal amino acid transport in the affected probands —G.F., J.P., B.B., and E.M. (a paternal first cousin of G.F., once removed, with cystinuria and renal lithiasis). None of the patients from the three pedigrees were able to transport significant quantities of lysine, arginine, or cystine. The results were identical to those noted for type I homozygotes (*3*). However, oral tolerance studies with cystine did reveal subtle differences in both G.F. and E.M. in that there were small, but perceptible rises in plasma cystine (Fig. 3), results which were midway between those of type III homozygotes and type I homozygotes (*3*).

Such results indicate that the genetic heterogeneity in human cystinuria is even more complex than supposed. The pedigrees (Fig. 2) indicate that subjects heterozygous for two different mutations causing cystinuria are phenotypically indistinguishable from documented homozygotes, and thus the question of allelism must be considered.

Let us assume that cystinuria, like other disorders characterized by autosomal recessive inheritance, results from defective synthesis of a specific membrane polypeptide which catalyzes transport of the dibasic amino acids in gut and kidney. We can make certain pre-

Table 1. Uptake of dibasic amino acids by jejunal mucosa. Results are expressed as the ratio of number of counts per minute of C^{14} or S^{35} per milliliter of cell water to that in the incubation medium (*8*). The initial concentration of the medium was 0.065 m*M* for L-lysine and L-arginine and 0.03 m*M* for L-cystine. All incubations were carried out for 45 minutes in Krebs-bicarbonate buffer, *p*H 7.4 at 37°C. Numbers in parentheses refer to number of subjects or, for G.F., E.M., J.P., and B.B., to the number of determinations. Roman numeral designations in brackets refer to probable genotypes for G.F., E.M., J.P., and B.B.

Subjects	Distribution ratio ± 1 standard deviation		
	L-Lysine	L-Arginine	L-Cystine
	Controls		
	11.2 ± 1.6 (10)	28.3 ± 1.3 (4)	7.0 ± 1.4 (12)
	Cystinurics		
Type I	1.0 ± 0.3 (9)	0.9 ± 0.2 (4)	1.1 ± 0.2 (9)
Type II	1.0 [0.9, 1.1] (2)		2.4 [2.3, 2.5] (2)
Type III	4.2 ± 3.0 (4)	6.6 ± 3.3 (3)	4.1 ± 2.8 (4)
G.F. [I–III]	0.9 (3)	0.6 (2)	1.1 (2)
E.M. [I–III]	.9 (4)	.7 (2)	1.3 (2)
J.P. [I–II]	.9 (2)	.7 (2)	1.4 (2)
B.B [II–III]	1.0 (2)	.4 (2)	

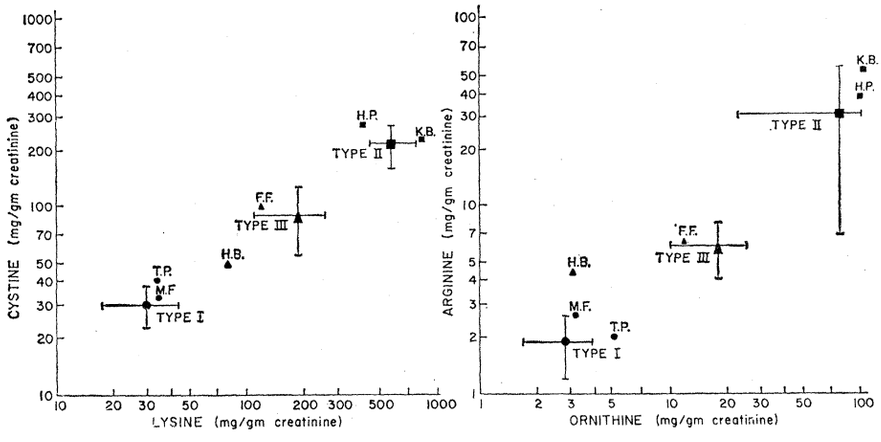
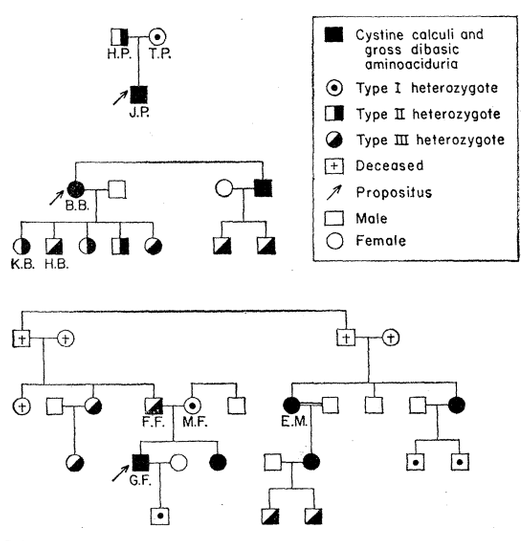


Fig. 1. Urinary dibasic amino acid excretion in heterozygotes for type I, II, and III cystinuria (mean ± standard deviation) and for pertinent heterozygotes from pedigrees presented in Fig. 2. Log-log plottings are used in both graphs. The mean values and standard deviations are based on previous results (*4*).

dictions about the genetic heterogeneity in cystinuria by considering the current definition of alleles as homologous segments of DNA on a single pair of chromosomes which direct the synthesis of a single polypeptide chain. If the dibasic amino acid transport system is pictured as a series of steps each under the direction of a separate pair of alleles, the observed heterogeneity could be due to mutations at any of the involved steps, the severity of the transport defects being dependent on the most rate-limiting step in the reaction sequence. By this scheme, however, a double heterozygote for type I and type II cystinuria would synthesize approximately 50 percent of the normal quantity of the polypeptides controlled by the I and II loci, and thus would not be expected to be any more seriously affected than a heterozygote for either type, barring complicating assumptions. The findings in G.F., E.M., J.P., and B.B. are contrary to this hypothesis and suggest an alternate explanation.

If the three major types of cystinuria result from different mutations at the same locus, as with hemoglobins A, S, and C (*6*), two consequences would follow. First, the transport differences in homozygotes and heterozygotes could be explained by postulating different structural or configurational alterations in the carrier protein or enzyme. Second, subjects heterozygous for two different mutations, like patients with hemoglobin S-C disease, would synthesize no normal protein and hence, might be expected to be as



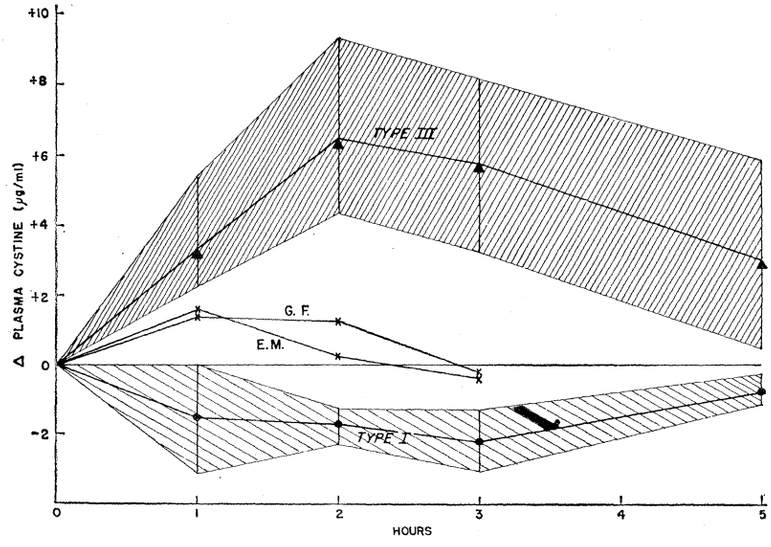


Fig. 2 (left). Pedigrees demonstrating intrafamilial heterogeneity. Types I, II, and III heterozygote designations are based on results described (*3, 4*) and summarized in Fig. 1. Fig. 3 (right). Results of oral cystine tolerance tests in G.F. and E.M. Values for types I and III homozygotes represent mean and range of three and four observations, respectively.

seriously affected clinically as a homozygote. The clinical and urinary findings in G.F., E.M., J.P., B.B. and their respective families are compatible with this formulation and provide the first direct evidence that the three known mutations responsible for the genetic heterogeneity in cystinuria are allelic. Additional families must be studied to test this hypothesis. The failure of others (*1, 2, 5*) to identify pedigrees with more than a single type of heterozygote may reflect either small sample size (*5*), or, as suggested by Harris (*7*), differences in newer methods of amino acid analysis.

Previous results of the intestinal transport and absorption studies indicate that cystinuric subjects with a proposed III–III genotype retained the ability to transport dibasic amino acids by the intestine, in contrast to subjects with a I–I genotype. Thus, it was not surprising that G.F. and E.M., whose genotypes appear to be I–III, responded to an oral cystine tolerance test with findings intermediate between those observed with I–I and III–III subjects. The transport studies in vitro, however, failed to distinguish G.F. and E.M. from type I homozygotes, an indication either that our techniques were not sensitive enough to detect such subtle differences or that the polypeptides coded by the involved loci are subunits of more complex protein molecules whose configuration is altered sufficiently by the presence of type I polypeptide to be essentially completely inactivated. Resolution of this most important question will demand identification and characterization of the postulated peptide carriers or enzyme molecules.

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Anaphase Delay after Inhibition of Protein Synthesis between Late Prophase and Prometaphase

Abstract. *The relationship between protein synthesis and mitosis was studied in* Physarum polycephalum, *a plasmodial slime mold whose nuclear divisions are synchronous. Results of studies with actidione (cycloheximide), an inhibitor of protein synthesis, indicated that the essential structural proteins for mitosis and nuclear reconstruction were completed prior to the dissolution of the nucleolus in prophase. Proteins that determine the duration of the transition from metaphase to nuclear reconstruction were synthesized from late prophase to prometaphase. It is proposed that these proteins are concerned with the transformation of chemical energy into the mechanical work of mitosis.*

In a study concerning the effect of actidione [cycloheximide, an inhibitor of protein synthesis (*1*)] on mitosis (*2*) it was shown that proteins necessary for mitosis were synthesized until late prophase, which is the time of nucleolar dissolution, but after that time morphologically normal mitosis and nuclear reconstruction occurred in the absence of protein synthesis. Most mitoses and nuclear reconstructions proceeded at or near the normal rate during inhibition of protein synthesis, but a significant proportion of the cultures were delayed in their transition from metaphase to nuclear reconstruction. We have tried to establish the relationship between the time of treatment with actidione and this delay in anaphase.

We have designed a procedure for critically timing metabolic events relative to the highly synchronous metaphase of the plasmodial slime mold *Physarum polycephalum*. Cultivation of *Physarum* and preparation of synchronous cultures were as described by Mittermayer, Braun, and Rusch (*3*). We began each experiment with a compact, vigorously growing plasmodium to insure good synchrony in all parts of the plasmodium. When late prophase was detected (*4*), the plasmodia were cut into quarters, and at set intervals three of the quarters were transferred to petri dishes and treated with 30 μg of actidione per milliliter; the fourth quarter served as an untreated control. Treatment with actidione preceded metaphase in every case. Smear preparations were made from all of the quarters at different intervals, and these smears were stored for microscopical observation and scoring at the end of the experiment. The scoring procedure permitted determination of the precise time of metaphase in the control quarter; this value was used as a reference point for the treated quarters. When untreated plasmodial quarters from a single plasmodium were compared according to a procedure identical to the experimental one, the time of metaphase varied by no more than two minutes.

Our data (Table 1) confirm the earlier observations on prophase blockage (*2*) (note the last line of Table 1) and clarify variations in the speed of transition from metaphase to reconstruction. They also show that treatment with actidione during very late prophase causes a slow transition from metaphase to reconstruction, whereas actidione treatment during prometaphase has no effect on the duration of mitosis. In both cases, nevertheless, the reconstructed nuclei are morphologically identical to the nuclei in untreated plasmodia. Our observations (Table 1) also indicate that the effect of actidione on complete prophase blockage is expressed rapidly because treatment 13 minutes before metaphase (just before the nucleoli dissolved) totally blocks mitosis, while treatment 11 to 12 minutes before metaphase (the period

Table 1. The effect of treatment with actidione in prophase and prometaphase.

Start treatment (minutes before metaphase)	Plasmodia observed (No.)	Time between metaphase and early reconstruction* (min)
	Prometaphase	
1	2	5, 5
2	2	5, 5
3	1	5
4	2	5, 10
5	3	5, 5, 20
	Late prophase	
7	2	30–60, 60–120
8	1	60–120
10	1	60–120
11	1	60–120
12	4	60–120, 60–120, P†, P
	Prophase	
13	4	P, P, P, P

* The time of transition in untreated plasmodia is normally 5 minutes. † P represents prophase blockage.