

Transport and Metabolism of Sarcosine in Hypersarcosinemic and Normal Phenotypes

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ABSTRACT An adolescent male proband with hypersarcosinemia was discovered incidentally in a French-Canadian family; no specific disease was associated with the trait. The hypersarcosinemia is not diminished by dietary folic acid even in pharmacologic doses (30 mg/day). The normal absence of sarcosine dehydrogenase in cultured human skin fibroblasts and in leukocytes was confirmed, thus eliminating these tissues as useful sources for further investigation of mutant sarcosinemic phenotypes and genotypes.

The response in plasma of sarcosine and glycine, after sarcosine loading, distinguished the normal subject from the subjects who were presumably homozygous and heterozygous for the hypersarcosinemia allele. Sarcosine clearance from plasma was delayed greatly ($t_{1/2}$, 6.1 hr) in the presumed homozygote and slightly ($t_{1/2}$, 2.2 hr) in the presumed heterozygote, while plasma glycine remained constant in the former and rose in the latter. Normal subjects clear sarcosine from plasma rapidly ($t_{1/2}$, 1.6 hr) while their plasma glycine trend is downward. The phenotypic responses suggest that hypersarcosinemia is an autosomal recessive trait in this pedigree.

Renal tubular transport of sarcosine was normal in the proband even though he presumably lacked the sarcosine oxidation which should normally occur in kidney. Sarcosine catabolism is thus not important for its own renal uptake.

Sarcosine interacts with proline and glycine during its absorption in vivo. Studies in vitro in rat kidney showed that sarcosine transport is mediated, saturable, and energy dependent. Sarcosine has no apparent transport system of its own; it uses the low K_m transport sys-

tems for L-proline and glycine to a minor extent and a high K_m system shared by these substances for the major uptake at concentrations encountered in hypersarcosinemia. Intracellular sarcosine at high concentration will exchange with glycine on one of these systems, which may explain a paradoxical improvement in renal transport of glycine after sarcosine loading in the hypersarcosinemic proband.

INTRODUCTION

Hypersarcosinemia with sarcosinuria was first reported in 1965 by Gerritsen and Waisman (2), and subsequently by Hagge, Brodehl, and Gellissen (3) and Scott, Clark, Teng, and Swedberg (4). Only four patients are represented by these reports, but many additional patients have since been reported to Gerritsen.¹ We wish to describe the results of our investigation of another subject with sarcosinemia (1), in whom there is no apparent disease associated with the biochemical disorder, thus affirming the now prevailing impression¹ that sarcosinemia is a "nondisease." We found no expression of the presumed block at sarcosine dehydrogenase (sarcosine:O₂ oxidoreductase EC 1.5.3.1) in fibroblasts or leukocytes from our patient, since this enzyme is not active in these tissues normally. Since tetrahydrofolate is apparently involved in the transfer of the formaldehyde group from sarcosine (5), we also evaluated the patient's response to treatment with folic acid in vivo; the trait was not folate responsive in this pedigree. A partial impairment of sarcosine clearance from plasma was demonstrated in the presumed heterozygotes for this trait, indicating that the inheritance of sarcosinemia in man is probably autosomal recessive.

Hereditary hypersarcosinemia provides a valuable opportunity to study the renal transport of sarcosine, since

¹Gerritsen, T. 1970. Personal communication.

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oxidation of this amino acid, which occurs normally in mammalian kidney will not contribute to measurements of its renal uptake *in vivo* in the patient with hypersarcosinemia. We were able to evaluate the characteristics of sarcosine transport both *in vivo* in the proband and *in vitro* by using rat kidney cortex slices. Although sarcosine appears to have no membrane transport system of its own, it readily enters renal tissue on the membrane systems used by imino acids and glycine (6).

METHODS

Studies in vivo. Endogenous renal clearance rates and net tubular reabsorption rates of sarcosine and other amino acids were evaluated by the methods described previously (7, 8).

Loading tests were performed with sarcosine (free base, 100 mg/kg body wt) taken as a solution in apple juice at 9 a.m. after an overnight fast. Heparinized venous blood was drawn from the antecubital vein just before the load (0 hr) and at 1, 2, 3, 4, and 5 hr after the load. Plasma was deproteinized immediately with sulfosalicylic acid (3% w/v; plasma: acid, 1:5). Timed urine collections were made before and during the sarcosine load.

Amino acids were measured by elution chromatography on ion exchange resins according to the methods of Spackman, Stein, and Moore (9) using a Beckman-Spinco model 120 analyzer, modified for rapid multiple analyses (10).

Studies in vitro: transport. Female Long-Evans rats, weighing about 160 g, were killed by decapitation. For studies of sarcosine transport, the kidneys were removed immediately, and cortex slices were prepared and incubated in Tris-electrolyte-glucose buffer (6). The measurements of amino acid uptake and determinations of sarcosine metabolism and oxidation were carried out by methods described previously (6).

Tissue culture studies. Skin fibroblasts from normal subjects were subcultured according to the method of Hayflick (11). About 5×10^6 cells were incubated in a Warburg flask in isotonic medium, pH 7.3, containing cold and ^{14}C -labeled sarcosine final concentration 0.5 mM. Induction of sarcosine dehydrogenase was tested by culturing cells for 18 hr in the presence of sarcosine (1 mM and 10 mM). Intact and sonicated cells were used in all experiments to determine whether uptake limited their capacity for sarcosine oxidation.

Leukocyte studies. Mixed leukocytes were isolated from venous blood by the method of Zucker and Cassen (12). The recovery of mixed leukocytes is 60–70% by this method. Cells were sonicated (22 kc/sec for 3–5 sec at 0°C) and incubated for 45 min as described for fibroblasts.

Homogenates. Rat tissues were homogenized in 0.25 M sucrose with a Potter-Elvehjem homogenizer. The centrifuged homogenate (0.1 ml) was then resuspended and incubated for 60 min in Warburg flasks containing Tris buffer and substrate.

Sarcosine dehydrogenase activity and sarcosine oxidation. Oxidation was measured with unlabeled sarcosine (1 mM) and sarcosine- ^{14}C (0.1 mM); $^{14}\text{CO}_2$ was collected on filter paper (2 cm square soaked with saturated KOH (50 μl)). The papers were dried under vacuum and counted in scintillation fluid. The efficiency of CO_2 collecting by this method was $57.7 \pm 4.9\%$. Sarcosine dehydrogenase was assayed specifically using methyl- ^{14}C -labeled sarcosine as

substrate, by the isotope method of Rehberg and Gerritsen (13). Pyruvate dehydrogenase was used as a control assay (14) for cell viability in these studies.

Chemicals. Sarcosine- ^{14}C (SA 2 mCi/mmmole) and sarcosine- $^{14}\text{CH}_3$ (SA 3.5 mCi/mmmole) were purchased from New England Nuclear Corp., Boston, Mass. Their radiochemical purity was confirmed by partition chromatography and high voltage electrophoresis in several systems. Unlabeled amino acids were obtained from Mann Research Labs. Inc., New York. The scintillation mixture for isotope counting contained 4.0 g 2,5-diphenyloxazole (PPO) and 0.1 g 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) in 1 liter of toluene; both scintillants were obtained from the Packard Instrument Co., Inc., Downers Grove, Ill.

Counting was performed in a Unilux II Nuclear-Chicago liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.) operating at 40% efficiency.

Calculations. The half-life for sarcosine disappearance from plasma was calculated by plotting the plasma response on log scale against time (arithmetic scale) and fitting the best straight line to the points by the minimum root mean square method; half-time for disappearance was obtained directly from the slope.

Data for distribution ratios in transport studies indicate an isotope distribution ratio (counts per minute per unit time per milliliter intracellular fluid: counts per minute per milliliter of initial incubation medium) unless stated otherwise. Net uptake velocity was corrected for the nonsaturable component by the method of Akedo and Christensen (15). The steady-state conditions used in our studies allow satisfactory estimates of the Michaelis constant for uptake of an amino acid (16), and under these conditions biphasic uptake kinetics were observed when the uptake velocity of sarcosine was examined in relation to its concentration in the medium. Assuming that the observed sarcosine uptake was the sum of one or more components, then:

$$u_{\text{observed}} = u_1 + u_2 \dots + u_n \quad (1)$$

V_{max} and K_m for each component can then be determined by solving:

$$u = \frac{V_{\text{max}_1}[S]}{K_{m_1} + S} + \frac{V_{\text{max}_2}[S]}{K_{m_2} + S} + \frac{V_{\text{max}_n}[S]}{K_{m_n} + S} \quad (2)$$

A revised computer method (17), adapted from our earlier work (6, 18), was used to solve this equation.

RESULTS

The proband

L. V. was born 8 August 1959, the fifth son of non-consanguineous French-Canadian parents. He developed normally to the age of 13 months, when an episode, diagnosed as poliomyelitis led to a mild spastic paraplegia. Investigation performed in 1969 during an orthopedic admission to another hospital included chromatographic examination of amino acids in urine. This showed the presence of a ninhydrin-positive substance subsequently identified as sarcosine. Sarcosine was also present in blood in concentration varying from 0.18 to 0.76 $\mu\text{moles/ml}$.

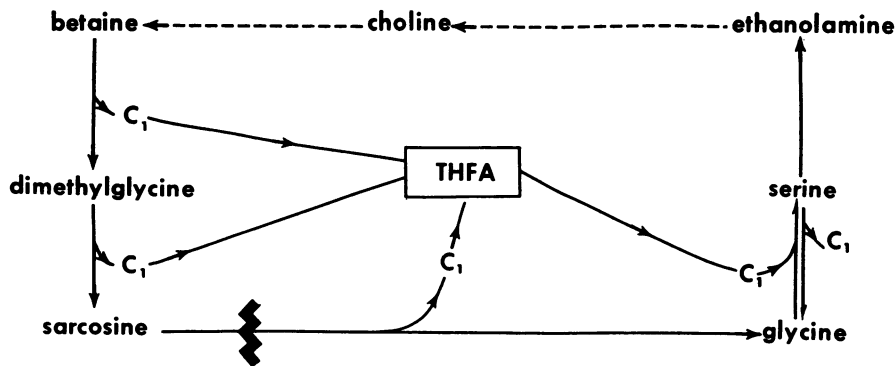


FIGURE 1 Scheme depicting sarcosine metabolism. THFA indicates the presumed N^5 , N^{10} -methylenetetrahydrofolate derivative which serves transfer of the 1-carbon formaldehyde group formed during oxidation of sarcosine to glycine. The presumed block in sarcosine metabolism is indicated.

Identity of sarcosine

The identification of sarcosine in urine and plasma was performed by partition chromatography on Whatman filter paper No. 4 in three different solvent systems (water-saturated phenol; lutidine:water, 2.2:1; and butanol:acetic acid:water, 12:3:5); by high voltage electrophoresis on Whatman filter paper No. 3 MM soaked in formic, acetic buffer pH 2, and by column chromatography on ion exchange resins. Sarcosine (free base) was run in all systems to identify its position. The unknown spot and the standard cochromatographed in the five systems and yielded identical color reactions with ninhydrin in the partition and electrophoretic systems; the 570 $m\mu$ to 440 $m\mu$ OD ratio after elution chromatography was identical for unknown and standard. There was no other ninhydrin-positive material in equivalent amounts in the patient's urine.

Sarcosine metabolism

Response to folic acid *in vivo*. The proband was given an excess of folic acid (30 mg/day; normal requirement about 1 mg/day) for 3 consecutive days. The rationale for this trial is indicated in Fig. 1. The methyl group is transferred as formate from sarcosine, presumably as an N^5 , N^{10} -methylenetetrahydrofolate derivative of folic acid. Fasting concentrations of sarcosine in plasma were 0.18 and 0.34 μ moles/ml before and after folate supplementation, respectively. The latter value falls within the range for plasma sarcosine in this patient when no folate was administered. The values for Δ -sarcosine in plasma after a sarcosine load by mouth (the sum of the difference between values at 1, 2, 3, 4, and 5 hr after the load, and the preload value) were +1.02 μ moles/ml·5 hr before and +2.18 μ moles/ml·5 hr after folate, indicating that folate did not influence sarcosine catabolism in this patient.

Response to sarcosine loading. Sarcosine loading by mouth was performed on the proband, his parents, and four normal adult subjects. The half-time for plasma sarcosine disappearance rates in each individual in the three groups and the simultaneous change in glycine, the product of sarcosine conversion, are shown in Fig. 2.

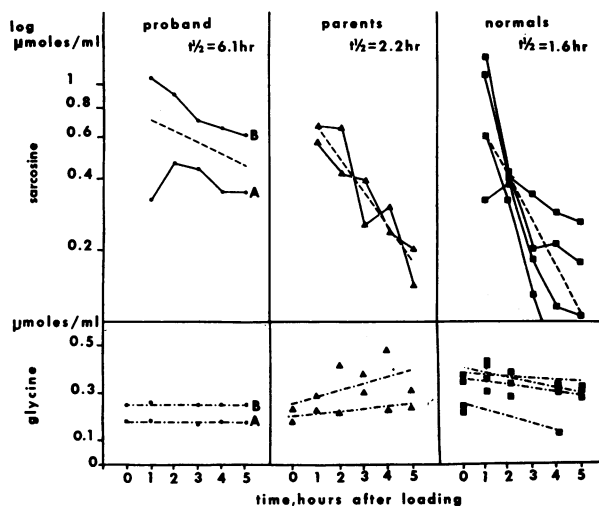


FIGURE 2 Response of sarcosine and glycine in plasma to sarcosine loading (100 mg/kg body wt given by mouth at 9 a.m.). Upper portion: A and B indicate studies in proband before and after folic acid supplement, respectively. Half-life for sarcosine clearance from plasma ($t_{1/2}$) was calculated as described under Methods; the interrupted line is the average decay slope for each group studied. The initial (preload) concentration of sarcosine in plasma was: zero in both parents and all normal subjects, and 0.18 μ M and 0.34 μ M in studies A and B, respectively, in the proband. Lower portion: Change in plasma glycine with time after sarcosine loading. Dotted lines define the average trend for each subject in the group. Changes in serine are not shown since they paralleled glycine.

TABLE I
Sarcosine Oxidation and Dehydrogenase Activity
in Tissues of Man and Rat

Tissue source	Oxidation to CO ₂ * dpm/hr per g wt
Human	
Skin fibroblast	0
Mixed leukocytes from blood	0
Rat	
Liver†	15,750
Kidney‡	6,375
Muscle	0
Brain	0
Spleen	0

* Sarcosine dehydrogenase activity was also examined by a specific assay (13). Oxidation and specific dehydrogenase activities complemented each other in all tissues examined.

† Organ weights (average from 10 rats): liver, 6.5 g; both kidneys, 1.6 g.

Normal subjects showed rapid disappearance of sarcosine from plasma ($t_{1/2}$, 1.6 hr). The concentration of glycine in plasma actually declines modestly during this period. In the proband, sarcosine clearance from plasma was greatly delayed ($t_{1/2}$, 6.1 hr), and there was no change in plasma glycine. Both parents had a slightly delayed disappearance of sarcosine ($t_{1/2}$, 2.2 hr), but the most striking finding in them was a modest but steady rise in the concentration of glycine in plasma after sarcosine loading. Changes in the concentration of serine in plasma in these studies paralleled those of glycine.

Sarcosine metabolism in vitro. No oxidation of sarcosine or evidence for specific dehydrogenase activity

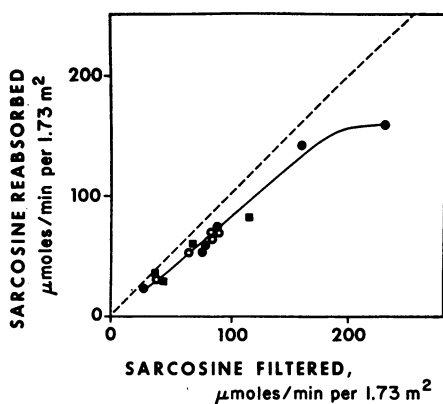


FIGURE 3 Renal tubular reabsorption of sarcosine in man, plotted in relation to its filtered load: in proband (●); in the patient of Brodehl and associates (3)^a (○); and in a normal subject (■). Plasma sarcosine was raised by venous infusion method, and glomerular filtration was monitored with inulin (8).

was detected in skin fibroblasts cultured for at least 14 passages from explants grown from the proband and two normal subjects (Table I). No activity was induced by exposure of cells for 18 hr to sarcosine (1.0 mM and 10 mM in the growth medium). Neither intact nor sonicated cells exhibited dehydrogenase activity under any of these conditions.

Leukocytes obtained from venous blood of normal subjects and the patient contained no specific sarcosine dehydrogenase activity. Measurement of sarcosine oxidation to CO₂ yielded similar negative results.

Tissue specificity of sarcosine oxidation (in the rat). The tissue specificity of sarcosine oxidation was examined in the Long-Evans rat. Significant oxidation of sarcosine was found only in liver and kidney (Table I). When the relative weights of liver and kidney were taken into account, we found that kidney accounts for 10% of total body oxidation of sarcosine in the rat, a value close to that reported for a different strain of rat by Rehberg and Gerritsen (13).

If it is assumed that man resembles the rat in his ability to oxidize sarcosine, we should expect sarcosine accumulation to occur initially in liver and kidney in the "blocked catabolic mutant" we know as hypersarcosinemia. Therefore, we examined the transport of sarcosine in kidney to determine whether it is impaired in this metabolic derangement.

Sarcosine transport in human kidney in vivo

Tubular absorption. The filtered renal load of sarcosine in the proband varied between 54.2 and 119.7 μmoles/min per 1.73 m² under fasting conditions; the corresponding value is almost zero in normal subjects. The equivalent endogenous renal clearance rates for sarcosine in the proband were about 8 ml/min per 1.73 m², indicating that the majority of filtered sarcosine experiences net tubular absorption in the sarcosinemic phenotype. Tubular reabsorption of sarcosine in our patient is probably a saturable phenomenon, although true saturation was not demonstrated. The T_m for sarcosine was estimated to be at least 160 μmoles/min per 1.73 m² (Fig. 3). Data on tubular absorption of sarcosine in another sarcosinemic patient (3) were obtained from Dr. Johannes Brodehl. Sarcosine transport at equivalent filtered loads was similar in both sarcosinemic probands and in our control subject (Fig. 3).

Interaction with other amino acids. Interaction between sarcosine, proline, and glycine was observed during renal transport in vivo. Intravenous infusion of sarcosine in the proband and in the normal subject altered the urinary excretion of proline and glycine. This phenomenon could not be explained by changes in the plasma concentration of the latter substances, and it was clearly related to the rising concentration of

TABLE II
Renal Response to Sarcosine Loading: Excretion and Absorption of Sarcosine, Glycine, and Proline

Subject and procedure	Sarcosine		Glycine		Proline	
	Before	After	Before	After	Before	After
Sarcosine infusion*						
Proband						
Urinary excretion, $\mu\text{moles}/\text{min per } 1.73 \text{ m}^2$	6.8	128	3.94	3.68	0	1.03
Net tubular absorption, % of filtered load	94	68	89	94	100	97
Control						
Urinary excretion, $\mu\text{moles}/\text{min per } 1.73 \text{ m}^2$	0	38	1.0	8.05	0	0
Net tubular absorption, % of filtered load	0	65	96.7	80.7	100	100
Sarcosine load by mouth, † urinary excretion, $\mu\text{moles}/\text{min per } 1.73 \text{ m}^2$						
Proband: on folate	6.35	10.3	2.75	2.27	0	0
off folate	2.83	9.15	2.76	1.81	0	Tr. §
Father	0	9.70	1.73	3.75	0	Tr.
Mother	0	9.42	1.10	4.10	0	Tr.
Controls, adult						
R. V.	0	3.62	0.17	1.52	0	0
F. G.	0	50.6	1.02	4.61	0	0
J. N.	0	7.25	0.27	3.30	0	Tr.
D. W.	0	18.4	1.54	7.91	0	0

* Data taken from periods of control and highest filtered load of sarcosine as shown in Fig. 3.

† Load, 100 mg/kg at 9 a.m. after overnight fast.

§ Indicates a detectable change but less than 0.1 $\mu\text{moles}/\text{min per } 1.73 \text{ m}^2$.

sarcosine in plasma and urine. Tubular reabsorption of glycine in the proband increased from 89 to 94% of its filtered load (Table II), but decreased from normal to 81% in the control subject. Net tubular absorption of proline clearly decreased during sarcosine infusion in the patient, but was not changed in the control subject.

When sarcosine was administered by mouth to the proband, his parents, and four normal adults, its concentration increased in plasma and urine of all subjects (Table II). At this time in the proband, there was a minimal increase in proline excretion whereas glycine excretion actually diminished. On the other hand, the urinary excretion of glycine clearly increased in all normal subjects and in the parents, while there was no comparable effect on proline excretion.

Rapid elevation of plasma and urinary sarcosine thus produced consistent, but divergent, effects on the urinary excretion of glycine, in particular, in the sarcosinemic proband when compared with subjects who do not have impaired sarcosine metabolism. An explanation for this was sought by the study of sarcosine transport in kidney in vitro. Rat kidney cortex slices were used for this work, since it has been shown that there are many homologies in the transport of *N*-substituted amino acids and glycine in the kidney of rat and man (6).

Sarcosine transport in rat kidney in vitro

Time course of uptake. The time course of sarcosine uptake was evaluated at 0.3 and 2.1 mM (Fig. 4). Sarcosine at both concentrations accumulated against an isotope gradient at the steady state; the latter is achieved within 40 min of incubation.

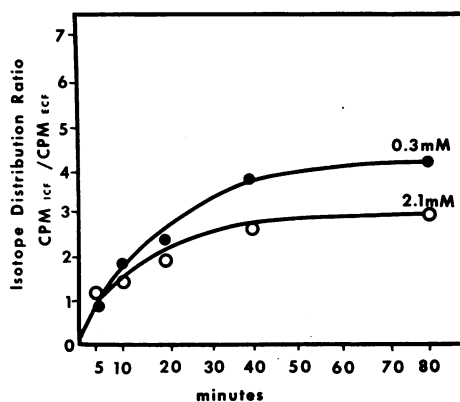


FIGURE 4 Time course of sarcosine uptake into rat kidney cortex slices at two initial concentrations in the medium. Uptake is expressed as an isotope distribution ratio. Additional studies (see text) show that the sarcosine (chemical) distribution ratio is only slightly less than isotope distribution ratio and unequivocally greater than 1.0 at steady state.

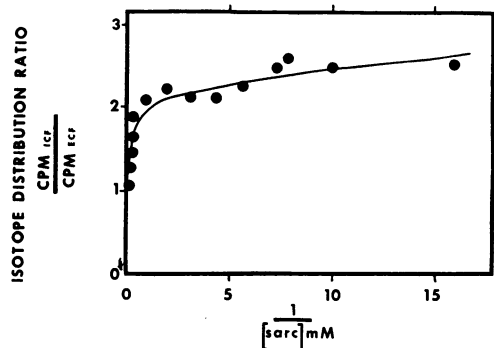


FIGURE 5 Akedo-Christensen plot (15) of sarcosine uptake by rat kidney cortex slices, at various external concentrations of substrate, under steady-state conditions. Uptake of sarcosine is saturable over the whole concentration range employed.

The effect of metabolic conversion of sarcosine (0.1 mM) on its uptake ratio was examined. ^{14}C label from sarcosine had appeared in other water-soluble metabolites after 40 min incubation; 14% of the soluble label was present in glycine, 7% in serine, and the remainder was predominantly sarcosine. The sarcosine (chemical) distribution ratio during uptake under steady-state conditions was then calculated from the isotope distribution ratio after correcting for the conversion of sarcosine to other labeled forms. The corrected ratio is greater than 3.0 for 0.3 mM sarcosine, indicating that sarcosine is transported in kidney against a true chemical gradient.

When the isotope distribution ratio is also corrected for loss of label by oxidation to CO_2 , the net uptake ratio obtained by adding soluble counts to counts in CO_2 is greater than depicted in Fig. 4. The adjusted "uptake ratios" are 6.8 and 3.4 at 0.3 mM and 2.1 mM sarcosine, respectively, after 40 min incubation.

Exposure of slices to cyanide (10^{-3} M NaCN) or anaerobic conditions, completely abolished concentrative uptake indicating that the mechanisms for sarcosine uptake are apparently coupled to energy metabolism.

Concentration-dependent uptake. When sarcosine is present at 0.3 mM in the initial medium, the steady-state isotope distribution ratio is greater than when the starting concentration is 2.1 mM (Fig. 4). As the external substrate concentration is increased, the steady-state distribution ratio approaches 1.0 (Fig. 5); this behavior indicates that sarcosine uptake in kidney at concentrations equivalent to those encountered in the proband occurs on a saturable mediation.

The kinetics of sarcosine uptake on the saturable component under steady-state conditions were examined by the Eadie and Augustinsson transformation (u vs. u/S) of the Michaelis equation (Fig. 6). Biphasic up-

take kinetics were revealed by this method, indicating that more than one component probably exists for sarcosine uptake. This behavior was also observed when the Lineweaver and Burk transformation ($1/u$ vs. $1/S$) was used to examine the same uptake data. At low external substrate concentrations, the apparent K_m for sarcosine uptake under steady-state conditions is about 0.1 mM, while at high substrate concentrations it is about 3 mM. These values are comparable with those which describe the uptake of glycine and proline by rat kidney (6).

If it is assumed that more than one type of membrane system accommodates sarcosine transport in kidney, the theoretical contribution of each component to the observed (total) uptake can be calculated. This was accomplished substituting the values for uptake, at 12 different sarcosine concentrations between 0.1 mM and 12.1 mM into equation 2. When the results were drawn as a Michaelis plot (Fig. 7), it became evident that at extracellular concentrations of sarcosine above 0.1 mM, the major fraction of its uptake takes place on a high capacity system. The revised K_m values for sarcosine transport were 0.1 mM on the low capacity system and 3.1 mM on the high capacity system.

Specificity of sarcosine uptake. Glycine and L-proline inhibit sarcosine uptake in vitro under steady-state conditions (Table III). The effect of these inhibitors was shown to be competitive. Glycine and L-proline did not have any additional effect on sarcosine oxidation in kidney, and their effect on net sarcosine uptake was restricted to interaction at the transport site.

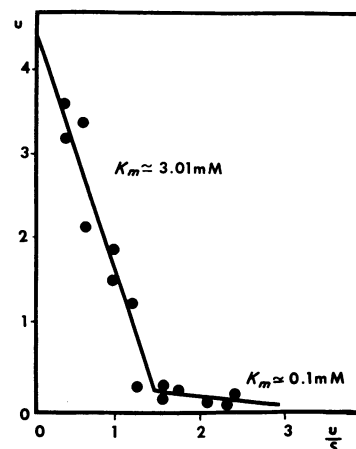


FIGURE 6 Eadie-Augustinsson plot of saturable sarcosine uptake under steady-state conditions in rat kidney cortex slices. Uptake occurs on more than one system for which first approximation K_m values are shown. When corrections were made for simultaneous uptake of sarcosine on multiple systems (viz. equation 2), the corrected K_m values were not significantly different, since uptake on the low K_m system is extremely small.

TABLE III
Comparative Interactions between Sarcosine, Glycine,
and L-Proline during Uptake by Rat
Kidney Cortex Slices

Substrate	Inhibitor	Inhibition of uptake at	
		5 min	40 min
<i>mM</i>	<i>20 mM</i>		%
Sarcosine 0.3	L-Proline	74	76
	Glycine	44	79
Sarcosine 2.0	L-Proline	88	92
	Glycine	71	85
Glycine 0.01	Sarcosine		80
Glycine 2.0	Sarcosine		(-27)*
L-Proline 0.01	Sarcosine		55
L-Proline 2.0	Sarcosine		82

Slices were incubated at 37°C in pH 7.4 Tris-electrolyte-glucose buffer for specified times. The effect of the second amino acid (at 20 mM) upon uptake of the first is expressed as per cent inhibition in relation to uptake of substrate in paired slices incubated without the second amino acid in the medium. All values are mean of triplicate observations. Inhibition was significant ($P < 0.01$ by Student's *t* test) in all cases. Per cent inhibition was comparable when measurement of uptake included or excluded label lost as $^{14}\text{CO}_2$.

* Indicates significant stimulation of uptake.

Sarcosine competitively inhibits the uptake of glycine and L-proline (Table III) when the latter are present in the medium at concentrations low enough to assign most of their transport to the respective substrate-

TABLE IV
Effect of Preloading with Sarcosine upon Uptake of Amino
Acids in Rat Kidney Cortex Slices

Substrate uptake medium	Concn of sarcosine in preload medium*	Isotope distribution ratio at 10 min†	
		Control	Preloaded
<i>mM</i>	<i>mM</i>		
Sarcosine 0.1	1	1.80	1.52
Sarcosine 1.1	10	1.22	1.34§
Glycine 0.012	20	1.75	1.17
Glycine 2.0	20	1.30	1.95§
L-Proline 0.011	20	0.36	0.42§
L-Proline 2.0	20	0.38	0.28

* Slices were preloaded during 40 min incubation in buffer containing unlabeled sarcosine, followed by removal, rinsing, and blotting. The slices were then transferred to fresh medium containing ^{14}C -labeled substrate.

† Uptake of substrate was measured at 10 min and compared with uptake by control slices carried through preincubation in the absence of sarcosine. Values are the mean of at least triplicate observations.

§ Indicates significant stimulation ($P < 0.02$).

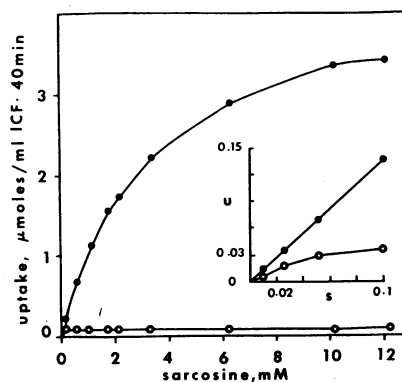


FIGURE 7 Michaelis plots of sarcosine uptake assigned to low K_m (○) and high K_m (●) systems in rat kidney; the K_m values are 0.1 mM and 3 mM, respectively. It is evident that the major fraction of sarcosine uptake is achieved on a high K_m system, at all external concentrations of the substrate.

specific, low K_m , low capacity systems which accommodate their uptake in kidney (6). On the other hand, the uptake of 2 mM glycine was actually stimulated by external sarcosine, whereas the uptake of 2 mM L-proline was still inhibited. At this concentration the renal uptakes of glycine and L-proline occur predominantly on a high capacity system shared by proline and glycine (6).

Sarcosine uptake and exchange with other amino acids. The likelihood that sarcosine uptake occurs on more than one system and thus might enter by one system and exchange on another, was investigated further by methods described previously (6). In slices preloaded with sarcosine, the uptake of 1.1 mM sarcosine, 2 mM glycine, and 0.01 mM L-proline was enhanced (Table IV). Uptake at other concentrations of these external substances was not stimulated by high concentrations of internal sarcosine.

Renal metabolism of sarcosine; effect on transport kinetics in vitro. We examined whether renal oxidation of sarcosine or lack thereof as in the blocked catabolic mutant, influenced its transport kinetics in that tissue. $^{14}\text{CO}_2$ from sarcosine was collected during incubations by the technique described earlier (6); counts appearing in CO_2 were then either included or omitted when calculating the net uptake rate. The K_m for sarcosine binding by the transport system(s), with or without oxidation, was the same (Fig. 8).

DISCUSSION

Sarcosine metabolism. The conversion of sarcosine to glycine is catalyzed by a mitochondrial oxidase system which has two components (19, 20). One is a soluble sarcosine dehydrogenase; the other is a particu-

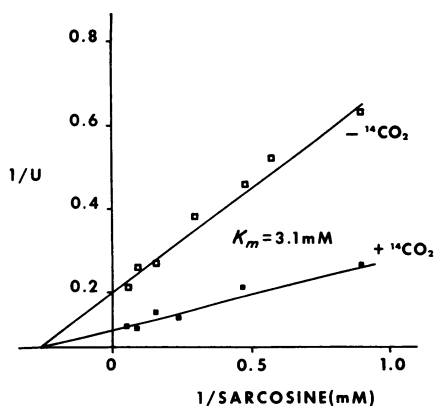


FIGURE 8 Effect of oxidation on uptake kinetics for sarcosine in rat kidney cortex slices. ¹⁴C counts lost from slice in CO₂ were included (■) or excluded (□) from calculation of uptake (u) at various substrate concentrations (S). Results presented as Lineweaver-Burk plot. Oxidation of sarcosine has no effect on its affinity for the uptake system, since the K_m value is not changed when the effect of oxidation on observed uptake is considered or neglected.

late electron transfer system. Sarcosine-specific dehydrogenase can be further fractionated to yield the dehydrogenase and a flavoprotein. Mitochondrial oxidation of the *N*-methyl group of sarcosine thus requires an electron transfer flavoprotein that accepts electrons from the substrate-specific flavoprotein dehydrogenase (20). A formaldehyde group is formed during sarcosine conversion to glycine, and by reaction with tetrahydrofolate, the intermediate *N*⁵,*N*¹⁰-methylene tetrahydrofolate is presumably formed (21). The 1-carbon fragment can be reutilized from the activated folic acid intermediate, to form the carbon of serine by condensation with glycine (22, 23).

The nature of the enzyme defect in human hypersarcosinemia is still unknown, but the weight of available evidence indicates a block in the conversion of sarcosine to glycine, presumably involving the substrate-specific dehydrogenase. Because this enzyme is neither active nor inducible in normal human fibroblasts or leukocytes as shown in the present and in previous studies (4, 13), it will not be possible to evaluate the enzymatic basis of the hypersarcosinemic trait further without recourse to organ biopsy. Sarcosine dehydrogenase activity in mammalian tissues is largely restricted to liver and kidney, as shown in the present work and in that of others (13).

The failure to ameliorate the hypersarcosinemic phenotype in the proband with pharmacologic doses of folic acid, suggests that the trait in this pedigree at least does not involve a reversible defect in the formation of "activated" formaldehyde from the specific dehydrogenase reaction. The potential responsiveness of

mutant biochemical phenotypes to vitamin supplements is an important consideration in those hereditary aminoacidopathies where a vitamin is the precursor of one of the reactants in the enzymatic conversion of metabolites (24). The proband appeared to become more hypersarcosinemic after sarcosine loading while receiving folate, when compared with the response in the absence of folate. We believe this variation is not significant since the patient's plasma sarcosine concentration was quite variable anyway. However the possibility that folate in some way enhanced intestinal absorption of sarcosine in this patient should be considered.

Loading studies: interpretation of phenotype. Sarcosine disappearance from plasma should be delayed and plasma glycine should not change after a sarcosine load given to subjects with deficient conversion of sarcosine to glycine; this response was obtained in our proband. Brodehl and coworkers (3)² observed a similar response after comparable sarcosine loading in their proband. Sarcosine clearance from plasma was delayed in the patients studied by Gerritsen and Waisman (2) and by Scott et al. (4). In the latter study (4) glycine rose in the proband's plasma but not until the 4th hr after loading; this could have reflected conversion of sarcosine to glycine by bacteria in the intestinal lumen, rather than an endogenous response. The plasma glycine response after sarcosine loading was not reported by Gerritsen and Waisman (2).

The heterozygote for hypersarcosinemia should have only modest impairment of sarcosine clearance from plasma after loading, and one anticipates the initial plasma concentration of sarcosine to be normal under fasting conditions. We found this to be the case in both parents of our proband. The rise in their glycine after sarcosine loading was a response opposite to that obtained in normal subjects. The renal response to sarcosine loading did not account for observed changes in plasma glycine of parents and control subjects.

Sarcosine clearance from plasma and the concomitant glycine response provide a useful distinction between normal subjects, parents, and proband in the present sarcosinemic pedigree. Moreover, they appear to be more reliable indices of the presumed genotype than sarcosine excretion data, which, as shown in Table III, are poor indices, contrary to earlier conclusions (2). On the basis of the plasma amino acid response to sarcosine loading, we tentatively suggest that the sarcosine trait is autosomal recessive in this French-Canadian pedigree.

Clinical significance of the sarcosinemic trait. Hypersarcosinemia appears to be a benign condition. Our pro-

² Brodehl, J. 1970. Personal communication.

band had no disease which could be directly attributed to his hypersarcosinemia. The same can be said for the hypersarcosinemic sibling of the first reported proband (2) and for another reported proband (3). A larger series¹ apparently also indicates that no consistent disease state accompanies hypersarcosinemia, and thus, as with other aminoacidopathies such as hydroxyprolinemia, hyperprolinemia, and cystathioninemia, hypersarcosinemia appears to be a "nondisease" in medical terms.

The likelihood is great that the sarcosinemic trait in man will exhibit genetic heterogeneity. For this reason we must be cautious in assuming that sarcosine dehydrogenase activity is necessarily deficient in our proband or that he is exactly similar to others described in the literature. However, we believe our assumption about deficient renal sarcosine dehydrogenase activity to be reasonable, in view of the particular nature of amino acid reabsorption in the proband and considering how these observations may be related to what we have learned about sarcosine transport in kidney.

Sarcosine transport. Sarcosine is oxidized in mammalian kidney, but hypersarcosinemia, due to sarcosine dehydrogenase deficiency, offers an opportunity to study its renal transport in man independent of its metabolism. Renal transport of proline and of phenylalanine has been investigated to some extent in man under the equivalent conditions of the blocked catabolic phenotypes found in hyperprolinemia and phenylketonuria, respectively. In neither case did the hereditary impairment in metabolism of the amino acid appear to influence its uptake by kidney in vivo (8, 25, 26). In fact, should intracellular amino acid at high concentration exchange with intraluminal amino acid, it is possible that renal transport could be enhanced as may be the case in phenylketonuria (25). We have now shown that renal transport of sarcosine is similar in the subject with hereditary hypersarcosinemia and in normal subjects. We were able to confirm in vitro that metabolism of transported sarcosine plays no role in determining its binding kinetics to membrane carrier in kidney.

Sarcosine apparently does not have its own transport system. It is a nonessential amino acid concerned in only a limited repertoire of intracellular metabolism being synthesized only from an intracellular precursor (dimethylglycine). Although bound sarcosine has been identified in human glycopeptides (27), it is believed to achieve this form by *N*-methylation of peptide-linked glycine. Our studies indicate that sarcosine is transported in vivo primarily on the tubular transport system(s) used by proline, hydroxyproline, and glycine in human kidney (6, 8, 28). The kinetics of sarcosine uptake in vitro and the inability of proline or glycine transport to resist sarcosine inhibition at low or high concentrations, clearly reveal that more than one transport site serves

its uptake in mammalian kidney. We have tentatively identified the systems used in sarcosine transport as the low K_m system for proline, the low K_m system for glycine, and the high K_m system shared by both; this constellation of sites has been characterized in detail (6, 8, 28-30), using the same methods employed in the present investigation. The in vitro studies suggest that the principle carrier available for sarcosine entry into kidney is probably the high K_m , high capacity system shared by proline, hydroxyproline, and glycine. This corroborates earlier studies of sarcosine transport in hamster intestine (31).

The data which describe sarcosine transport in rat kidney in vitro are likely to be informative about the nature of sarcosine transport by human kidney in vivo, since it has already been shown that the principle system for sarcosine transport has many homologies in human and rat kidney (6). The different responses in urinary amino acid excretion obtained in proband and control subjects after sarcosine loading can be explained by applying these insights.

The sarcosine concentration in kidney should be unusually high after sarcosine loading in the sarcosinemic proband; under such conditions, sarcosine could exchange with glycine in vivo as it will in vitro. The ability of sarcosine to stimulate glycine uptake under certain conditions presumably arises because the first amino acid is allowed to enter by one system and exchange with the second amino acid on another (32). Glycine reabsorption could improve in the proband when the intracellular sarcosine pool is further expanded by loading. On the other hand, renal oxidation of sarcosine can occur in normal subjects thus preventing a sustained high concentration in kidney after loading. In this circumstance sarcosine can exert its inhibitory effect on glycine entry on the low K_m system to an extent which would outweigh the opportunity for exchange on the high K_m system.

Significance of studies. The foregoing information can be used to counsel future sarcosinemic probands in the likelihood that this autosomal recessive trait is harmless. The mechanism by which glycine alters its steady state in response to sarcosine loading awaits clarification. The further demonstration of parasitic transport wherein membrane systems serving "primary" substrates are used under certain circumstances by "secondary" substrates (in this case sarcosine) illustrates the value to be gained from designing pharmacologically active compounds which can be accommodated on directed transport systems in tissues.

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REFERENCES

- Glorieux, F. H., F. Mohyuddin, D. T. Whelan, and C. R. Scriver. 1970. Hypersarcosinemia: new observations. *Pediat. Res.* 4: 450. (Abstr.)
- Gerritsen, T., and H. Waisman. 1966. Hypersarcosinemia. An inborn error of metabolism. *N. Engl. J. Med.* 275: 66.
- Hagge, W., J. Brodehl, and K. Gellissen. 1967. Hypersarcosinemia. *Pediat. Res.* 1: 409. (Abstr.)
- Scott, C. R., S. H. Clark, C. C. Teng, and K. R. Swedberg. 1970. Clinical and cellular studies of sarcosinemia. *J. Pediat.* 77: 805.
- Meister, A. 1965. Biochemistry of the amino acids. Academic Press, Inc., New York. 2nd edition. 2: 658.
- Mohyuddin, F., and C. R. Scriver. 1970. Amino acid transport in mammalian kidney: multiple systems for imino acids and glycine in rat kidney. *Amer. J. Physiol.* 219: 1.
- Scriver, C. R., and E. Davies. 1965. Endogenous renal clearance of free amino acids in pre-pubertal children. *Pediatrics.* 36: 592.
- Scriver, C. R., M. L. Efron, and I. A. Schafer. 1964. Renal tubular transport of proline, hydroxyproline and glycine in health and in familial hyperprolinemia. *J. Clin. Invest.* 43: 374.
- Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* 30: 1190.
- Scriver, C. R., E. Davies, and P. Lamm. 1968. Accelerated selective short column chromatography of neutral and acidic amino acids on a Beckman-Spinco analyzer, modified for simultaneous analysis of two samples. *Clin. Biochem.* 1: 179.
- Hayflick, L. 1965. The limited "in vitro" lifetime of human diploid cell strains. *Exp. Cell Res.* 37: 614.
- Zucker, R. M., and B. Cassen. 1969. The separation of normal human leukocytes by density and classification by size. *Blood.* 34: 591.
- Rehberg, M. L., and T. Gerritsen. 1968. Sarcosine metabolism in the rat. *Arch. Biochem. Biophys.* 127: 661.
- Blass, J. P., J. Avigan, and B. W. Uhlendorf. 1970. A defect in pyruvate decarboxylase in a child with an intermittent movement disorder. *J. Clin. Invest.* 49: 423.
- Akedo, H., and H. N. Christensen. 1962. Nature of insulin action on amino acid uptake by the isolated diaphragm. *J. Biol. Chem.* 237: 118.
- Tenenhouse, A., and J. M. Quastel. 1960. Amino acid accumulation in Ehrlich ascites carcinoma cells. *Can. J. Biochem.* 38: 1311.
- Neal, J. 1971. Analysis of non-linear Lineweaver-Burk plots. *J. Theor. Biol.* In press.
- Scriver, C. R., and F. Mohyuddin. 1968. Amino acid transport in kidney: heterogeneity of alpha aminoisobutyric uptake. *J. Biol. Chem.* 243: 3207.
- Frisell, W. R., and C. G. Mackenzie. 1962. Separation and purification of sarcosine dehydrogenase and dimethyl-glycine dehydrogenase. *J. Biol. Chem.* 237: 94.
- Frisell, W. R., J. R. Cronin, and C. G. Mackenzie. 1962. Coupled flavoenzymes in mitochondrial oxidation of N-methyl groups. Purification of the electron transfer flavoprotein. *J. Biol. Chem.* 237: 2975.
- Mackenzie, C. G. 1955. Conversion of N-methyl glycines to active formaldehyde and serine. In A Symposium on Amino Acid Metabolism, Sponsored by the McCallum-Pratt Institute of the Johns Hopkins University. W. D. McElroy and B. Glass, editors. The Johns Hopkins Press, Baltimore. 684.
- Blakley, R. L. 1957. The interconversion of serine and glycine: some further properties of the enzyme system. *Biochem. J.* 65: 342.
- Mackenzie, C. G., and W. R. Frisell. 1958. The metabolism of dimethylglycine by liver mitochondria. *J. Biol. Chem.* 232: 417.
- Rosenberg, L. E. 1969. Inherited amino acidopathies demonstrating vitamin dependency. *N. Engl. J. Med.* 281: 145.
- Brodehl, J., K. Gellisen, and W. P. Kass. 1970. The renal transport of amino acids in untreated infants with phenylketonuria. *Acta Paediat. Scand.* 59: 241.
- Scriver, C. R. 1969. The use of human genetic variation to study membrane transport of amino acids in kidney. *Amer. J. Dis. Child.* 117: 4.
- Cherian, M. G., and A. N. Radhakrishnan. 1965. A new glycopeptide containing hydroxyproline and sarcosine in human urine. *Biochim. Biophys. Acta.* 101: 241.
- Scriver, C. R. 1968. Renal tubular transport of proline, hydroxyproline, and glycine. III. Genetic basis for more than one mode of transport in human kidney. *J. Clin. Invest.* 47: 823.
- Wilson, O. H., and C. R. Scriver. 1967. Specificity of transport of neutral and basic amino acids in rat kidney. *Amer. J. Physiol.* 213: 185.
- Baerlocher, K., C. R. Scriver, and F. Mohyuddin. 1970. Ontogeny of iminoglycine transport in mammalian kidney. *Proc. Nat. Acad. Sci. U. S. A.* 65: 1009.
- Hagihira, H., T. H. Wilson, and E. C. C. Lin. 1962. Intestinal transport of certain N-substituted amino acids. *Amer. J. Physiol.* 203: 637.
- Jacquez, J. A. 1967. Competitive stimulation: further evidence for two carriers in the transport of neutral amino acids. *Biochim. Biophys. Acta.* 135: 751.