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THE AETIOLOGY OF PRIMARY HYPEROXALURIA

BY

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The natural history of primary hyperoxaluria (Archer, Dormer, Scowen, and Watts, 1957a) is one of progressive bilateral calcium oxalate urolithiasis, nephrocalcinosis, and recurrent infection of the urinary tract: the latter accelerates the renal parenchymal destruction initiated by the nephrocalcinosis, and death occurs in childhood or early adult life from renal failure and hypertension. There are no characteristic overt biochemical abnormalities except for a continuous high urinary oxalate excretion, which is unrelated to exogenous factors; and urinary oxalate determinations are necessary to distinguish these patients from cases of calcium oxalate urolithiasis without hyperoxaluria. Calcium oxalate deposits in the parenchyma and interstitial tissue of the kidneys, and in other organs (oxalosis), are characteristic histological findings. The renal deposits are not necessarily related to areas of post-pyelonephritic scarring.

Primary hyperoxaluria is a rare disease, and we have been able to find only 10 recorded cases that can be regarded as reasonably definite examples of the condition (Table I). The apparent occurrence of the condition among siblings (Newns and Black, 1953; Aponte and Fetter, 1954) suggests that it results from an inborn error, although lack of urinary oxalate determinations among the supposedly affected siblings except in two of the three cases reported by Aponte and Fetter (1954) makes a certain retrospective diagnosis impossible. Myers (1957), in reviewing the aetiology of urolithiasis in childhood, mentions two families in which cases of what he termed "familial oxaluria" occurred. We have investigated the first of these in which twin girls and their sibling brother suffered from renal calculi; the affected members, their unaffected brother, both parents, and a paternal uncle were all found to excrete normal amounts of oxalate. These cases cannot, therefore, be regarded as examples of primary hyperoxaluria and should not, in our opinion, be classified with Newns and Black's (1953) case, although the presence of some other genetically determined factor which predisposes to urolithiasis in this family cannot be excluded on the basis of this finding.* The second family discussed by Myers (1957) is the one which was reported by Newns and Black (1953) (see Table I).

Lund and Reske-Nielsen (1956) reported two cases of calcium oxalate nephrolithiasis and nephrocalcinosis with oxalosis. These differed from the cases recorded in Table I in that they presented at relatively advanced ages (16 and 35 years) and survived to the ages of 35 and 40 respectively. The urinary oxalate excretion by these patients was not determined. Vischer (1947), Ostry (1951), and Mulloy and Knutti (1951) described cases in which patients with calcium oxalate nephrocalcinosis died from renal failure at the ages of 5 months, 11 years, and 28 years respectively; data concerning the urinary oxalate excretion levels and the occurrence of oxalosis are lacking from these case reports also. Arons, Christensen, and Sosman (1955) mention a case diagnosed as chronic glomerulonephritis with secondary hyperparathyroidism and urinary calculi in which calcium oxalate crystals were found in the kidneys, lymph nodes, and lungs at necropsy. This may have been a case of primary hyperoxaluria, although the occurrence of calcium oxalate nephrolithiasis only late in the evolution of the disease would be atypical, and again no urinary oxalate determinations were apparently performed.

We have met with two accounts of calcium oxalate nephrocalcinosis in patients who did not die from renal failure: Lepoutre (1925) noted calcium oxalate crystals in a renal biopsy from a child aged 4½ years (the cause of death in this case is not stated), and Laas (1941) reported a similar finding in a man dying accidentally at the age of 25 years. Whether these additional cases where information concerning the urinary oxalate excretion level or the occurrence of oxalosis is lacking should be regarded as examples of primary hyperoxaluria is uncertain.

Newns and Black (1953) reported that in the case they described some of the oxalate in the urine was present in non-ionized form but could be rendered precipitable with calcium ions by acid hydrolysis. We have been unable to confirm this (Table II).

The fundamental abnormality in primary hyperoxaluria might theoretically be a low renal oxalate threshold, excessive gastro-intestinal oxalate absorption, or increased endogenous oxalate synthesis. The

*We are indebted to Dr. R. E. Bonham-Carter and Professor M. L. Rosenheim for their help in tracing this family and for giving us access to their case records.

TABLE I.—*Reported Cases which are Regarded as Examples of Primary Hyperoxaluria*

Author	Sex	Presentation	Age at 1st Symptom (Years)	Age at Death (Years)	Urinary Oxalate Excretion (mg.$(COOH)_2.2H_2O$/24 hr.)	Renal Oxalate Deposits	Oxalosis
Davis *et al.* (1950)	M	Acute febrile illness (nature?). Polyuria. Radiological evidence of renal calculi	3	12	—	Present	Present
Chou and Donohue (1952)	M	Haematuria	1 11/12	6 11/12	—	,,	,,
Zollinger and Rosenmund (1952)	M	Urinary tract infection. Renal failure	3 6/12	4 6/12	—	,,	,,
Newns and Black (1953)	F*	Passage of urinary calculi	2	8 7/12	95–182	No necropsy	No necropsy
Aponte and Fetter (1954)	M* †	Increased frequency of micturition	5	16	200	Present	Present
	M* †	Haematuria	10	13 6/12	180	,,	,,
Burke *et al.* (1955)	M	,,	3	11 10/12	—	,,	,,
Dunn (1955)	F	,,	4	15	—	,,	,,
Neustein *et al.* (1955)	M	Passage of urinary calculi	1 3/12	5	—	,,	,,
Lund (1957)	M	,, ,, ,,	3	22	—	,,	,,
Archer *et al.* (1957a)	M	Haematuria, passage of urinary calculi	1	Alive (22)	162–290	—	—
	F	Renal pain, haematuria	4	Alive (10)	110–265	Present ‡	—

* The pedigrees of these cases each contain one other sibling in whom the clinical data strongly suggest the condition but on whom neither necropsy data nor the level of the urinary excretion are known.
† Twins.
‡ In tissue removed at operation.

TABLE II.—*Effect of Hot Acid Hydrolysis on the Amounts of Oxalate Which Can be Precipitated as Calcium Oxalate*

Exp. No.	Subject	Oxalate Content (mg./100 ml.)	
		Unhydrolysed	Hydrolysed
1	Primary hyperoxaluric	8·4 (3)	8·2 (3)
2	Normal	3·8 (3)	3·6 (3)
3	,,	1·9 (4)	1·9 (4)

The figures in parentheses indicate number of replicate determinations upon which the cited mean value is based.

In each experiment the hydrolysed urine was prepared by adding concentrated HCl (5% by volume) to a measured portion of a 24-hour urine collection and boiling under reflux for half an hour. The original volume was restored by the addition of an appropriate volume of water, and most of the urinary pigments were removed by treatment with a weighed amount of charcoal ("norit") for 1½ hours at room temperature. The charcoal was filtered off and replicate oxalate determinations were performed on the filtrates. The same procedure was followed for the unhydrolysed samples, except that water was added in place of concentrated HCl and boiling was omitted.

experiments reported in the present paper were undertaken in order to investigate some of these possibilities.

The confusing and often uncritical earlier literature dealing with the possible physiological and pathological significance of oxalic acid and its salts was reviewed by Jeghers and Murphy (1945). It is now generally believed that oxalate plays no part in the intermediary metabolism of animals (see, for example, Curtin and King, 1955), but glyoxylate appears as an intermediate in one of the pathways of glycine metabolism (Weinhouse and Friedmann, 1951; Nakada and Weinhouse, 1953; Nakada, Friedmann, and Weinhouse, 1955; Weinhouse, 1955), and the conversion of glyoxylate to oxalate in the intact rat has been demonstrated (Weinhouse and Friedmann, 1951). Trampetti and Vantaggi-Cozzari (1948) reported the conversion of glycine to glyoxylate and oxalate *in vitro* by heart muscle preparations, and Nakada *et al.* (1955) demonstrated the incorporation of the glycine α-carbon atom into oxalate by liver slices when glyoxylate was added to the incubation medium. Although the conversion of glycine to formate and carbon dioxide via glyoxylate is probably not the main normal pathway of glycine metabolism (Arnstein, 1954), it suggests a possible source other than a simple dietary one from which urinary oxalate might arise.

Detoxication of the benzoate ion by hippurate formation *in vivo* results in depletion of the free glycine metabolic pool (Arnstein and Neuberger, 1951; Simkin and White, 1957), and sodium benzoate was administered in the present work in order to produce this effect.

Illustrative Cases

A detailed clinical description of the two cases on which the present studies were made has been given elsewhere (Archer *et al.*, 1957a).

Patient 1.—A man aged 22 had a history of recurrent urinary calculi beginning at the age of 1 year. Seven major surgical operations, including a partial nephrectomy, were required for the removal of urinary tract calculi; a staphylococcal urinary infection which lasted 18 months occurred when he was 12 years old; and repeated studies designed to demonstrate any disorder of calcium and phosphorus meta-

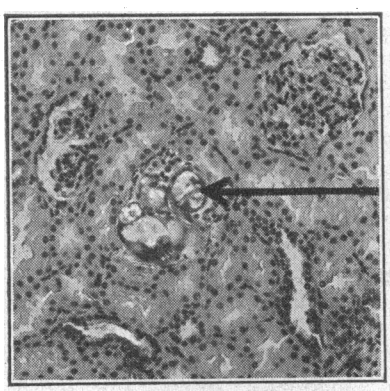

FIG. 1

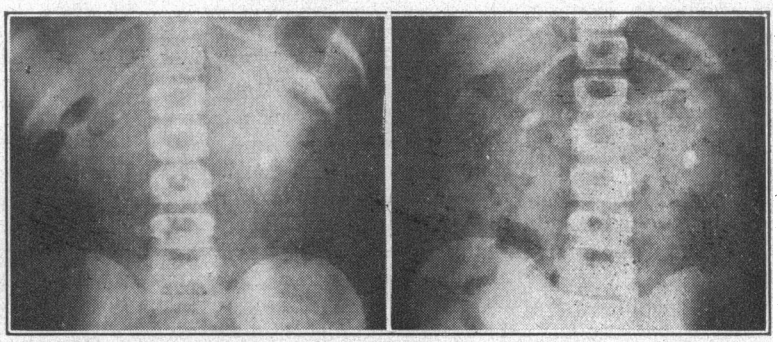

FIG. 2

FIG. 1.—Photomicrograph of a histological section prepared from surgically excised kidney tissue from Case 2, viewed under polarized light. A typical well-defined calcium oxalate crystal is seen, probably lying in a renal tubule. Similar crystals may be found in the interstitial tissues of the kidney, and, in oxalosis, occur in the extrarenal tissues also. FIG. 2.—Plain abdominal radiographs of Case 2 taken at an interval of approximately four months and illustrating the very rapid growth of multiple calculi characteristic of primary hyperoxaluria.

bolism have proved negative. The urinary oxalate excretion was first determined in July, 1955, and repeated determinations since have given results in the range 162–290 mg. of $(COOH)_2.2H_2O$ per 24 hours as opposed to the normal value of <40 mg. of $(COOH)_2.2H_2O$ per 24 hours (Archer *et al.*, 1957b). At the time of writing he had multiple small renal calculi and hydronephrotic changes in both kidneys. The urine was sterile, there was continuous microscopic and occasional macroscopic haematuria, and small calculi were occasionally passed per urethram. There was evidence of impaired renal function (poor concentration of radio-opaque dyes, blood urea 62 mg./100 ml., urea clearance 32% of the average normal value) with compensatory polyuria, and renal hypertension (blood pressure 145/110 mm. Hg). He was able to perform full-time clerical work and to engage in some competitive sport. There was no family history of urinary calculi. Re-examination of the only available histological section of the tissue removed from his left kidney six years ago showed only the effects of pyelonephritis.

Patient 2.—A girl aged 11 years had a seven-year history of recurrent urolithiasis, for which three major surgical operations had been performed with, at the present time, multiple small bilateral renal stones, hydronephroses, continuous microscopic haematuria, but relatively little evidence of parenchymal renal damage (blood urea 43 mg./100 ml., urea clearance 62.5% of the average normal value, blood pressure 130/80 mm. Hg). The only other abnormality was the grossly increased urinary oxalate excretion—110–265 mg. of $(COOH)_2.2H_2O$ per 24 hours—which has been found on each occasion that it has been measured during the past 2½ years. There is no family history of urinary calculi. The appearance of calcium oxalate crystals in histological preparations examined with polarized light is well shown in sections of this patient's kidney (Fig. 1). The characteristic rapid reaccumulation of multiple renal calculi after surgical treatment was illustrated by the serial abdominal radiographs (Fig. 2).

Calculi from both patients have been analysed on several occasions and shown to consist entirely or almost entirely of calcium oxalate.

Analytical Methods

Urinary oxalate was determined as previously described (Archer *et al.*, 1957a, 1957b). Both patients had microscopic haematuria, and Patient 2 experienced occasional episodes of macroscopic haematuria during the course of the present study. The determinations also had to be made in the presence of considerable amounts of hippurate and probably also of benzoate in the urine. It was shown that neither blood (0.005 ml. of blood per ml. of urine) nor hippurate and benzoate in the concentrations encountered in the present experiments interfered with these determinations.

Urinary creatinine determinations, using a standard procedure based on the Jaffe reaction (Hawk *et al.*, 1947), were employed as a check on the completeness of the 24-hour urine collections.

Urinary amino-nitrogen was determined by formal titration, allowance being made for the amount of NH_3 present (Hawk *et al.*, 1947).

Urinary Hippuric Acid.—When large amounts of sodium benzoate were being administered, hippuric acid had crystallized spontaneously by the time that the 24-hour urine collections were available for analysis. The crystals were filtered off, 50-ml. portions of the filtrate were removed without delay, and their hippuric acid content was determined by a standard procedure (Hawk *et al.*, 1947). The hippuric acid removed by the initial filtration was dissolved in water and determined titrimetrically.

Diets and Food Analyses (for Oxalate and Calcium).—A repetitive standard diet was employed except where a low-protein intake was required (see below). It was not practicable to analyse every day's food, so determinations were made on representative days of each experimental period; the results cited in Table III are average values. (For details of the standard diet and of the methods of sampling and analysis see Archer *et al.*, 1957b.)

TABLE III.—*Oxalate and Calcium Contents of Diets*

Patient	Diet	Oxalate (mg.$(COOH)_2.2H_2O$/day)	Calcium (mg. Ca/Day)
1	Standard ..	1,100	1,250
	Low protein ..	950	800
2	Standard ..	800	900
	Low protein ..	750	425

Experimental and Results

One group of experiments was designed to determine whether patients suffering from primary hyperoxaluria excrete supernormal amounts of oxalate in the urine following the ingestion of this anion. Other experiments were designed to test indirectly the hypothesis that glycine is the precursor of at least some of the oxalate which these subjects excrete. The latter experiments comprised a study of the effect of (1) a low-protein diet, (2) the administration of large doses of sodium benzoate, and (3) the administration of large amounts of glycine on the urinary oxalate excretion. A limited number of comparable observations have been made on two normal subjects.

Effect of Oral Administration of Sodium and Calcium Oxalates and of Expressed Rhubarb Juice on Urinary Oxalate Excretion in Primary Hyperoxaluria

Sodium and calcium oxalates were given in increasing daily doses (Fig. 3). The same amounts of sodium oxalate, calculated on the basis of unit body weight, were given as in our previously reported studies on normal subjects (Archer *et al.*, 1957b); the total amounts of calcium oxalate administered were, however, larger. Sodium oxalate was given as an aqueous solution (2.66 g. of $(COONa)_2$ per litre), and calcium oxalate in rice-paper cachets (0.5 g. of $Ca(COO)_2.H_2O$ per cachet). The expressed rhubarb juice contained an amount of oxalate equivalent to about 4 g. of $(COOH)_2.2H_2O$ per litre, and 1,000 g. of fresh rhubarb stem yielded approximately 900 ml. of juice. The daily doses (Fig. 3) were divided into four equal portions, given after meals. Patient 1 received total amounts of sodium and calcium oxalates and of rhubarb juice equivalent to 13.5 g.,

TABLE IV.—*Proportion of the Administered Dose of Sodium Oxalate and of the Oxalate Present in Rhubarb Juice which was Subsequently Excreted in Urine*

Patient	Mean Urinary Oxalate Excretion During Control Periods (mg.$(COOH)_2.2H_2O$ per 24 hr.)	Calculated "Basal" Urinary Oxalate Excretion during Experimental Periods (mg.$(COOH)_2.2H_2O$ per 24 hr.)		Observed Total Oxalate Excretion during Experimental Periods (mg.$(COOH)_2.2H_2O$)		Calculated Increase in Urinary Oxalate Excretion Associated with Administration of			
						$(COONa)_2$		Rhubarb Juice	
		$(COONa)_2$ Administration	Rhubarb Juice Administration	$(COONa)_2$ Administration	Rhubarb Juice Administration	mg. $(COOH)_2.2H_2O$	% of Dose	mg. $(COOH)_2.2H_2O$	% of Dose
1	191	1,146	784	1,477	972	331	2·4	208	1·9
2	219	1,314	638	1,721	598	407	5·4	40	1·2

The average daily urinary oxalate excretion during the control periods, shown in Fig. 3, has been computed for each patient and the "basal" level of oxalate excretion during the experimental periods (periods of sodium oxalate or of rhubarb juice administration) is assumed to be equal to this value. See text for the total amounts given.

34.5 g., and 11.2 g. of $(COOH)_2.2H_2O$ respectively. The total doses of sodium and calcium oxalates and of rhubarb juice given to Patient 2 were equivalent to 7.5 g., 25 g., and 3.4 g. of $(COOH)_2.2H_2O$ respectively.

The time course of the increase in the urinary oxalate excretion associated with sodium oxalate feeding (Fig. 3) closely resembles that found in the comparable experiments with normal subjects (Archer *et al.*, 1957b). The fraction of the total sodium oxalate dose which was excreted in the urine (see Table IV) also agreed with the corresponding values obtained previously (2.3%, 3.3%, 4.5%, and 3.1% respectively for four normal individuals) (Archer *et al.*, 1957b). The evanescent increase in the urinary oxalate excretion which occurred when rhubarb juice was administered did not exceed that which might have been expected on the basis of the oxalate content of the juice (Table IV).

Calcium oxalate administration did not materially alter the level of urinary oxalate; normal secretion of gastric HCl was demonstrated in both patients, thus excluding hypochlorhydria as a cause for the difference between the effect of sodium oxalate and of calcium oxalate feeding.

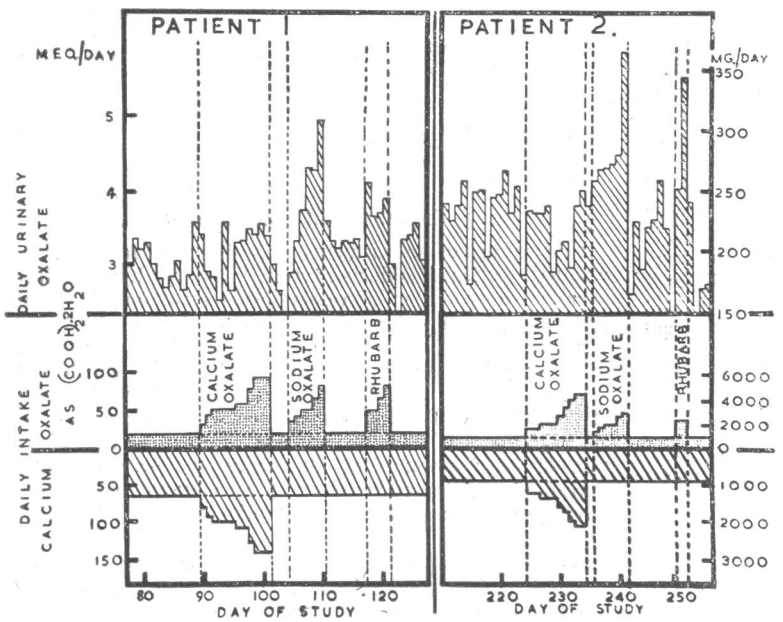

FIG. 3.—Daily urinary oxalate excretion and the total daily intakes of calcium and oxalate by Patients 1 and 2 during a period when supplements of sodium and calcium oxalates and expressed rhubarb juice were being administered.

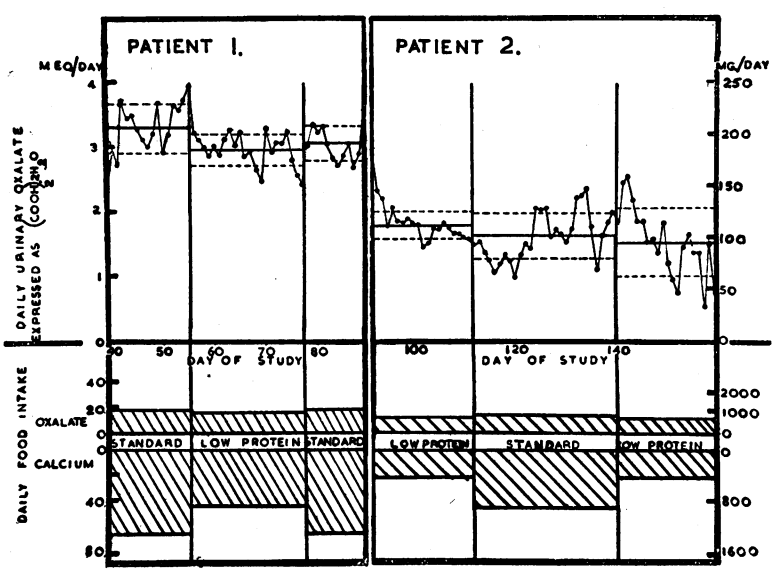

FIG. 4.—Daily urinary oxalate excretion by Patients 1 and 2 when a low-protein diet was being administered and during suitable control periods. The average value for the individual results in each experimental and control period is represented by an uninterrupted horizontal line. The interrupted horizontal lines demarcate the range ±1 standard deviation about each mean value.

Effect of a Restricted Dietary Protein Intake on Urinary Oxalate Excretion

The daily urinary oxalate excretion by the patients during periods of low-protein intake and during control periods when the protein intake was not restricted are shown in Fig. 4. In the case of Patient 1, whose daily protein intake was 35 g. and 70 g. during the experimental and control periods respectively, the difference between the average daily urinary oxalate excretion during the experimental and the combined control periods is such that $0.01<P<0.02$, and the difference between the average daily excretion for the two control periods is such that $0.05<P<0.1$. In the case of Patient 2 the difference between the average urinary excretion for the control period (dietary protein intake 50 g. daily) and the combined experimental periods (dietary protein intake 17 g. daily) shown in Fig. 4 is such that $P>0.1$.

Effect of Sodium Benzoate Administration on Urinary Oxalate Excretion

Sodium benzoate administration was associated with a decrease in the urinary oxalate excretion by both patients (Figs. 5 and 6). The magnitude of the response was smaller in Patient 1 than in Patient 2, although there is no doubt of its significance. The difference between the average urinary oxalate excretion during the period of sodium benzoate feeding and that during the combined control periods before and after the experimental period in Patient 1 is such that $P<0.001$. The urinary excretion of hippuric acid indicated that up to 200 mMol (15 g.) and 100 mMol (7.5 g.) of glycine were excreted each day in this form by Patients 1 and 2 respectively during the periods of sodium benzoate administration.

A third period of sodium benzoate feeding in the case of Patient 2, this time combined with a restricted protein intake (17 g. daily), lowered the average daily urinary oxalate excretion to approximately the same level as that shown during the second period of sodium benzoate administration in Fig. 6. There was thus no evidence that the effect of sodium benzoate could be enhanced appreciably by combining it with a low-protein diet. An attempt to lower the urinary oxalate excretion in Patient 2 permanently by means of prolonged sodium benzoate administration (20 g. per day) was unsuccesful; after about four weeks the excretion of oxalate rose gradually to high levels. Here, as with the data presented in Fig. 6, the average level of urinary oxalate excretion after sodium benzoate administration was higher than during the previous "control period." Renal colic and macroscopic haematuria occurred intermittently towards the end of, and for a period of about 10 days after, both of the longer periods of sodium benzoate administration in the case of Patient 2.

Two normal subjects (a man aged 35 and a girl aged 12) were given similar amounts of sodium benzoate without a significant alteration in their urinary oxalate excretion (Table V). The hippurate excretion by these subjects was not determined, but large amounts of hippuric acid crystallized from the acidified urine on standing at room

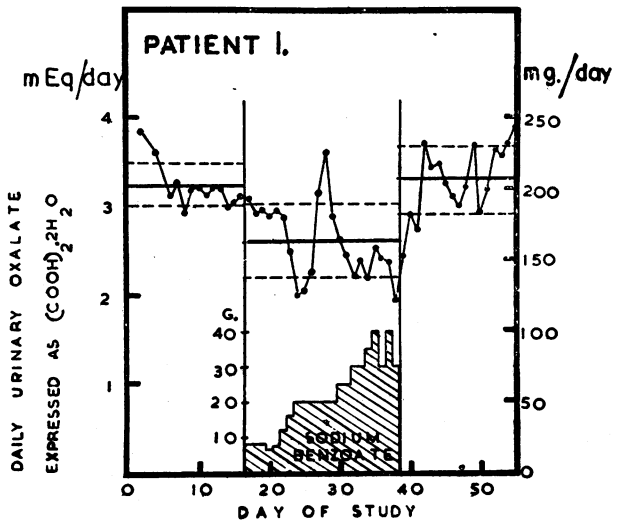

FIG. 5.—Daily urinary oxalate excretion by Patient 1 before, during, and after a period of sodium benzoate administration. The average value for the individual results in each experimental and control period is represented by an uninterrupted horizontal line. The interrupted horizontal lines demarcate the range ± 1 standard deviation about each mean value.

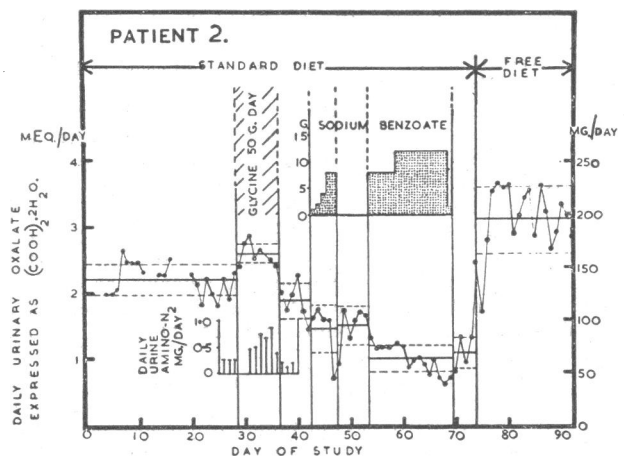

FIG. 6.—Daily urinary oxalate excretion by Patient 2 during periods of glycine administration and of sodium benzoate administration, together with the results obtained during the corresponding control periods. The average value for each experimental and control period is represented by an uninterrupted horizontal line, and the range ± 1 standard deviation about each mean value is indicated by the interrupted horizontal lines. The results of daily urinary amino-nitrogen determinations performed in association with the experiment in which glycine was administered are also shown. The period marked "free diet" indicates an interval during which the patient was not kept on a strictly repetitive diet owing to the deterioration in her general condition associated with severe attacks of renal colic; macroscopic haematuria and the passage of small calculi occurred at this time.

temperature, and from this it is concluded that considerable amounts of glycine were being withdrawn from the freely available glycine pool, as was the case in the experiments on patients with primary hyperoxaluria.

General malaise, impaired power of concentration, and a sensation of "remoteness from his surroundings" were reported by Patient 1 when he was receiving more than 20 g. of sodium benzoate per day. Our normal adult volunteer noticed similar symptoms when taking 30–40 g. of sodium benzoate per day.

Changes in Urinary Oxalate Excretion which were Associated with Oral Administration of Glycine

Patient 2 was given 4.5 g. of glycine four times daily for eight days. She vomited on several occasions soon after taking the amino-acid and there is therefore some doubt concerning the exact dose retained, although the increased excretion of urinary amino-nitrogen and the appearance of a prominent glycine spot on the filter-paper chromatogram of the urine suggests that at least some of the additional glycine was being absorbed. The results of the urinary oxalate determinations before, during, and after the period of glycine administration are shown in Fig. 6.

TABLE V.—*Urinary Oxalate Excretion by Two Normal Subjects Before, During, and After a Period of Sodium Benzoate Administration. (Subject A was a Man Aged 35 Years; Subject B was a Girl Aged 12 Years)*

Subject	Day of Experiment	Sodium Benzoate Dosage (g./day)	Urinary Oxalate Excretion (mg.$(COOH)_2.2H_2O$ per day)
A	1–8	0	9–25 Mean 20 S.D.7
	9	20	—
	10	20	8
	11	30	16
	12	27·5	12
	13	40	33
	14	40	20
	15–20	0	16–26 Mean 20 S.D. 4
B	1–12	0	[illegible]–15 Mean 9 S.D. 3
	13	4	/
	14	8	3
	15	16	10
	16	20	9
	17	20	9
	18	20	8
	19	20	7
	20	20	7
	21–26	0	4–10 Mean 9 S.D. 4

We do not at the present time wish to stress the significance of the rise in urinary oxalate excretion which occurred when glycine was fed to Patient 2, because it was impracticable to repeat the experiment on this patient, and we considered that it would be unwise to do so on Patient 1 in view of his impaired renal function.

A normal subject (aged 12) was given large oral doses of glycine (20–100 g. daily, divided into four equal and equally spaced doses) for six days. The daily urinary oxalate excretion during the period of glycine administration was equivalent to between 2 and 12 mg. of $(COOH)_2.2H_2O$, and the corresponding values for a four-day control period before and a three-day control period after the administration of glycine were 4 and 10 mg. and 8 and 12 mg. of $(COOH)_2.2H_2O$ per 24 hours respectively.

Discussion

It is assumed, in the interpretation of the present results, that there is no appreciable change in the oxalate content of urine during its passage through the collecting tubules of the kidney and the urinary passages. Although there can be little cause to doubt the validity of this in normal subjects, it might not necessarily apply to patients with urinary calculi or nephrocalcinosis, and we have aimed to employ sufficiently long experimental and control periods to allow for the spontaneous fluctuations in the day-to-day urinary oxalate excretion which occurred in our patients.

Variations in the intake of oxalate from the usual dietary sources do not provide an adequate explanation for the high levels of urinary oxalate excretion encountered in primary hyperoxaluria (Archer *et al.*, 1957a). There appears to be no tendency for patients with primary hyperoxaluria to excrete a larger proportion of a test dose of oxalate than normal subjects, and it is concluded that there is also no tendency for these patients to absorb oxalate from the gastro-intestinal tract excessively. It should be pointed out, however, that an increased oxalate absorption would be masked in this test if there were simultaneously a tendency for oxalate to be retained in the tissues or if the renal excretion of the anion were limited. The changes in the urinary oxalate excretion which were associated with the ingestion of expressed rhubarb juice indicate that this material does not tend to produce hyperoxaluria out of proportion to its oxalate content in patients with primary hyperoxaluria.

The observation that the magnitude and the time course of the increase in the urinary oxalate excretion did not differ

materially from that observed in comparable experiments with normal subjects also suggests that the fundamental abnormality in primary hyperoxaluria is not a low renal oxalate threshold, although completely conclusive evidence on this problem cannot be obtained until satisfactory methods for determining the blood oxalate have been developed (cf. Barber and Gallimore, 1940; Barrett, 1943).

Some previously published evidence which suggests that there may be a connexion between glycine metabolism and oxalate production under certain conditions has been mentioned above. The relevant metabolic interrelationships of glycine and glyoxylate are summarized in Fig. 7. The determination of the relative importance of alternative mechanisms for the metabolism of a given substance *in vivo* is a major problem in contemporary biochemistry, and it may be that different mechanisms are responsible for different fractions of the total metabolism of a given substance in different tissues (see, for example, the general review by Potter and Heidelberger (1950), and the studies of Bloom, Stetten, and Stetten (1953), dealing specifically with the relative importance of the glycolytic and non-glycolytic pathways of glucose metabolism).

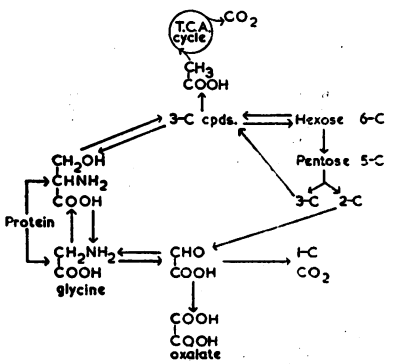

FIG. 7.—Some metabolic interrelationships of glycine, with particular reference to the possible formation of oxalate from glycine in primary hyperoxaluria. The symbols "1-C," "2-C," etc., indicate the number of carbon atoms in the compounds or "active residues" concerned. These "skeleton formulae" have been employed in order to make the general pattern of the reaction sequences more obvious than is the case if the full structural formulae are used.

The relative importance of the two possible routes of glycine metabolism shown in Fig. 7, and hence the importance of the reaction sequence glycine→glyoxylate→formate+CO_2 in the overall metabolism of glycine *in vivo*, is controversial (Arnstein, 1954; Weinhouse, 1955; Arnstein and Keglević, 1956). It appears, however, that the individual reactions are thermodynamically possible *in vivo* and that, if glyoxylate oxidation to formate and carbon dioxide becomes a rate-limiting step in the reaction sequence, oxalate synthesis might occur as it does in these circumstances in the rat liver *in vitro* (Nakada *et al.*, 1955). This circumstance would arise *in vivo* if a greater proportion of the total glycine metabolism occurred via glyoxylate than normally without any corresponding increase in the ability to degrade glyoxylate or if the degradation of glyoxylate was deficient owing, perhaps, to a deficiency of the enzyme system, or systems, necessary for its conversion to formate and carbon dioxide. Quantitatively, a metabolic lesion such as this would need to involve only a relatively small portion of the total daily glycine turnover* to result in the production of the 1–2 mMol of additional oxalate excreted by subjects of primary hyperoxaluria.

There is a small but statistically significant difference ($0.01<P<0.02$) between the average daily urinary oxalate excretion during the low-protein-feeding period and that during the corresponding control periods in the case of Patient 1. The difference between the average values for the two control periods is not statistically significant ($0.05<P<0.1$). It is apparent, therefore, that low-protein feeding was associated with a decrease in the daily urinary oxalate excretion in this patient. If this is in fact a cause-and-effect relationship it could be explained on the basis of the hypothesis that glycine is a precursor of at least some of the urinary oxalate in patients with primary hyperoxaluria. This effect was not observed in the studies on Patient 2. The reason for this discrepancy is not clear, although it may have been due to the fact that the day-to-day fluctuations in the urinary oxalate excretion level are generally larger in this patient than they are in Patient 1, and this would tend to obscure small differences between the average urinary oxalate excretion during the experimental and the control periods.

It is reasonable to assume that large doses of the benzoate ion which combines specifically with glycine produces a greater depletion of the free glycine metabolic pool than can be achieved by the administration of a low-protein diet. We feel that the changes in the daily urinary oxalate excretion which occurred when massive doses of sodium benzoate were administered provide experimental support for the hypothesis that glycine is the precursor of the urinary oxalate in primary hyperoxaluria.

The observation that sodium benzoate administration did not lower the urinary oxalate excretion by two normal subjects suggests that the urinary oxalate may not normally be derived from the same sources as it is in cases of primary hyperoxaluria. It should, however, be pointed out that the apparently spontaneous fluctuations in the daily oxalate excretion by normal subjects (Archer *et al.*, 1957b) are such as to mask any *small* changes which may have occurred, so a partial origin of the normal urinary oxalate from glycine via glyoxylate is not excluded by this finding.

The rate of glycine synthesis in mammals does not greatly surpass that required for growth, and in the growing rat it is insufficient for both growth and the detoxication of more than 1–2 mMol of benzoate per day (Arnstein and Neuberger, 1953). No comparable data are available for man.

The rise in the excretion of oxalate by Patient 1 which occurred when the dose of benzoate remained steady at 20 g. per day (Fig. 6) and our failure to produce permanent lowering of the oxalate excretion in Patient 2 by prolonged benzoate administration may have been due to the mobilization of biosynthetic sources of glycine involving metabolic pathways which normally contribute only a minor fraction of the glycine available for conjugation with benzoate, or to a compensatory increase in the proportion of the total dose of benzoate which was detoxicated by some alternative mechanism (for example, glucuronide formation).

There have been grounds for suggesting that ascorbic acid, which can be quantitatively converted to oxalic and L-threonic acids by chemical oxidation (Herbert, Hirst, Percival, Reynolds, and Smith, 1933), may be a precursor of the urinary oxalate. Lamden and Chrystowski (1953, 1954) found that the ingestion of large doses (4 g. or more daily) of ascorbic acid increased the urinary oxalate excretion by normal subjects, and Curtin and King (1955) demonstrated the conversion of ascorbic acid-1-^{14}C to oxalate-^{14}C in the intact rat, 2% of the injected ^{14}C appearing as oxalate-^{14}C in 24 hours. It is apparent from the stoicheiometry of ascorbic acid oxidation that the production of the amount of oxalate (of the order of 1–2 mMol per day) which was excreted by patients with primary hyperoxaluria would require the complete oxidation of at least 1–2 mMol (176–352 mg.) of ascorbic acid to oxalic and L-threonic acids per day. This is more than the usual daily dietary intake of the vitamin, and it appears improbable on quantitative grounds, therefore, that ascorbic acid can be the source of all the urinary oxalate in cases of primary hyperoxaluria; whether or not it contributes to the normal urinary oxalate excretion remains, however, an open question.

*Arnstein and Stanković (1956) estimated the daily biosynthesis of glycine by the rat and guinea-pig to be 2.7 and 4 mMol/100 g. body weight respectively, and Arnstein and Neuberger (1951) estimated the rat's free glycine pool to be of the order of 0.1 mMol per 100 g body weight. A preliminary attempt to measure the size of the free glycine pool in a normal man yielded a result of similar order of magnitude, although the rate of turnover of glycine in the pool appeared to be somewhat slower (Crawhall and Watts, unpublished data).

We are unaware of any information concerning the mechanism by which the oxalate ion is excreted by the kidney, whether by glomerular filtration only or by a combination of glomerular filtration and renal-tubular excretion. In the latter case it is, at least theoretically, possible that either the hippurate ion or the benzoate ion, or both these, might compete with the oxalate ion for common transport mechanisms in the renal tubule epithelial cells, and that if the former ionic species were present in sufficiently high concentrations such competition would produce a decrease in the urinary oxalate excretion. The results of the experiments in which sodium benzoate was administered to normal subjects are not in accord with this explanation of the decrease in the urinary oxalate excretion which was observed when sodium benzoate was given to patients with primary hyperoxaluria. This tentative conclusion requires confirmation by the determination of the renal oxalate clearance, a procedure which will remain impracticable until the blood oxalate level can be satisfactorily determined (see above).

Primary hyperoxaluria appears to be a rare disease (Archer *et al.*, 1957a). It should, however, be considered as a possible diagnosis in patients with a history of renal calculi dating from early childhood. Confirmation of the diagnosis by measurement of the 24-hour urinary oxalate excretion (Archer *et al.*, 1957a, 1957b) is a relatively simple procedure which is within the scope of any routine chemical pathology laboratory. Early diagnosis is of importance in planning the lifelong combined medical and surgical supervision which these patients require, and the detection of additional cases would encourage further study of the possible underlying metabolic abnormality.

Summary

The natural history of primary hyperoxaluria is briefly reviewed and the previously reported cases which appear to be examples of the disease are summarized. A number of incompletely reported cases which present some features of the condition are mentioned.

Experimental studies which were performed on two cases of primary hyperoxaluria with the object of elucidating the nature of the defect from which these patients suffer, together with a limited number of comparable investigations which were carried out on two normal subjects, are described.

The persistently high urinary oxalate excretion which characterizes the condition is not due to excessive gastrointestinal oxalate absorption, nor does it appear, in the present state of our knowledge, to result from a low renal-oxalate threshold.

Depletion of the free glycine metabolic pool lowered the urinary oxalate excretion temporarily in the patients with primary hyperoxaluria. This is discussed in the light of contemporary knowledge concerning some aspects of glycine metabolism. It is suggested that the findings are compatible with the hypothesis that at least some of the urinary oxalate in patients with primary hyperoxaluria is derived from glycine, and that there may be a failure to degrade this amino-acid normally via glyoxylate to formate and carbon dioxide in these cases.

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ABNORMAL RESPONSES TO MUSCLE RELAXANTS IN CARCINOMATOUS NEUROPATHY

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In recent years there has been increasing interest in the group of neurological disorders associated with carcinoma at different sites, but without the presence of metastases in the nervous system. Subacute cerebellar degeneration occurring with carcinoma outside the nervous system was reported by Greenfield (1934) and by Brain, Daniel, and Greenfield (1951), while Denny-Brown (1948) described sensory neuropathy in patients with bronchial carcinoma. Henson (1953), in a discussion on unusual manifestations of carcinoma of the bronchus, reported a group of cases with carcinoma of the lung in which a motor neuropathy and myopathy had developed. Several of these patients had a history of muscular fatigability suggestive of myasthenia. Henson, Russell, and Wilkinson (1954) published a clinicopathological study of 19 cases with various types of carcinomatous neuropathy and myopathy. Eight of these patients had proximal atrophic weakness of the limbs; some also had involvement of ocular and bulbar muscles. Four patients in this group exhibited myasthenic features, including improvement with neostigmine in some cases. Since patients with myasthenia gravis are abnormally sensitive to drugs which interfere with the function of the myoneural junction, it was to be expected that patients with carcinomatous neuropathy who had evidence of myasthenia might also be abnormally sensitive to the various muscle relaxants used in modern anaesthesia.