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THE METABOLIC ERROR IN PRIMARY HYPEROXALURIA*

BY

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Knowledge of the incidence of a disease in successive generations of a family has value beyond the basis it provides for prediction of the health of future generations. Should the incidence suggest inheritance according to simple Mendelian rules, the disease probably has its origin in abnormality of a single genetic particle, though this has never been directly demonstrated. Garrod (1908) linked the occurrence of metabolic errors with the inheritance of Mendelian characteristics. Abnormalities inherited according to Mendelian laws were shown to result from inactivity of a single enzyme, first in moulds (Beadle and Tatum, 1941) and later in human disease (e.g. deficiency of erythrocyte diaphorase in one type of methaemoglobinaemia (Gibson, 1948)). Alteration of only one amino acid in a protein is sufficient to cause disease, as in the haemoglobinopathy of sickle-cell anaemia (Ingram, 1957).

In the many common diseases with a familial tendency that are, at least partially, genetically determined (e.g. essential hypertension, diabetes mellitus, schizophrenia), simple Mendelian rules do not apply, and summation of the actions of several genes has been evoked. In seeking the mechanisms through which inherited flaws are manifest, it seems simpler to study conditions with clear-cut Mendelian inheritance despite their rarity.

The evidence that 'primary hyperoxaluria' is inherited as a Mendelian recessive characteristic will be discussed, and taken as the foundation for a search for an enzymatic fault. All patients with apparently similar metabolic abnormalities will not necessarily have the same metabolic defect, nor will all alterations in one enzyme protein be identical or have the same effect on function. Thus, in the glycogenoses, excess of liver glycogen may be associated with any one of four different enzyme deficiencies (Cori, 1957), and at least five different single amino acid abnormalities of haemoglobin are known. At present only those cases of inherited disease that occur within one family can be considered identical in origin.

The Clinical Condition and its Inheritance

Primary hyperoxaluria (Archer, Dormer, Scowen, and Watts, 1957) or oxalosis (Ying Chou and Donohue, 1952) is a rare and serious disease, which usually presents before the age of 10 years and often causes death from renal failure before the age of 20. It is characterized by excessive deposition of calcium oxalate in the kidneys, recurrent calcium oxalate stones in the urinary tract, excessive urinary excretion of oxalate not caused by excessive ingestion of oxalate, and the finding at necropsy of calcium oxalate crystals throughout the body. None of these abnormalities alone is pathognomonic of the disease, since each occurs in conditions other than primary hyperoxaluria, as has been discussed by Hockaday, Clayton, Frederick, and Smith (1964). On the other hand, the presence of all three abnormalities is not essential in the diagnosis of primary hyperoxaluria. In the absence of any critical *in vitro* test, this is best illustrated by members of a sibship of whom some show all the classical features and some do not. Dr. E. G. Hall has kindly given permission for reference to a further member, born in 1962, of family No. 4 of Hall, Scowen, and Watts (1960). This child was in renal failure in his first month and died in his first year, yet never had renal calculi despite renal and bony oxalate deposits. Four of his sibs, who also had hyperoxaluria, had recurrent oxalate calculi.

The boy, a mongol of birth weight 6 lb. 9 oz. (2,976 g.), was admitted to hospital at the age of 1 month because of a 3-day history of 'convulsions'—in fact, tetany. The

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blood urea was 115 mg./100 ml. and the serum calcium 7·6 mg./100 ml. Urinary excretion of oxalate was around 50 mg./24 hr. The blood urea concentration rose to 250 mg./100 ml., but later he improved slowly and the blood urea decreased. On radiological examination the kidneys had a uniformly increased density but did not contain calculi. At the age of 7 months the boy died at home from aspiration of food. At necropsy the kidneys were rather small and had a smooth surface. There were no calculi but both cortex and medulla were speckled with minute gritty granules. Histological examination showed deposits of oxalate throughout the kidneys and scattered in the bones, but little evidence of oxalate elsewhere.

The first proven case of primary hyperoxaluria was in a 7-year-old girl troubled by urinary calculi since the age of 2 years (Newns and Black, 1953). Apart from this first measurement of the abnormally high urinary excretion of oxalate, this case was important because the patient's elder brother had died at 8½ years from renal failure consequent to multiple calcium oxalate stones and another sib had died shortly after premature birth. Aponte and Fetter (1954), in a report of 3 brothers, 2 of whom were identical twins, described the first cases to show all the features now thought typical of the disease. Thereafter 'primary hyperoxaluria' and 'oxalosis' were generally considered to be the same condition.

Inheritance of primary hyperoxaluria as a recessive characteristic is based upon 16 families in which sibs were affected (Buri, 1962; Hockaday *et al.*, 1964). The most extensive surveys of urinary oxalate excretion by the relatives of patients (Archer, Dormer, Scowen, and Watts, 1958b; Scowen, Watts, and Hall, 1959) showed abnormally high values in some sibs, but never in parents or other relatives. Likewise, no parent has had a medical history typical of primary hyperoxaluria, though parents or other close relatives may have had renal stones in middle age. Parental consanguinity has been reported in four cases.

The following brief description of two patients and their families, previously reported only in summary (Hockaday *et al.*, 1964), provides additional evidence that primary hyperoxaluria is inherited as a Mendelian recessive characteristic.

Case Reports

Case 1. J.M., a 13-year-old girl, had passed gravel in her urine and had had slight haematuria when less than 1 year old. These symptoms, together with flank and abdominal pain, had recurred frequently. Despite repeated urinary infections, recently controlled by continual antibiotic treatment, the girl is healthy, of normal size, and menstruates regularly. Her urinary oxalate excretion varied from 200 to 240 mg./24 hr. (the upper limit of normal is 60 mg./24 hr.).

Her parents, not consanguineous, have had 6 other children. One healthy daughter, age 23, is married with a healthy child, and one son age 7 is healthy. A 4½-year-old boy, though physically healthy, is mentally retarded. One son died age 7 years, after pain, vomiting, fever, haematuria, and the passage of gravel had begun in his second year. A kidney removed at operation shortly before his death contained many deposits of calcium oxalate. Another son died at 13 years and a daughter died at 5½ years; both had had abdominal pain, haematuria, and passage of gravel since infancy. The parents are healthy and without evidence of disease of the urinary tract.

Case 2. T.S., an 18-year-old college student, has had recurrent urinary calculi since the age of 9. In the past she had had episodes suggestive of renal colic, more frequently dull pain in both renal angles, and episodes of urinary frequency and nocturia. Although she has been relatively free of symptoms during the past 2 years, radiological examination has shown stones in both kidneys. Her urinary excretion of oxalate was 190 and 230 mg./24 hr. on two separate occasions. She is a normally developed girl and no abnormalities were found on abdominal examination, except for tenderness on palpation posteriorly over the left renal angle.

The girl has 6 sibs, 4 of whom are healthy. A 6-year-old brother has had recurrent renal stones, and both he and another brother age 5 years have increased urinary excretion of oxalate. The parents, from unrelated families, are healthy and have no history of renal disease.

Claims that primary hyperoxaluria is inherited as a dominant trait are based on study of a few families only. The evidence has consisted of slight increase of urinary oxalate excretion by relatives in previous generations (Shepard, Krebs, and Lee, 1958), of the occurrence of renal stones in previous generations, but without hyperoxaluria or a typically severe clinical course (McLaurin, Beisel, McCormick, Scalettar, and Herman, 1961), or of case histories in which death from uraemia and increased urinary excretion of oxalate went with other features suggestive of a primary renal disorder (Lagrue, Laudat, Meyer, Sapir, and Milliez, 1959; Dempsey, Forbes, Melick, and Henneman, 1960).

No consistent abnormality has yet been detected in the parents of patients, who are presumed heterozygous for the condition.

Metabolic Investigations

Neither increased alimentary absorption nor decreased destruction of oxalate is likely to be the cause of oxalosis and primary hyperoxaluria. Archer, Dormer, Scowen, and Watts (1958a) showed that patients absorbed oxalate normally. Injected ^{14}C-labelled oxalate can be almost completely recovered from urine and does not contribute to

expired carbon dioxide (Elder and Wyngaarden, 1960), which indicates that oxalate is metabolically inert in humans.

Increased formation of oxalate within the body, the result of the postulated enzyme defect, seems a more probable mechanism of the disease. The only known precursor of oxalate is glyoxylate (Fig. 1), though carbon atoms 1 and 2 of ascorbic acid may be converted to oxalate without prior formation of glyoxylate (the results of Atkins, Dean, Griffin, Scowen, and Watts (1963) agree with this possibility). Possible precursors of glyoxylate are glycollate, glycine, and γ-hydroxy-α-ketoglutarate, a metabolite of hydroxyproline (Goldstone and Adams, 1962). Since increased urinary excretion of glycollate is as characteristic of the disease as is hyperoxaluria (Frederick, Rabkin, Ritchie, and Smith, 1963), deficient conversion of glyoxylate to glycollate cannot be its cause. Also, γ-hydroxy-α-ketoglutarate may be metabolized more readily by way of malate than by decomposition to glyoxylate and pyruvate (Payes and Laties, 1963). The evidence for the formation of glyoxylate from glycine comes from *in vitro* experiments, in which high concentrations of glyoxylate were used (Nakada and Weinhouse, 1953). Because such concentrations inhibit tissue respiration the findings may apply only to tissues with such an abnormal metabolism. Other experiments have demonstrated that glycine is an important precursor of oxalate *in vivo*. Crawhall, de Mowbray, Scowen, and Watts (1959a) and Crowhall, Scowen, and Watts (1959b), in studies of two hyperoxaluric patients, showed that the proportion of urinary oxalate formed from glycine was normal in both.

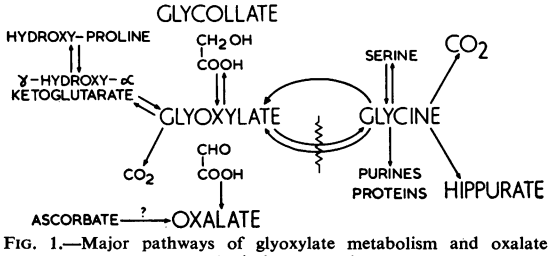

FIG. 1.—Major pathways of glyoxylate metabolism and oxalate synthesis in mammals.

Many transaminase enzymes are present in both mitochondria and the cytoplasmic sap (Baudhuin, Beaufay, Rahman-Li, Sellinger, Wattiaux, Jacques, and de Duve, 1964). The equilibrium position for the reversible interconversions of glycine and glyoxylate catalysed by transaminase enzymes is strongly in favour of glycine, but glycine oxidase (Ratner, Nocito, and Green, 1944) catalyses an irreversible formation of glyoxylate.

The mode of oxidation of glyoxylate to carbon dioxide is uncertain, though one reaction involves glutamate or α-ketoglutarate; its exact mechanism is unknown (Nakada and Sund, 1958; Crawhall and Watts, 1962a). Glycine is sometimes assumed to be oxidized principally via glyoxylate, but conversion of glyoxylate to carbon dioxide after transamination to glycine is equally possible. Glyoxylate and glycollate are readily converted to glycine *in vivo* in rats (Weinhouse and Friedmann, 1951) and in pigeons (Weissbach and Sprinson, 1953), and glycine may be oxidized to carbon dioxide mainly by reactions involving serine, pyruvate or hydroxy-pyruvate, δ-amino-laevulinic acid, or amino acetone, but not glyoxylate.

The metabolism of glyoxylate, by mitochondria from liver biopsy specimens from hyperoxaluric patients, was normal in its conversion to glycine, oxalate, and carbon dioxide (Crawhall and Watts, 1962b). In contrast, Frederick *et al.* (1963), after they injected ^{14}C-carboxyl-labelled glyoxylate intravenously, recovered much less isotopic carbon dioxide from patients than from normal subjects.

Our Recent Findings

The results of our recent studies on the defective metabolism in primary hyperoxaluria are summarized in this section according to the type of measurement made.

The time-honoured method used for metabolic investigations is the measurement of the amount of a substance excreted in the urine of a subject both under normal conditions and after administration of a possible precursor in large amount. Urinary glyoxylate concentration had not been measured previously by a quantitative method. Using the principle of isotope dilution, a method was developed for the measurement of urinary glyoxylate (Hockaday, Frederick, Clayton, and Smith, 1965). Patients with primary hyperoxaluria excreted more glyoxylate in the urine than did normal children (Hockaday *et al.*, 1964), in line with previous findings for oxalate and glycollate. The significance of the values obtained for glyoxylate is uncertain because the reactivity of glyoxylate with a number of compounds in urine makes it likely that the amount of glyoxylate in voided urine is not the same as that of urine in the renal calyces.

The effect of the ingestion of large amounts of ascorbic acid, glycine, or 1-hydroxyproline on the urinary excretion of glyoxylate, glycollate, and oxalate was observed in both healthy subjects and in patients with primary hyperoxaluria (Table 1). A change was seen only after 1-hydroxyproline (200 mg./kg.), when urinary excretion of glyoxylate and

TABLE 1

URINARY EXCRETION OF OXALATE, GLYOXYLATE, AND GLYCOLLATE AFTER ORAL ADMINISTRATION OF GLYCINE AND HYDROXYPROLINE IN PRIMARY HYPEROXALURIA

	Oxalate (μ mole/hr.)			Glycollate (μ mole/hr.)			Glyoxylate (μ mole/hr.)		
	Normal	Patient 1	Patient 2	Normal	Patient 1	Patient 2	Normal	Patient 1	Patient 2
Glycine, 400 mg./kg.									
Before dose	15	48	162	30	59	244	0·43	0·24	0·78
After dose, 0-3 hr.	19	52	116	52	96	119	0·68	0·36	0·51
3-6 hr.	17	73	113	70	56	106	0·92	0·34	0·62
6-9 hr.	12	47	110	61	179	124	0·64	0·23	0·52
l-Hydroxyproline, 200 mg./kg.									
Before dose	21	55	120	39	68	106	0·35	0·15	0·48
After dose, 0-3 hr.	32	91	121	238	253	172	1·26	0·92	0·33
3-6 hr.	23	94	106	272	402	139	1·35	0·51	0·69
6-9 hr.	29	90	127	174	386	298	1·01	0·61	0·12

N.B. Patient 1 is Case 2 (age 11 years) of Frederick *et al.* (1963) and Patient 2 is their Case 1 (age 14 years) when some impairment of renal function (blood urea nitrogen = 34 mg./100 ml.) was already evident.
After 3 g. ascorbic acid, urinary excretion of oxalate, glyoxylate, and glycollate was unchanged in both a patient and his mother.

glycollate (but not oxalate) increased. Previously, repeated observations had shown that urinary oxalate excretion is unchanged by large amounts of glycine or ascorbic acid (Elder and Wyngaarden, 1960).

A second method of investigation is to follow the entry into various metabolites of an isotopic carbon atom from a compound concerned in oxalate metabolism. After intravenous injection of carboxyl-labelled ^{14}C-glycollate, the entry of ^{14}C into expired carbon dioxide and urinary oxalate and glycollate showed the same difference between hyperoxaluric patients and control subjects as when ^{14}C-glyoxylate had been injected: there was a low recovery of ^{14}C in carbon dioxide and increased recovery in urinary oxalate and glycollate. The urinary glyoxylate of patients also contained more ^{14}C than that of control subjects. Urinary hippurate and glycine, like carbon dioxide, contained less ^{14}C when collected from patients than from healthy subjects or even the parent of a patient (Table 2, Fig. 2). The patients showed no evidence of renal failure (blood urea nitrogen concentrations and creatinine clearances were normal).

This decreased recovery of injected ^{14}C as urinary hippurate and glycine from patients compared with controls was confirmed for ^{14}C-carboxyl-labelled glyoxylate on its intravenous infusion for 6 hours, instead of the single, rapid injection used in previous studies (Frederick *et al.*, 1963). Observations on expired carbon dioxide were made hourly during the last 3 hours of the infusion, when an approximately steady state had been reached, and these (as well as measurements on urine for 24 hours from the start of the infusion) are listed in Table 3 and illustrated in Fig. 3. The pattern in patients of decreased recovery of isotope as urinary hippurate and glycine, as well as respiratory carbon dioxide, was maintained, as was the increase in isotope found as urinary glycollate, glyoxylate, and oxalate.

These observations on urinary hippurate and glycine strongly suggest a defect in the conversion of glyoxylate to glycine in primary hyperoxaluria. They can be easily reconciled with the observations

TABLE 2

RECOVERY OF RADIOACTIVITY AFTER INTRAVENOUS INJECTION OF SODIUM GLYCOLLATE, $CH_2OH \cdot {}^{14}COONa$
(1μ mole/kg. body weight and 20 μc. given to each)

Subjects	Percentage Recovery of Administered Radioactivity in Two Periods (Time in Hr.)											% Recovery Administered
	Expired CO:	Urinary Oxalate		Urinary Glyoxylate		Urinary Glycollate		Urinary Hippurate		Urinary Glycine		
	0–2	0–2	2–24	0–2	2–24	0–2	2–24	0–2	2–24	0–2	2–24	Counts
Normal	22·3	0·7	0·11	0·07	0·05	1·8	0·14	3·5	0·53	0·11	0·02	29·3
Normal 2	14·2	1·1	0·14	0·11	None	2·7	0·05	1·4	0·51	0·15	0·06	19·4
Heterozygote	20·5	1·1	0·07	0·16	None	2·8	0·13	5·8	0·42	0·15	0·15	31·3
Patient 1 (L.E.)	2·1	3·8	13·7	0·45	0·14	9·5	27·0	0·18	0·08	0·01	0·02	57·0
Patient 2 (D.M.)	3·0	4·4	11·0	0·08	0·09	14·9	20·1	0·39	0·35	0·01	0·02	54·4

L.E. was an 8-year-old girl and D.M. was a 5-year-old boy. Both have had recurrent urinary oxalate calculi since the age of 2-3 years and have increased urinary excretion of oxalate. Though both had renal calculi on radiographs, there was no significant impairment of renal function in either.

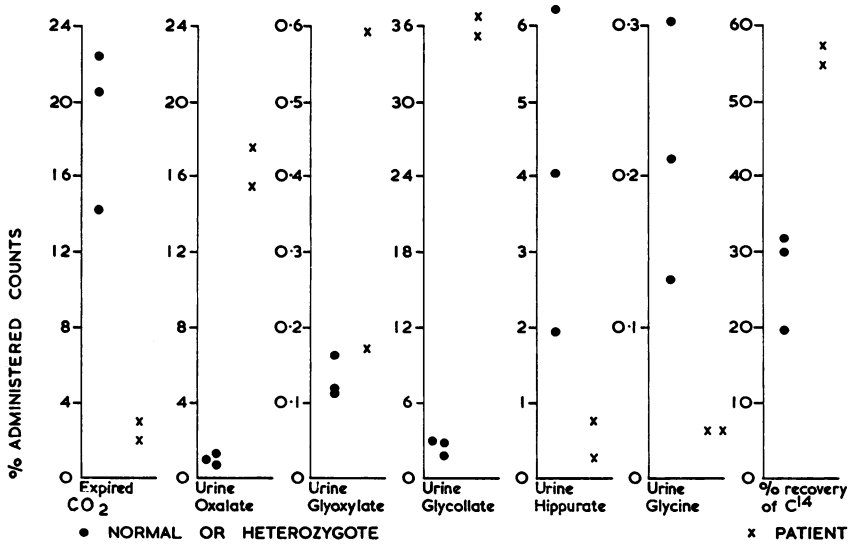

FIG. 2.—Metabolism of carboxyl-labelled glycollate-^{14}C in man. The recovery is shown of injected ^{14}C in respiratory CO_2 (2 hours), 5 urinary compounds (24 hours), and these 6 compounds combined after rapid intravenous injection of sodium glycollate (1μmole/kg. body weight).

suggesting impaired conversion of glyoxylate to carbon dioxide only if it is accepted that glyoxylate is converted to carbon dioxide primarily via glycine. It must be emphasized that the specific activities of the excreted labelled compounds were not measured.

The third and most crucial method of investigation is the *in vitro* demonstration of defective metabolism by tissue from a patient, The hypothesis of defective conversion of glyoxylate to glycine is not new and has been tested *in vitro* with tissue from patients. Neither Shepard, Krebs, Lee, and Johnson (1960) nor Crawhall and Watts (1962b) found any abnormality in transamination. However, a defect in the conversion of glyoxylate to glycine might be difficult to detect because of the number of enzymes that can catalyse this reaction. In studies of rat liver, Nakada (1964) recently reported the purification of a specific glyoxylate-glutamate transaminase confined to the cytoplasmic sap and different from the main mitochondrial glutamate transaminases. The mitochondria are also the main site of ω-transaminase, for which ornithine is the most reactive donor (Meister, 1954; Peraino and Pitot, 1963), while the greater part of the alanine and aspartate-aminotransferase activity is in the cell sap (Baudhuin *et al.*, 1964). Transamination

TABLE 3

RECOVERY OF ^{14}C IN URINE ON INTRAVENOUS INFUSION OF SODIUM GLYOXYLATE† ($CHO \cdot C^{14}OONa$) FOR 6 HOURS

† (1μ mole/kg. body weight and 25 μc. given to both)

Subject	Times (hr.)	% Urinary Counts Recovered As					% Urine Counts Recovered	% Administered Counts Recovered in Urine
		Oxalate	Glyoxylate	Glycollate	Hippurate	Glycine		
Heterozygote ..	0–3 Infusion	61·6	4·7	8·4	0·50	0·045	75·0	44·2
	3–4 Infusion	54·7	2·7	11·8	0·44	0·042	69·7	
	4–5 Infusion	65·6	3·8	25·1	0·37	0·080	95·1	
	5–6 Infusion	76·1	0·9	7·7	0·63	0·071	85·4	
	6–8	64·6	0·3	6·3	0·49	0·089	71·9	
	8–10	69·4	0·6	5·5	4·91	0·014	80·4	
	10–12	64·6	0·4	4·4	0·80	0·003	70·2	
	12–23	59·9	1·6	Zero	0·39	Zero	61·9	
Patient (J.M.) ..	0–3 Infusion	58·0	22·5	18·3	0·18	0·004	99·0	57·2
	3–4 Infusion	68·4	34·9	13·2	0·12	0·004	116·6	
	4–5 Infusion	63·0	24·4	26·2	0·06	0·010	113·7	
	5–6 Infusion	43·8	11·1	21·0	0·02	0·004	75·9	
	6–8	48·8	10·0	16·9	0·03	0·020	75·7	
	8–10	56·4	2·3	14·4	0·08	0·011	73·2	
	10–12	38·0	1·5	50·8	0·41	0·007	90·7	
	12–21	29·4	Zero	22·2	0·24	0·006	51·8	

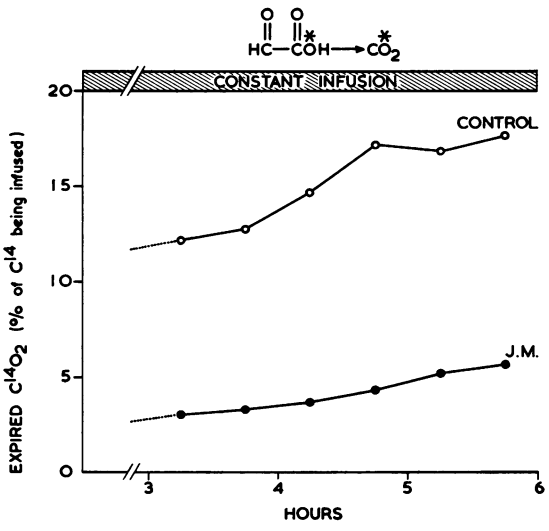

FIG. 3.—Metabolism of carboxyl-labelled glyoxylate-^{14}C in man. Its conversion to respiratory $^{14}CO_2$ is expressed as percentage of isotope being infused during the collection period, and is compared in a patient with primary hyperoxaluria and in her mother. One μ mole/kg. body weight of glyoxylate was infused throughout a 6-hour period (from Hockaday *et al.* (1964) with permission of the publishers).

may also be associated with deamidation, as when glutamine or asparagine is the donor (Meister, Sober, Tice, and Fraser, 1952).

Attempts to demonstrate defective glycine formation from glyoxylate *in vitro* have so far been unsuccessful. Enzymatic transamination of glyoxylate was not found in intact erythrocytes, nor haemolysates, intact or sonicated leucocytes, or plasma. A cytoplasmic glyoxylate-glutamate transaminase in human liver exhibits similar characteristics to those recently described for this enzyme in rat liver (Nakada, 1964). In a single study this enzymatic activity was normal in liver obtained *post mortem* from a typical patient with primary hyperoxaluria (tissue obtained with the help of Dr. O. Wrong). The transamination reactions that convert glyoxylate to glycine are being studied at present, and this work must be extended to all the transaminase enzymes active with glyoxylate.

Treatment

The primary aim of understanding the pathogenesis of a disease is to provide a basis for treatment. Admittedly identification of the protein, abnormality of which underlies a metabolic error, has rarely, as yet, been followed by an improvement in methods of treatment. Two possible ways of treating hyperoxaluria are to increase the urinary solubility of oxalate and to manipulate metabolism so that the amount of oxalate formed in the body and the amount excreted in the urine are reduced. Apart from avoidance of foods with a particularly high oxalate content (e.g. spinach, tomato, rhubarb, chocolate, coffee, and tea) and drinking a large volume, dietary measures are not likely to help. A low protein diet does not reduce urinary oxalate.

Because vitamin B6 provides a co-factor for glyoxylate-glycine transamination and for the interconversion of glycine and serine, and because hyperoxaluria is found in B6-deficient animals (Gershoff, Faragalla, Nelson, and Andrus, 1959), pyridoxine treatment has been tried for hyperoxaluria, though B6 deficiency in infants is not accompanied by clinical or biochemical evidence of hyperoxaluria (Scriver and Hutchison, 1963). However, B6 supplements, either alone or combined with folic acid, have had no consistent effect on either urinary oxalate excretion or on the clinical course of hyperoxaluria. A number of other agents, including ascorbic acid, *d*-glutamate, and histidine, have been given, also without apparent effect. A reduction in urinary oxalate has often followed ingestion of sodium benzoate (Archer *et al.*, 1958a; Daniels, Michels, Aisen, and Goldstein, 1960), but the improvement lasted for only two weeks despite continued treatment. Presumably the increased conversion of glycine to hippurate associated with excess benzoate provokes a compensatory hypertrophy of mechanisms producing glycine. This idea is supported by the 'rebound' to unusually high levels of urinary oxalate excretion that occurs immediately administration of benzoate ceases.

Summary

The clinical features of primary hyperoxaluria are recurrent renal calculi and nephrocalcinosis in the young. Inheritance of this condition as a Mendelian recessive characteristic prompts search for a defective enzyme in patients with this disease.

The metabolism of oxalate and glyoxylate is reviewed briefly, together with previous metabolic observations on patients with hyperoxaluria.

The detailed results are given of the recovery of ^{14}C as expired carbon dioxide and urinary oxalate, glyoxylate, glycollate, glycine, and hippurate after intravenous injection of carboxyl-labelled glycollate or glyoxylate. There was less ^{14}C in carbon dioxide, glycine, and hippurate from the patients than from normal subjects or parents of patients.

These results suggest defective transamination of glyoxylate to glycine in patients with primary hyperoxaluria. No support for this belief has come

from preliminary observations *in vitro* with blood cells and liver tissue from patients.

No satisfactory treatment of this condition has been found, though the usual symptomatic treatment for oxalate calculi should be given.

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