LIVER, PANCREAS, AND BILIARY TRACT

Oral Calcium Phosphate: A New Therapy for Crigler–Najjar Disease?

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Background & Aims: Calcium phosphate binds unconjugated bilirubin in vitro, and dietary calcium phosphate supplementation reduces the serum bilirubin level in rats with hereditary unconjugated hyperbilirubinemia (Gunn rats). The aim of this study was to evaluate the effect of oral calcium phosphate supplementation on plasma bilirubin levels in patients with Crigler–Najjar disease. **Methods:** A placebo-controlled, double-blind, crossover design was used. Eleven patients, 2–42 years of age, participated. The group included 5 patients with type I disease who were all treated with phototherapy and 6 patients with type II disease who were primarily treated with phenobarbital. In addition to plasma bilirubin levels, dietary intake and urinary and fecal excretion of calcium and phosphate were evaluated. **Results:** A modest but significant decrease in serum bilirubin was observed in patients with type I disease ($18\% \pm 6\%$, $P = 0.03$) but not in patients with type II disease during treatment with calcium phosphate. Urinary output of calcium and phosphate did not change during the treatment period. **Conclusions:** Oral calcium phosphate may be a useful adjuvant to phototherapy in Crigler–Najjar type I disease.

Crigler–Najjar disease, first described in 1952,¹ is an autosomal recessive inherited disorder caused by a deficiency of bilirubin–uridine 5'-diphosphate–glucuronosyltransferase (bUGT) in the liver. Patients affected by this disease have unconjugated hyperbilirubinemia. Two types of the disease are distinguished.² Patients with type I disease are the most severely affected: the enzyme activity of bUGT is absent, bile analysis shows only traces of bilirubin conjugates, plasma bilirubin

levels are $>350 \mu\text{mol/L}$ (20.5 mg/dL), and plasma bilirubin level does not decrease after treatment with phenobarbital. Patients with type II disease have less severe disease because of residual activity of the bUGT. In these patients, plasma bilirubin can be reduced partly by treatment with phenobarbital. The prevalence of Crigler–Najjar disease is extremely low, approximately 1:1,000,000.³ Until about 20 years ago, most patients with type I disease died at an early age because of the development of kernicterus, and only few patients with type II disease were reported to live without complications into the sixth decade.⁴ Since the introduction of phototherapy during the early 1970s,⁵ most patients survive childhood, but development of neurological disorders at a later age has been described.⁶ In The Netherlands, 19 patients with this disease are known, 9 of them with type I and 10 with type II disease. The patients with type I disease (age, 1–22 years) are treated with home phototherapy for 8–12 h/day, and those with type II disease (age, 8–42 years) are treated with either phenobarbital or a combination of phototherapy and phenobarbital. Three patients with type II disease do not receive any treatment. The neurological condition of all patients is good.

Despite the fact that the immediate risk of kernicterus and death is greatly diminished since the introduction of phototherapy, a lot of problems remain for the patients. For instance, all type I patients have skin problems caused by the phototherapy. The skin is fragile, easily injured, and heavily pigmented despite the fact that only

Abbreviations used in this paper: ACP, amorphous calcium phosphate; bUGT, bilirubin–uridine 5'-diphosphate–glucuronosyltransferase; UCB, unconjugated bilirubin.

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blue light is used. The long-term effects of phototherapy are unknown, but it has been described that DNA damage is induced in human cells exposed to phototherapy light, and this effect is enhanced by bilirubin.⁷ These patients cannot live away from home because they need daily phototherapy. Furthermore, although the sedative effect of phenobarbital in many patients is transient, some patients with type II disease complain of lethargy and diminished mental activity. In addition, phenobarbital affects other enzymes in the liver, such as cytochrome P450s, and thereby influences the metabolic breakdown of drugs and nutrients.

Gunn rats and patients with Crigler–Najjar disease excrete normal amounts of bilirubin and bilirubin breakdown products in the feces despite extremely low excretion of bilirubin in bile.^{8,9} Thus, intestinal excretion of bilirubin has been postulated as an alternative pathway for disposal of bilirubin during unconjugated hyperbilirubinemia. The effectiveness of this excretory route is reduced by reabsorption of unconjugated bilirubin (UCB) in the intestine.^{10–12} Therefore, it should be possible to decrease the plasma bilirubin level by intestinal trapping of bilirubin. The feasibility of this approach has been proven in Gunn rats, the animal model for Crigler–Najjar disease. Oral administration of 5% (wt/wt) activated charcoal decreases bilirubin levels by approximately 40%.¹³ Aspecific binding of a broad range of organic and inorganic compounds renders the applicability of activated charcoal impossible for prolonged use. We have reported that UCB binds to amorphous calcium phosphate (ACP) *in vitro*¹⁴; in line with this, we have demonstrated that oral supplementation of calcium phosphate reduces the plasma bilirubin levels in Gunn rats.¹⁵ The dose of calcium (administered as calcium phosphate) needed to achieve a decrease of approximately 40% was only four times the normal ingested amount of calcium. Based on these results, we initiated a placebo-controlled, double-blind, crossover study of the effect of oral calcium phosphate in 11 patients with Crigler–Najjar disease.

Patients and Methods

Medication

In the human gut, substantial amounts of phosphate are present. As we have shown, free phosphate inhibits the binding of UCB to ACP *in vitro*.¹⁴ Therefore, the phosphate already present in the gut should be used in the formation of ACP. This can be achieved by administration of calcium carbonate. Consumption of up to 50 mmol of calcium carbonate/day leads to the formation of ACP, as has been shown by fecal analysis of human adults receiving calcium supplementation. Higher intake of calcium carbonate does not lead to more ACP formation. To produce more ACP in the intestine, supplementation of calcium phosphate is needed.¹⁶

The effect of 100 mmol calcium (50 mmol CaCO₃ and 50 mmol CaHPO₄ · 2H₂O)/day in adult patients with Crigler–Najjar disease was investigated. Children (<40 kg body wt) received 2.5 mmol calcium/kg body wt. The molar ratio of carbonate vs. phosphate was identical in adults and children.

Patients

Eighteen patients and their parents received written and oral information about the trial and were asked to participate in the study. A 1-year-old patient was excluded. The only other exclusion criterion was impaired renal function as determined by a serum creatinine above the age-dependent normal value. All patients had normal renal function. Reasons for withdrawal were an increase in plasma bilirubin level above 450 µmol/L (26.3 mg/dL), sustained constipation during the experimental period, a decrease in phenobarbital levels under the therapeutic range (only for type II patients), and the wish of the patient or parents to stop. The study was approved by the medical ethical committees of both the Academic Medical Center of University Hospital of Amsterdam and the Sophia Children's Hospital connected to the University of Rotterdam. Eleven patients, 10 from The Netherlands and 1 from Belgium, were willing to participate. Patients older than 18 years gave written informed consent; for patients younger than 18 years, the parents gave informed consent. In patients 1–9, the trial was performed during spring (April to June) and in patients 10 and 11 during autumn (September to November).

Study Design

A placebo-controlled, double-blind, crossover study was performed. Cellulose was used as placebo because, in Gunn rats, a diet containing 40% (wt/wt) Cellulose had no effect on plasma bilirubin levels (unpublished results, December 1991 to February 1992).

The duration of the trial was 10 weeks. The usual therapy was continued. Blood was collected by venipuncture once a week. The intraindividual variation in plasma bilirubin level was determined in three blood samples during a control period. During the next 3 weeks, patients ingested either the calcium medication or the placebo and blood was collected three times (treatment period or placebo period). The daily dosage was divided into four portions and ingested with the meals and before bedtime. Then a washout period of 2 weeks without treatment followed. At the end of this period, bilirubin was analyzed again. Subsequently, the patients received either the placebo or the calcium medication (placebo or treatment period). Thus all patients received one course of calcium medication and one course of placebo and could be used as their own control.

Patients were allowed to eat their normal diet, but the use of vitamin D was prohibited. Food intake was assessed three times, once in each period. Patients and parents were instructed by a dietician to keep a detailed record for 3 successive days, ending the first day of the weekend. During the same weekend, the patients collected 24-hour urine and 48-hour feces, except in the control period. The dietary records were coded for analy-

sis by a dietician and nutrient intake was calculated from the computerized Netherlands food composition table.

Blood samples were assayed for bilirubin both in the local hospital and in our own laboratory. Directly after venipuncture, the tubes were protected from light. Bilirubin, hemoglobin, and hematocrit were analyzed within 30 minutes. The rest of the plasma was placed on dry ice and handed to the researcher. It was analyzed further in the laboratory of the Academic Medical Center, Amsterdam, the next day.

Urine and feces were stored at 4°C and -20°C, respectively, immediately after collection and handed to the researcher within 4 days. Urine was processed the next day, and feces were stored at -20°C until analyzed. Patients or parents were asked to keep a record about the frequency of bowel movements and possible side effects.

In vitro binding of phenobarbital and estrogen to calcium phosphate was analyzed and found to be undetectable. However, patients using oral contraceptives were advised to use additional methods during the last 7 weeks of the trial.

Analytical Procedures

Blood. Bilirubin, calcium, magnesium, phosphate, haptoglobin, hemoglobin, hematocrit, lactate dehydrogenase, sodium, potassium, urea, creatinine, and phenobarbital levels were determined using routine clinical chemical methods on a Hitachi 747 analyzer (Hitachi, Tokyo, Japan).

Urine. Urine was acidified by adding 10 mL of 4 mol/L HCl to the flask before collection was started. Urinary calcium, phosphate, creatinine, sodium, potassium, and urea were analyzed by standard clinical chemical methods.

Feces. After determination of wet weight, the 48-

hour collection of stools was diluted 1:1 with bidistilled water and homogenized. Three different samples of approximately 1 g were taken from each collection of stool and weighed. Ten milliliters of 1 mol/L HNO₃ was added to the samples, and the mixture was boiled for 5 minutes. The volume was adjusted to exactly 50 mL with bidistilled water and mixed. Five milliliters was removed and centrifuged at 1500g. The supernatant was analyzed for calcium and phosphate. Calcium was determined by flame spectrophotometry. Phosphate analysis of the supernatant was identical to the analysis of phosphate in urine.

Diet. The mean daily intake of calcium and phosphate was assessed by determining the intake over a period of 3 successive days. This was performed three times, once in each period.

Statistical Analysis

Results are expressed as means $\pm$ SD. For intraindividual differences, statistical analysis was performed using a one-way paired *t* test. For analysis of the group as a whole, a nonparametric paired test was performed, using the mean of the treatment period (*n* = 3) and the mean of control values plus values in the placebo period (*n* = 7) after it was shown that the values in the placebo period were not significantly different from the control values. A level of 0.05, one-tailed, was used for significance.

Results

The clinical parameters of the included patients are described in Table 1. The age of the patients ranged from 2 to 42 years. Five patients had type I and 6 patients type II disease. Four patients were treated with photo-

Table 1. Clinical Parameters of the Patients Included in the Trial

Patient	Age (yr)	Weight (kg)	Sex	Crigler-Najjar type	Therapy	Other medication
1	23	74	F	II	PT: 12 bulbs of 140 W, 10 bulbs of 100 W; ^a 2 h/day PB: 35 mg twice daily	OCC
2	8	22	M	II	PB: 50 mg three times daily	—
3	23	81	M	II	PT: 24 bulbs of 140 W, ^a 2 h/day PB: 50 mg twice daily	Lactulose, 12 g daily Psyllium seed, 3.6 g daily
4	6	25	M	II	PT: 2 bulbs of 40 W, ^b 2 bulbs of 36 W, ^c 2 bulbs of 36 W, ^d 4 h/day PB: 50 mg twice daily	—
5	21	76	F	II	PB: 60 mg daily	OCC
6	5	19.5	M	I	PT: 7 bulbs of 100 W, ^a 12 h/day	—
7	2	11	F	I	PT: 8 bulbs of 20 W, ^a 15 h/day	—
8	6	21	M	I	PT: 6 bulbs of 100 W, ^a 12 h/day	—
9	10	32	F	I	PT: 10 bulbs of 100 W, ^a 9 h/day	—
10	25	63	F	I ^e	—	OCC
11	42	85	M	II	—	—

PT, phototherapy; OCC, oral contraception; PB, phenobarbital.

^aBlue light, Jansen type 52.

^bBlue light, Philips type 03.

^cGreen light, Philips type 17.

^dWhite light, Philips type 94.

^eType I diagnosis was based on reaction to phenobarbital trial only (no reaction at all; maximum dosage, 200 mg/day) and mean bilirubin level of 300 μ mol/L (17.5 mg/dL) without treatment.

Figure 1. Bilirubin level of patients with (A) type I and (B) type II Crigler-Najjar disease during control period, placebo period, and treatment period. Results are expressed as means $\pm$ SD ($n = 3$).

therapy, 2 with phenobarbital, and 3 with a combination of phototherapy and phenobarbital; 2 patients were not treated at all. The intensity of phototherapy differed considerably among the patients. The patients continued their usual therapy during the study. All 11 patients completed the study. Stool frequency was unchanged during the trial. The parents of patients 4 and 7 stated that their child protested more often against one of the two drugs, which afterward proved to be the calcium medication. No other complaints or side effects were mentioned. Bilirubin levels decreased in 9 of 11 patients, but the effect in individual patients reached statistical significance only in patients with type I Crigler-Najjar disease (Figure 1 and Table 2). In patient 4, the bilirubin level was higher during the treatment period than during the placebo period ($P = 0.03$).

The intraindividual variation in plasma bilirubin levels ranged from 1% to 18%. A paired nonparametric test was used to evaluate the effect of the calcium medication in the type I group. A statistically significant decrease in mean plasma bilirubin level was found in the type I group during the treatment period ($18\% \pm 6\%$; $P = 0.03$). In the type II group, no significant difference was found. No differences were observed in plasma calcium, magnesium, phosphate, haptoglobin, hemoglobin, hematocrit, lactate dehydrogenase, sodium, potassium, urea, creatinine, and phenobarbital levels.

As was anticipated, the dietary intake of calcium and phosphate varied among the patients, but less expected, individual patients also consumed highly different

amounts of calcium and phosphate during the three different periods (Table 3). Nevertheless, the total amount of calcium ingested was three to eight times higher during the treatment period than during the placebo period in all patients. The ingested calcium to phosphate ratio was also increased, which is favorable for the formation of amorphous calcium phosphate in the gut.

Urinary output of calcium and phosphate was similar in the placebo period and the treatment period and reflects the lack of absorption of both compounds from the gut (Table 4). Urea and creatinine levels in urine were unaltered. Fecal output of calcium and phosphate was determined in 48-hour collections. In all patients, except patient 4, output of calcium and phosphate markedly increased during the treatment period and the calcium to phosphate ratio in feces increased as well, corresponding to the intake of both compounds (Table 4).

Total recovery of ingested calcium and phosphate in urine and feces varied considerably among patients (Table 5). During the placebo period, calcium recovery was $103\% \pm 31\%$ and phosphate recovery was $90\% \pm 26\%$. Overall recovery of calcium and phosphate was less during the treatment period. In patient 4, recovery of calcium and phosphate was extremely low during the treatment period. This patient therefore might have been noncompliant.

Discussion

To our knowledge, the combination of calcium carbonate and calcium phosphate that we prepared had

Table 2. Plasma Bilirubin Concentrations During Control, Placebo, and Treatment Periods and After Washout Period

Patient	Control (n = 3)		Placebo (n = 3)		Treatment (n = 3)		Washout (n = 1)		Effect of treatment (%)	P value
	$\mu\text{mol/L}$	mg/dL	$\mu\text{mol/L}$	mg/dL	$\mu\text{mol/L}$	mg/dL	$\mu\text{mol/L}$	mg/dL		
Type I										
6	240 $\pm$ 20	14 $\pm$ 1	240 $\pm$ 40	14 $\pm$ 2	190 $\pm$ 10	11 $\pm$ 0.5	269	15.7	-24	0.01
7	220 $\pm$ 20	13 $\pm$ 1	220 $\pm$ 10	13 $\pm$ 0.5	190 $\pm$ 20	11 $\pm$ 1	253	14.8	-14	0.03
8	210 $\pm$ 20	12 $\pm$ 1	200 $\pm$ 5	12 $\pm$ 0	150 $\pm$ 10	9 $\pm$ 0.5	213	12.5	-26	0.002
9	270 $\pm$ 20	16 $\pm$ 1	240 $\pm$ 10	14 $\pm$ 0.5	210 $\pm$ 10	12 $\pm$ 0.5	243	14.2	-15	0.01
10	300 $\pm$ 30	18 $\pm$ 2	300 $\pm$ 5	18 $\pm$ 0	270 $\pm$ 10	16 $\pm$ 0.5	336	19.6	-11	0.045
Mean $\pm$ SD	250 $\pm$ 30	15 $\pm$ 2	240 $\pm$ 30	14 $\pm$ 2	200 $\pm$ 40	12 $\pm$ 2	260 $\pm$ 40	15 $\pm$ 2	-18 $\pm$ 6	0.03
Type II										
1	195 $\pm$ 2	11.4 $\pm$ 0.1	200 $\pm$ 20	12 $\pm$ 1	180 $\pm$ 20	11 $\pm$ 1	227	13.3	-10	NS
2	197 $\pm$ 3	11.5 $\pm$ 0.1	190 $\pm$ 10	11 $\pm$ 0.5	210 $\pm$ 10	12 $\pm$ 0.5	205	12.0	6	NS
3	350 $\pm$ 20	20 $\pm$ 1	350 $\pm$ 10	20 $\pm$ 0.5	340 $\pm$ 30	20 $\pm$ 2	380	22.2	-5	NS
4	240 $\pm$ 40	14 $\pm$ 2	290 $\pm$ 10	17 $\pm$ 0.5	330 $\pm$ 50	19 $\pm$ 3	238	13.9	12	0.03
5	210 $\pm$ 6	12.3 $\pm$ 0.4	218 $\pm$ 5	12.7 $\pm$ 0.3	200 $\pm$ 20	12 $\pm$ 1	194	11.3	-4	NS
11	300 $\pm$ 20	18 $\pm$ 1	320 $\pm$ 20	19 $\pm$ 1	320 $\pm$ 10	19 $\pm$ 0.5	321	18.8	0	NS
Mean $\pm$ SD	250 $\pm$ 60	15 $\pm$ 4	260 $\pm$ 60	15 $\pm$ 4	260 $\pm$ 70	15 $\pm$ 4	260 $\pm$ 70	15 $\pm$ 4	1 $\pm$ 7	NS

NOTE. Values are expressed as means $\pm$ SD.

not been administered before. Therefore, it was first evaluated in 6 healthy human volunteers. This pilot trial focused on the best choice of preparation (capsules, suspension, tablets, or powdered), side effects, and tolerance. Calcium carbonate is often said to be a constipating agent, and this is potentially disadvantageous for Crigler-Najjar patients because they partly dispose of their UCB directly via the gut. No side effects were detected, and tolerance was good. The most acceptable way to ingest this amount of calcium (13.6 g calcium salts/day in adults) proved to be the powdered form.

A few reports have been published on the interruption of the enterohepatic cycling of bilirubin by agar or cholestyramine^{17,18} for treatment of Crigler-Najjar disease. The effect was found to be at best marginal, and

the significance of the observations was restricted by the fact that only 1 patient was treated in each study. We had the opportunity to perform a trial in 11 patients with Crigler-Najjar disease. The normal therapeutic management (phototherapy or phenobarbital) had to be continued for obvious reasons in this study. The mean bilirubin levels of the patients are relatively low under this regime, which could mask the efficacy of the drug tested in this study. The trial was performed during the same period in spring in almost all patients. During this season, bilirubin levels are substantially lower than in winter. Considering the variation in individual bilirubin levels and the limited number of patients, a placebo-controlled crossover design was selected so that each patient could be used as his or her own control. Because half of the patients received the placebo first and the calcium medication last, and the other half vice versa, we were able to exclude the influence of sunlight as a confounding factor.

Bilirubin levels decreased significantly but modestly in type I group (18% $\pm$ 6%) but not in the type II group. The apparent increase in bilirubin level during treatment in patient 4 probably was caused by deviating (low) control values because these were also significantly lower than values obtained during placebo treatment. In all patients, except patient 4, fecal output of calcium and phosphate was increased markedly during the treatment period. In patient 4, the fecal output of both calcium and phosphate hardly increased, which suggests that this patient was noncompliant.

It is important to note that, during the patients' current therapy, the average serum bilirubin levels in type I and II groups were not significantly different at the outset of the experiment (control period). Hence, under conditions of identical serum bilirubin levels, calcium

Table 3. Dietary Intake of Calcium and Phosphate and Oral Dosage During Treatment Period

Patient	Dietary intake (mmol/day)		Oral dosage during treatment (mmol/day)		Ca ²⁺ intake ratio of treatment period to placebo period
	Ca ²⁺	P	Ca ²⁺	P	
1	25 $\pm$ 4	41 $\pm$ 1	100	50	4.4
2	27 $\pm$ 2	42 $\pm$ 2	55	28	3.3
3	47 $\pm$ 9	61 $\pm$ 7	100	50	2.7
4	20 $\pm$ 8	35 $\pm$ 7	62	31	6.8
5	21 $\pm$ 2	36 $\pm$ 1	100	50	6.2
6	11 $\pm$ 4	22 $\pm$ 7	48	24	6.1
7	14 $\pm$ 5	20 $\pm$ 6	27	14	2.3
8	12 $\pm$ 3	18 $\pm$ 2	52	26	7.5
9	28 $\pm$ 2	43 $\pm$ 3	80	40	4.2
10	12 $\pm$ 7	31 $\pm$ 12	100	50	5.6
11	47 $\pm$ 7	69 $\pm$ 3	100	50	3.8

NOTE. Values are means $\pm$ SD.

ND, not determined.

Table 4. Urinary and Fecal Output of Calcium and Phosphate During Placebo and Treatment Periods

Patient	Placebo						Treatment					
	Calcium			Phosphate			Calcium			Phosphate		
	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
1	2.5	28.6	31.1	24.4	17.4	41.8	2.7	48.3	51.0	25.8	23.6	49.4
2	0.8	29.0	29.8	19.3	16.3	35.6	1.2	87.5	88.7	19.4	42.2	61.6
3	9.0	26.3	35.3	40.9	16.6	57.5	8.8	NC ^a		40.3	NC	
4	1.6	11.1	12.7	15.1	8.2	23.3	0.6	14.8	15.4	14.0	8.9	22.9
5	4.4	18.7	23.1	30.3	11.4	41.7	9.3	78.4	87.7	28.3	41.3	69.6
6	4.9	10.3	15.2	18.5	4.4	22.9	4.7	40.7 ^b	45.4	18.0	14.7	32.7
7	NC	NC		NC	NC		NC	NC		NC	NC	
8	NC	NC		NC	NC		NC	NC		NC	NC	
9	1.6	18.5	20.1	17.5	9.7	27.2	5.1	137.1	142.2	22.5		
10	1.5	NC		16.2	NC		2.5	96.6	99.1	17.4	47.0	64.4
11	6.0	22.4	28.4	26.3	12.3	38.6	5.5	ND		22.2	ND	

NOTE. Values are means $\pm$ SD expressed in mmol/day.

NC, not collected; ND, not determined.

^aMean bowel movement frequency, two per week.

^bOne of four bowel movements missing.

phosphate induces a more efficient pathway of bilirubin disposal in patients with type I disease compared with type II disease. The reason for this is unclear. Patients with type II disease excrete some bilirubin glucuronides in bile, and in all patients but 1 in this subgroup, this pathway was stimulated by phenobarbital treatment. Conjugated bilirubin is not reabsorbed in the gut.^{10,11} On the other hand, all patients but 1 (patient 10) with type I disease were treated with phototherapy, which induces disposal of a substantial amount of bilirubin by excretion of bilirubin photoproducts in the bile. Three different photochemical reactions of bilirubin have been shown to occur in vivo during phototherapy: configurational isomerization, photocyclization (lumirubin formation), and photo-oxidation.¹⁹ Only the configurational isomers can rearrange back to bilirubin IX α , which can diffuse back to the blood again. The latter step may be

prevented when bilirubin or its isomers are bound to calcium phosphate in the gut. Therefore, the differential effect of calcium phosphate treatment in patients with type I and type II disease may be caused by the fact that type I patients undergo extensive phototherapy and thus excrete UCB (in the form of isomers), whereas type II patients excrete conjugated bilirubin. If this hypothesis is true, the effect of calcium phosphate will be related to the intensity and duration of phototherapy, and calcium phosphate could be used only as an adjuvant to phototherapy. Three patients in type II group also received phototherapy; however, therapy was much less extensive than in type I group (Table 1). Two of these patients (patients 1 and 3) had a small but insignificant decrease in serum bilirubin level; the third (patient 4) was probably noncompliant as discussed above.

If our hypothesis is correct, a substantial amount of bilirubin should be excreted into bile in the form of configurational isomers during phototherapy. It has been postulated that formation and excretion of lumirubin (photocyclization) is the most important route for bilirubin elimination during phototherapy.^{20,21} This product is unstable but cannot revert to bilirubin IX α . Because of its exposed negative charges, this product is probably not reabsorbed by the gut, which means that oral calcium phosphate will not have any additional effect in the excretion of this compound. The relative contribution of the two photoisomerization pathways cannot be studied in patients because that would require bile sampling during phototherapy. However, it is known that the serum level of configurational isomers reaches a constant, high level during phototherapy, which may reflect the equilibrium

Table 5. Total Recovery of Calcium and Phosphate During Placebo and Treatment Periods

Patient	Placebo		Treatment	
	Ca ²⁺ (%)	P (%)	Ca ²⁺ (%)	P (%)
1	110	100	41	55
2	117	83	105	89
3	69	88		
4	106	82	19	34
5	117	116	71	82
6	160	133	79	77 ^a
9	77	60	131	101
10			90	79
11	70	56		

^aOne of four bowel movements missing.

between formation, on one hand, and reversion and enterohepatic cycling, on the other. Our data imply that formation of configurational isomers and excretion into bile could contribute to bilirubin disposal during phototherapy. Apparently, this pathway is inefficient and can be enhanced significantly by bilirubin trapping in the gut. In line with our data is the observation that treatment of jaundiced neonates with agar reduces the period of phototherapy required for normalization of the serum bilirubin level.^{22,23} Although there is no supporting evidence in our study for the assumption that the observed decline in plasma bilirubin level was caused by enhanced fecal excretion of the pigment as a bound complex with solid calcium phosphate, we have proven previously the correctness of this mechanism in the Gunn rat.¹⁵ Of course, we cannot exclude the possibility that at higher doses of calcium phosphate, there would also be an effect in patients with type II disease due to intestinal trapping of UCB, arising from hydrolyzation of conjugated bilirubin (by bacterial glucuronidases) or UCB that diffused from the blood compartment to the intestinal lumen.

Oral calcium phosphate was very effective in Gunn rats, although they were not treated with phototherapy.¹⁵ This implies that the pathway of increased bilirubin disposal in humans on combined calcium phosphate and phototherapy differs from that in calcium phosphate-treated rats. The diffusion of UCB directly across the intestinal wall may be quantitatively more relevant because these animals have a relatively much larger surface of intestinal mucosa than humans, because their intestinal tracts are longer relative to body size. Another reason might be the fact that Gunn rat bile contains more UCB than bile from patients with Crigler-Najjar type I disease.^{24,25} The relative contribution of photoisomer vs. diffusional excretion could be studied in Gunn rats undergoing phototherapy with or without oral calcium phosphate treatment.

The question arises, which dose of calcium phosphate and calcium carbonate can be administered without the development of adverse effects. Oral calcium has been administered to human volunteers in connection with the potentially beneficial effect of calcium phosphate in the prevention of colon carcinoma. Calcium was administered as calcium carbonate because, in humans, in contrast to the rat, sufficient phosphate is present in the intestine to precipitate calcium. Doses of 50–100 mmol calcium (2–4 g)/day were used in adults without any side effects.^{16,26} Ca^{2+} salts also have been used for many years as a dietary supplement in the treatment of deficiency states when intake is inadequate and as antacids.²⁷

Phosphate is absorbed from, and to a limited extent secreted into, the gastrointestinal tract. The presence of

large quantities of Ca^{2+} or Al^{3+} may cause formation of large amounts of insoluble phosphate that may diminish the net absorption of phosphate.²⁷ Obviously, the purpose in our trial was the formation of insoluble calcium phosphate in the gut, and therefore resorption of high amounts of calcium or phosphate would be undesirable. Urinary output of calcium and phosphate were similar during the placebo period and the treatment period, which is important because it reflects the lack of resorption of both compounds from the gut. It seems likely that it is possible to increase the dose without the development of adverse effects. It may be useful to change the ratio of carbonate to phosphate when higher amounts of calcium are administered because the daily intake of phosphate with the diet will be constant.

In conclusion, oral calcium phosphate can be a useful adjuvant for phototherapy in patients with Crigler-Najjar type I disease.

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