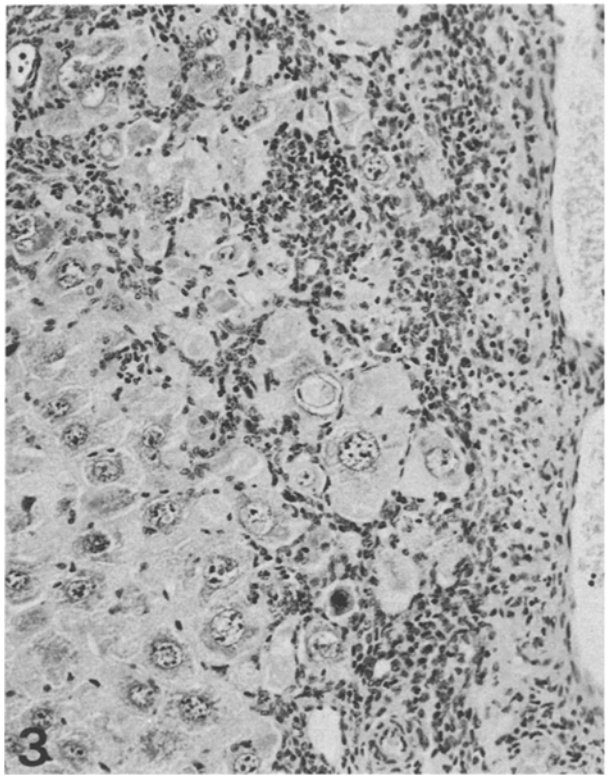

Figure 3. Higher power view showing extensive periportal piecemeal necrosis with trapping of surviving liver cells within the dense mononuclear infiltrate. Several liver cells show cellular and nuclear enlargement. H&E, ×156.

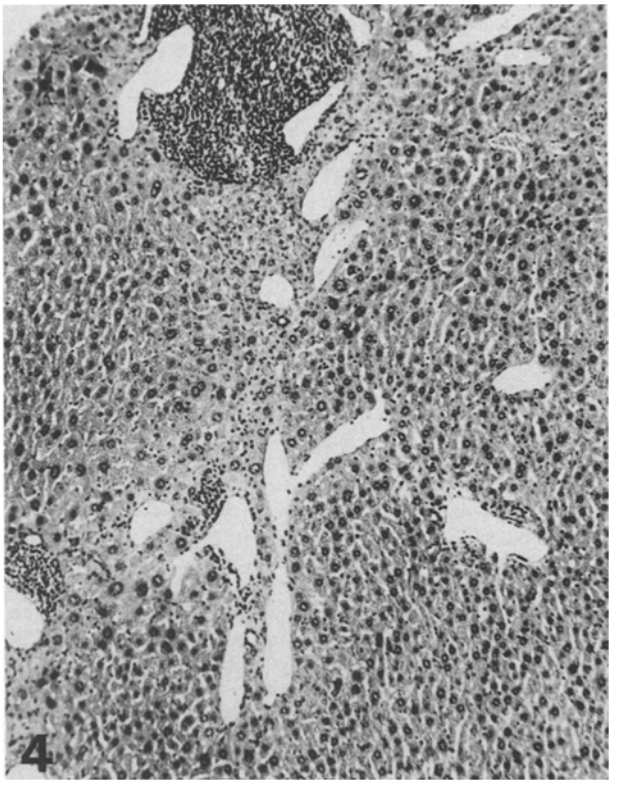

Figure 4. Liver of mouse fed 3-hydroxy-4-pyrone for 21 weeks followed by normal mouse pellets for the next 20 weeks shows regression of CAH with residual scarring, crowding of vascular structures and a persistent lymphoid infiltrate. Compare with figure 2. H&E, ×75.

immune system has been characterized in detail and is also the species in which several inbred, recombinant and congenic strains are available. Therefore the mouse model described here should provide a valuable tool for defining any immunological and/or genetic determinants of CAH.

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Metabolic implications in the elevation of serum activity of intestinal alkaline phosphatase in chronic renal failure

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Summary. The activity of intestinal isoenzyme of serum alkaline phosphatase was evaluated in 21 non-dialyzed patients with advanced renal failure and in 52 patients on regular hemodialysis. In patients without hepatopathy, a significant inverse correlation was found between the enzyme activity and serum calcium levels. Hepatopathy was the most significant variable influencing the enzyme activity in patients on dialysis. Secondary hyperparathyroidism and a decreased rate in enzyme elimination should be assessed for the above-normal activities of intestinal ALP in serum in chronic renal failure.

Elevation in the serum activity of intestinal isoenzyme of alkaline phosphatase (EC 3.1.3.1) has been described in patients on regular hemodialysis[1-3]. The present investigations were designed to determine the factors which influence the changes in the enzyme activity in chronic renal failure.

Patients and methods. Measurements were made in 21 non-dia-

Biochemical values in patients and in healthy adults (mean ± 2 SD range)

Variable		Chronic renal failure With hepatopathy		Without hepatopathy		Control group	
n (male/female)		37	(19/18)	36	(17/19)	40	(20/20)
ALP intestinal	U/l						
Blood group	O,B	16.9*	(2.4–50.0)	3.3	(0.7–15.5)	2.8	(1.1–7.2)
Blood group	A, AB	5.2*	(1.5–17.5)	2.1	(0.4–10.5)	1.2	(0.3–4.6)
ALP liver	U/l	27.0*	(7.1–103.4)	12.5	(6.9–22.7)	14.0	(9.3–20.8)
GMT male	U/l	92*	(13–658)	29	(6–149)	32	(10–99)
female	U/l	70*	(10–513)	21	(7–62)	28	(11–71)
Cholinesterase	U/l	3544*	(1834–6847)	4404	(2730–7675)	6067	(3600–10210)
Calcium	mmol/l	2.27*	(2.00–2.54)	2.20*	(1.91–2.49)	2.48	(2.25-2.72)
Phosphate	mmol/l	2.04*	(1.41–2.67)	1.77*	(0.85–2.69)	1.14	(0.65–1.62)
iPTH II	ng/ml	0.43*		2.06*	(0.53–8.03)	0.4	(0.1–1.0)
iPTH N-terminal	ng/ml				(0.17–2.94)	0.1	(0.0–0.3)
Creatinine	µmol/l	861*	(592–1252)	668*	(280–1592)	90	(67–120)

* $p < 0.005$, as compared with the control group. With exception of calcium and phosphate, the statistical analysis was applied to logarithmically transformed data.

lyzed patients with advanced renal failure (creatinine clearance mean ± 2 SD range, 35 ml/min, and 13–85 ml/min, respectively) and in 52 patients on regular hemodialysis. No patients received phosphate binders, vitamin D or its derivatives, anticonvulsants or corticosteroids (except for the transplant patients and patients with an active hepatitis). In 37 patients (35 on dialysis), a chronic hepatopathy was documented by a previous history of acute hepatitis and serial evaluation of liver tests. The dialysis calcium concentration was 2.3 mmol/l. Nine patients had a previous history of a non-successful renal transplantation. The control group consisted of 40 adults without evidence of renal, hepatobiliary, or bone disease. Blood samples were drawn in the morning after a fasting period of approximately 8 h, in patients on dialysis before the dialysis. Informed consent was obtained from all the patients.

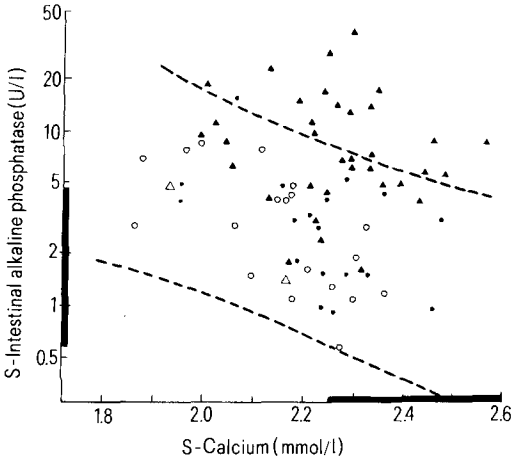
The activity of the intestinal, liver and bone isoenzyme of serum alkaline phosphatase (ALP) was determined with 4-nitrophenyl phosphate as substrate[4]. The total concentration of calcium in the serum was determined with methylthymol blue and corrected for individual variations in serum albumin concentration[5], inorganic phosphate in the serum was determined photometrically[6]. In all patients, determinations of the serum activity of alanine aminotransferase (EC 2.6.1.2), gamma-glutamyl transferase (EC 2.3.2.1, GMT) cholinesterase with butyryl thiocholine as substrate (EC 3.1.1.8), albumin, creatinine, and clearance of endogeneous creatinine, were performed. The serum immunoreactive parathyroid hormone (iPTH) was determined by radioimmunoassay, using Human Parathyroid Hormone PTH II and Human N-Terminal Parathyroid Hormone Kits (Immuno Nuclear Corp., MN, USA). The kits were kindly provided by DRG International, NJ, USA. Statistical analysis of the results was performed by commonly accepted methods using BMDP 1V, 1R, 6R, 9R, 6M and 7M programs of the Health Science Computing Facility, University of California, Los Angeles, USA[7].

Results. Mean values for the variables are shown in the table. The intestinal isoenzyme activity in patients without biochemical and clinical evidence of hepatopathy was most significantly influenced by serum calcium level (fig.). Accordingly, in these patients a significant positive correlation was found between serum iPTH and intestinal ALP activity (log intestinal ALP activity (log intestinal ALP = 0.21+0.72 log iPTH, $r = 0.61$, $n = 19$, $p < 0.01$).

The figure shows that the activity of the intestinal isoenzyme of serum ALP in 44% of the patients with hepatopathy fell out of the 95% confidence limits of the regression observed in patients without hepatopathy. No significant correlation was found between iPTH values and intestinal ALP activity in patients with hepatopathy. The multiple regression analysis showed that the activity of the intestinal ALP in serum of patients with hepatopathy was significantly influenced by blood group, liver ALP activity in serum and serum GMT activity.

Discussion. The results of the present study indicate that the liver functional state and the role of serum calcium should be considered as being involved not only in an increased entrance rate of the enzyme into the blood, but also in the removal of intestinal ALP from the circulation. In our patients who had normal liver function tests and normal serum calcium level, a normal activity of intestinal ALP was found.

In contrast to the other organ-specific forms of ALP, the adult intestinal ALP is an asialoglycoprotein[8]. A damage to the hepatocyte plasma membrane is known to interfere with the hepatic uptake of circulating desialylated glycoproteins and lead to their accumulation in serum[9], especially if the patients blood group is type O[10]. The binding activity of the asialoglycoprotein receptor of hepatocytes has been shown to depend on calcium ions[11]. However, hypocalcemia and a consequent secondary hyperparathyroidism might lead to an increased rate of entrance of the enzyme into serum as well. Birge and Gilbert[12] identified an ALP in rat intestine that was stimulated by parathyroid hormone. An augmented catalytic efficiency of the intestinal ALP could also account for its increased activity[13].



Relationship between serum calcium and intestinal isoenzyme of alkaline phosphatase in patients with chronic renal failure. ○, patients without hepatopathy; △, patients with evidence of hepatopathy. Open symbols, non-dialysed patients; black symbols, patients on dialysis. The solid bars indicate the normal ranges for our laboratory ± 2 SD. The dotted lines indicate the 95% confidence limits of the regression line in patients without hepatopathy (log intestinal ALP = 2.92–1.14 calcium, $r = -0.47$, $p < 0.003$).

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Enzymatic profile of *Kluyvera* species

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Summary. *Kluyvera*, a proposed genus formerly known as Enteric group 8, was found to have similar enzyme profiles among the present 3 groups. *Kluyvera* species group 3 showed the most heterogeneous enzyme reactions.

Kluyvera is a proposed genus of a group of organisms formerly known as Enteric group 8[2]. The organisms are gram-negative, oxidase-negative fermentative bacteria which were previously separated into 2 species[3] but have recently been divided into 3 groups. *K. ascorbata* is the first species and may be isolated from clinical specimens such as sputum. *K. cryocrescens* is the second species which also has been isolated from clinical specimens but may be commonly isolated from the environment. The third group, referred to as *Kluyvera* species group 3, is a heterogeneous group that is rarely isolated[2]. In an attempt to increase the available knowledge about these organisms, the present investigation determined their enzyme profiles to characterize their potential invasiveness and to determine whether the species could be further differentiated biochemically.

Methods. Because limited clinical isolates are available, only 20 strains of *Kluyvera* kindly provided by Geoge Morris, Centers for Disease Control, Atlanta, GA. were used in this study, including 9 strains of *K. ascorbata*, 6 strains of *K. cryocrescens* and 5 strains of *Kluyvera* species group 3. The strains were maintained on MacConkey Agar (BBL Microbiology Systems, Cockeyville, MD.) with incubation at 37 °C for 24 h before testing. After the addition of 3 ml of sterile saline to the cultures, heavy cell suspensions were removed and adjusted to a turbidity approximately equal to that of a McFarland No. 5 standard.

Enzyme assays were performed on each strain by using API ZYM System (Analytab Products, Plainview, NY.). Enzyme strips were used for the enzyme assays as previously described for other organisms[4].

Results and discussion. It was observed that enzyme reactions occurred uniformly in all strains for alkaline phosphatase, esterase lipase (C8), leucine aminopeptidase, valine aminopeptidase, acid phosphatase, phosphoamidase, β-galactosidase, α-glucosidase and β-glucosidase. Two variations were noted in group 3 for esterase (C4) and α-galactosidase.

All strains showed activity for an esterase lipase (C8) and phosphatases which may be involved in the virulence of *Kluyvera*. However, Farmer et al.[2] reported no activity for gelatinase and stated that these organisms are probably infrequent opportunistic pathogens and most common isolates from sputum are probably not clinically significant.

Another aspect shown in this study is the inability to differentiate the 3 *Kluyvera* groups by these 19 enzyme assays. A slight variation was found in group 3, further pointing out the heterogeneous reactions in this group. In addition, the colony growth characteristics of the members of the genus *Kluyvera* resemble that of *Escherichia coli*. The absence of β-glucuronidase activity among the *Kluyvera* strains could be used to differentiate them from *E. coli*. *E. coli* usually demonstrates β-glucuronidase activity, although several other biochemical assays can also be used to separate these genera including malonate utilization and growth in KCN[2].

Enzymatic reactions of 20 strains of *Kluyvera*

Enzyme[a]	*K. arcorbata*	*K. cryocrescens*	*K.* Group 3
Alkaline phosphatase	+	+	+
Esterase lipase (C8)	+	+	+
Leucine AP	+	+	+
Valine AP	+	+	+
Acid phosphatase	+	+	+
Phosphoamidase	+	+	+
β-Galactosidase	+	+	+
α-Glucosidase	+	+	+
β-Glucosidase	+	+	+
Lipase (C14)	−	−	−
Cystine AP	−	−	−
Chymotrypsin	−	−	−
Trypsin	−	−	−
N-Acetyl-β-glucosaminidase	−	−	−
α-Mannosidase	−	−	−
α-Fucosidase	−	−	−
β-Glucuronidase	−	−	−
Esterase (C4)	−	−	−[b]
α-Galactosidase	−	−	−[c]

[a] AP, Aminopeptidase; [b] 1 strain of Group 3 showed positive results for Esterase (C4); [c] 2 strains of Group 3 showed positive results for α-Galactosidase.

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