Electrophoretic separation of the different isoenzymes of alkaline phosphatase was done, using polyacrylamide gel electrophoresis. In some patients with liver disease, two isoenzymes of alkaline phosphatase were detected—one corresponding to liver and the other, to intestinal fraction. In 17 of 51 patients with nutritional cirrhosis and in 6 of 14 with chronic active hepatitis, the intestinal band was observed in their fasting sera. Intestinal isoenzyme was not found, however, in the fasting sera of 23 patients with viral hepatitis, 16 with metastatic disease to the liver, 11 with obstructive jaundice or 33 healthy volunteers. The presence of intestinal isoenzyme was not dependent on the value of total serum phosphatase activity.

Alkaline phosphatase is present in low concentrations in normal human adult serum and is derived from the liver. In children and adolescents, some circulating isoenzyme is also derived from bone (1, 2). In a variety of diseases of the liver and biliary tract, total serum alkaline phosphatase activity is elevated, which is attributed to increased amounts of circulating isoenzyme in the liver. Intestinal alkaline phosphatase has also been detected by electrophoretic technics in patients with liver disease as well as in some normal subjects (2–4). The presence of the intestinal fraction may be related to the ABO blood groups and to the ABH secretor status (4, 5). The purpose of this study was to investigate the presence and contribution of the intestinal alkaline phosphatase isoenzyme in patients with various diseases of the liver and biliary tract.
because intestinal isoenzyme has been reported in diabetics, and some of our first patients with elevated alkaline phosphatases had only diabetes mellitus.

Total serum alkaline phosphatase activity was determined according to the method of Bessey-Lowery (normal, < 2.3 units). Isoenzymes were separated electrophoretically on polyacrylamide gel slabs (3). Electrophoresis was run on 3-mm thick, 7 1/2% polyacrylamide gel slabs in an ice water-cooled vertical gel electrophoresis cell in a continuous buffer system of 0.2 M trisborate, pH 9.5, and magnesium chloride 2 mM. Ten-microliter samples of the test sera containing 0.5% bromphenol blue solution were applied to the gel, and electrophoresis was subsequently run for 3 1/2 hours. The gel was then incubated in a trismaleate solution of pH 9.8 containing 3 mM magnesium sulfate and 0.8 mM \( \alpha \)-naphthyl phosphate. Bands of alkaline phosphatase activity were stained by incubating them overnight with a solution of trismaleate containing 0.5% 4-amino-diphenylamine diazonium dye and then clearing with 5% aqueous glycerine. The contribution of the different isoenzymes was calculated as a percentage of the total serum alkaline phosphatase by densitometry of the gels labs, with the use of a Densicord apparatus.*

RESULTS

The isoenzymes of alkaline phosphatase were characterized by their different rates of migration. The band with the fastest mobility from the point of origin corresponds to the liver isoenzyme. A second, more diffuse band with a slightly slower mobility represents the bone isoenzyme. The slowest migrating band corresponds to the intestinal alkaline phosphatase isoenzyme (Fig 1) (3).

Table 1 shows the distribution of the subjects tested according to their clinical diagnosis. The intestinal isoenzyme was present in 17 of 51 patients with alcoholic cirrhosis, and in 6 of 14 patients with chronic active hepatitis. In contrast, the intestinal fraction was not detected in the sera of 23 patients with uncomplicated acute viral hepatitis, 10 patients with hepatic cell carcinoma without cirrhosis, 16 patients with metastatic tumors to the liver or 11 patients with obstructive jaundice. This isoenzyme was not found in the sera of the 33 healthy controls tested, but was present in 15 of 37 patients with diabetes mellitus. The intestinal isoenzyme was detected in the 3 patients who had both nutritional cirrhosis and diabetes.

Table 2 shows the presence of the intestinal isoenzyme in relation to total serum alkaline phosphatase activity. It was as frequently detected in those with elevated serum values as in those with normal total serum alkaline phosphatase activity. In both cases, the intestinal fraction con-

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* Densicord Recording Electrophoresis Densitometer, Photovolt Corp, New York City, NY.