Quantification of High-Density-Lipoprotein Cholesterol in Plasma from Hamsters by Differential Precipitation, Kurt W. Weingand1 and Bruce P. Daggy2 (1 Human & Environmental Safety Division and 2 Health & Personal Care Technology Division, The Proctor & Gamble Co., P.O. Box 398707, Cincinnati, OH 45239)

Hamsters are increasingly used for comparative study of cholesterol metabolism (1). To assess the validity of differential precipitation with Mg2+-phosphotungstate for quantification of plasma high-density-lipoprotein cholesterol (HDLC), we did a method-comparison with ultracentrifugal flotation. The precipitating reagent, 0.55 mmol/L phosphotungstic acid and 25 mmol/L magnesium chloride, was used as recommended by the manufacturer (Boehringer Mannheim Diagnostics, Indianapolis, IN). High-density-lipoprotein fractions in plasma were isolated in a potassium bromide/sodium chloride adjusted density range of 1.063–1.210 kg/L by analytical ultracentrifugation. Cholesterol concentrations were determined spectrophotometrically with an enzymatic cholesterol reagent containing microbial cholesterol esterase, in an Hitachi 705 clinical chemistry analyzer (Boehringer Mannheim Diagnostics).

Adult male Golden Syrian hamsters (n = 32) were fed a pelleted-grain-based diet (Purina Rodent Laboratory Chow, St. Louis, MO), either with or without 2 g of cholesterol added per kilogram. Half of the animals consuming each diet were fasted for 18 h before blood sampling.

Differential precipitation was highly associated (y = 1.02x + 0.02; r = 0.99) with ultracentrifugal flotation for isolation and quantification of HDLC (range 310–950 mg/L) regardless of feeding status or diet. The mean was 650 (SE 40) mg/L for differential precipitation and 640 (SE 30) mg/L for ultracentrifugation. The relative analytical error for the differential-precipitation method was 1.6%.

Differential precipitation with Mg2+-phosphotungstate evidently is a valid alternative to ultracentrifugation for measuring HDLC in plasma from hamsters.

We acknowledge the superb technical assistance provided by Carol L. Hudson and M. Karen Eichhold.

References

Identification and Treatment of Hypomagnesemia and Hypokalemia in Patients Receiving Digoxin, Charles C. G. Njinimbam,1 Kenneth W. Ryder,2 Melvin R. Glick,3 Starla J. Glick,3 and Robert Whang4 (1 Department of Pathology, Wishard Memorial Hospital, Indiana University Medical Center, Indianapolis, IN; and 2 Department of Medicine, Veterans Administration Medical Center, University of Oklahoma School of Medicine Oklahoma City, OK. Correspondence: Dr. Ryder, Dept. of Pathol., Wishard Memorial Hospital, 1001 West 10th St., Indianapolis, IN 46202)

Hypomagnesemia and hypokalemia affect the action of digoxin and should be corrected in patients receiving this medication (1). We assessed both the frequency of hypomagnesemia and hypokalemia in patients receiving digoxin and the frequency with which these electrolyte abnormalities were identified and appropriately treated. All serum specimens received in our hospital laboratory for digoxin analysis during two months were included in this study. Potassium and magnesium were assayed in each of these specimens, but the results were reported to the requesting physician only if potassium, magnesium, or both had also been ordered. During this interval, 90 requests for digoxin measurements were received for 58 different patients. Hypokalemia (potassium <3.6 mmol/L (2)) was seen in five samples from four of 58 patients. For all these five specimens a request for potassium measurement was received on the same day as the request for digoxin analysis and an appropriate follow-up (potassium supplementation or a repeat potassium analysis showing normal concentration) occurred in all cases.

By contrast, we identified hypomagnesemia (magnesium <0.75 mmol/L (3)) in 21 specimens from 13 patients. For only five of these 13 patients (38%) had a magnesium measurement been requested at any time during their hospitalization. When the decreased magnesium was identified, however, it was properly treated in most cases. Of the five patients known by their physicians to be hypomagnesemic, three received magnesium supplements, one patient’s magnesium measurement was repeated and found to be within the reference interval, and one test was repeated and confirmed to be <0.75 mmol/L, but no further action was taken.

Although hypomagnesemia has been shown to occur frequently in patients receiving digoxin (4), its assay was ordered by physicians much less frequently than was that for potassium. In this study, eight of 13 hypomagnesemic patients were not identified or treated. We think that routinely including magnesium with other “electrolyte” measurements (5, 6) or automatically including magnesium results with digoxin reports will assist in identifying additional patients at risk for the toxic effects of digoxin.

References
Fecal $\alpha_1$-Antichymotrypsin in Inflammatory Bowel Diseases, G. Huët, M. Baldyuck, C. Mizon, J. F. Colombel, and J. Mizon (1) Unité INSERM no. 16, Lille; 2 Lab. Biochim., Fac. de Pharmacie, Lille; 3 Clin. des Mal. de l'appareil digestif, Hôpital Cl. Huriez, CHR, Lille, France)

Fecal $\alpha_1$-proteinase inhibitor ($\alpha_1$PI) may be an endogenous marker for protein-losing enteropathies. We recently reported (1) that three different molecular forms of $\alpha_1$PI with apparent molecular masses of 38, 45, and 51 kDa can be characterized in feces. The fecal $\alpha_1$PI (38 kDa) was recovered in fecal extracts from healthy subjects and from some patients with inflammatory bowel disease (IBD). In contrast, the 45- and 51-kDa components were found only in patients with active IBD. We hypothesized that the 51-kDa $\alpha_1$PI resulted from the cleavage of native $\alpha_1$PI, at or near its reactive site, by proteinases involved in the inflammation of mucosa. This cleavage presumably would accompany a structural rearrangement that would reinforce the stability of the inhibitor, which would then become resistant to further pancreatic and bacterial digestion. Thus the fecal concentration of the 51-kDa $\alpha_1$PI should be a specific marker of intestinal inflammation.

We reasoned that $\alpha_1$-antichymotrypsin ($\alpha_1$CT) also involved in the inflammation process and present in stools of patients with IBD (2), could be similarly excreted in the feces in various different forms. We thus analyzed for $\alpha_1$CT in fecal extracts, using 10% SDS-PAGE as previously described (1). After transfer of the resulting pattern onto nitrocellulose, we performed immunodetection, using antibodies to either $\alpha_1$CT or $\alpha_1$PI. Figure 1 shows some patterns obtained.

As was true for $\alpha_1$PI, $\alpha_1$CT could be characterized in all samples and exhibited several mobilities in SDS-PAGE. Indeed, we noted correspondence between the simultaneous molecular forms of $\alpha_1$PI and $\alpha_1$CT. Accordingly, 58-kDa $\alpha_1$CT was detected in fecal samples that contained 51-kDa $\alpha_1$PI (Figure 1, lanes 1 and 3). Similarly, 55- and 35-kDa $\alpha_1$CT were present with 45- and 35-kDa $\alpha_1$PI (lane 2), and 36-kDa $\alpha_1$AC and 38-kDa $\alpha_1$PI were again simultaneously characterized in another sample (lane 4).

Because $\alpha_1$PI and $\alpha_1$CT belong to the general group of proteinase inhibitors referred to as "serpins" and show a reinforced conformational stability upon cleavage at their reactive sites (3), we suggest that a similar process might produce identical structural modifications of both inhibitors in the intestinal lumen. Indeed, both proteinase inhibitors have been detected immunohistochemically in epithelial cells of the small intestine (4). Recently, Perlmutter et al. (5) demonstrated the synthesis and secretion of functionally active $\alpha_1$PI by enterocytes. The antiproteinase activity of these inhibitors may represent a self-protection mechanism.