determination of salicylate in plasma. However, for economy many clinical laboratories still measure salicylate in plasma by Trinder's colorimetric procedure (*4*), even though Trinder's reagent contains mercuric salt, which is hazardous for the environment. Here we report an alternative for Trinder's reagent for rapid and economical determination of salicylate in plasma.

We mix by hand inversion 10 μL of sample (plasma or aqueous standard) with 10 mL of 0.1 mol/L ammonium hydroxide in glass-distilled water in a 16 × 100 mm glass tube. We then measure the fluorescence of the diluted sample at λex = 306 nm and λem = 400 nm, using a Model 650-15 fluorospectrophotometer (Perkin-Elmer, Norwalk, CT 06859-0012).

The observed fluorescence of aqueous and plasma salicylate standards is similar and is linearly related to salicylate concentrations from 0.1 to 8.0 mmol/L. The presence of increased concentrations of protein (12 g/L), bilirubin (35 μmol/L), hemoglobin (0.5 g/L), and triglycerides (5 mmol/L) did not lead to any decrease in the observed fluorescence in various plasma samples supplemented with 2 mmol of salicylate per liter. Analysis of 25 plasma samples known to be salicylate-free gave fluorescence values corresponding to salicylate concentrations of 0.05 to 0.2 mmol/L. Acidic drugs that, like salicylate, have relatively high therapeutic concentrations (e.g., acetaminophen, ibuprofen, tolmetin, sulindac, and diclofenac) gave insignificant values for salicylate when plasma supplemented with these drugs at a concentration of 1 g/L was analyzed by the described procedure in separate experiments. However, diflunisal, an analog of salicylate, gave a fluorescence response similar to that of salicylate. Chlorpromazine, one of the commonly prescribed phenothiazines, does not show any fluorescence response at a concentration of 1 g/L by this procedure. Acetylsalicylate in plasma is, for all practical purposes, present as salicylate and is measured as such.

To check reproducibility, we analyzed Therachem® low and high therapeutic drug controls (Fisher Scientific, Orangeburg, NY 10962). The results show acceptable precision and accuracy, comparing well with the values obtained by the Abbott TDx procedure:

	Therachem, low		Therachem, high	
	Mean, mmol/L 0.45 (0.61–0.29)[a]	CV, %	Mean, mmol/L 2.83 (3.29–2.37)	CV, %
Within batch	0.51	0.80	2.88	1.17
Between batch	0.49	6.48	2.83	5.9

n = 10 each.

[a] Mean values (and range) obtained by Abbott TDx as reported in Fisher's product insert.

Several fluorometric procedures for the determination of plasma salicylate have been described and applied for pharmacokinetic studies of salicylate because of the high sensitivity and specificity provided by fluorescence procedures. In most cases, samples have been prepared for fluorescence measurement by extraction with organic solvents and back-extraction into the aqueous phase (*5*); in some cases, proteins have been removed by precipitation with tungstic–sulfuric acid reagent (*6*). Recently, a direct fluorometric procedure in the presence of EDTA and terbium salts and without the removal of proteins has been described (*7*). The procedure we describe is economical and compatible with the environment.

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Screening for Paucialbuminuria with Frozen Urine Samples, *Bernhard K. Krämer, Christiane M. Erley, Inge Lindena, and Teut Risler* (Section of Nephrol. and Hypertension, III. Dept. of Internal Med., Univ. of Tübingen, Otfried-Müller-Str. 10, D-7400 Tübingen, F.R.G.)

Paucialbuminuria ("microalbuminuria"), a strong predictor of future overt nephropathy in type I diabetic patients, can be stopped or even reversed by control of blood pressure and (or) blood glucose, thus probably slowing or preventing development of overt diabetic nephropathy (*1*). A decrease in albumin concentration in samples stored at −20 °C (*2, 3*) and uncertain effects of centrifugation before assay (*4, 5*) have recently been reported. Therefore our aim in this study was to examine what effects storage at −20 °C, centrifugation before assay, or urinary pH might have on urinary albumin concentration.

We studied 43 urine specimens after storage at −20 °C for two weeks and three months. Albumin concentration was measured by enzyme-linked immunosorbent assay as described previously (*6*). Albumin concentrations decreased significantly (statistical evaluation by analysis of variance, Student–Newman–Keuls test) after three months of storage but not after two weeks (Table 1). Coating the storage tubes with casein to prevent adsorption of albumin to the tube as well as centrifugation before assaying the albumin concentration had no consistent effect on the decrease of albumin concentrations (Table 1). The pH was significantly lower ($P < 0.02$) in samples in which the albumin concentration decreased compared with that in samples with no decrease (pH 5.75 ± 0.23 vs 6.46 ± 0.13, respectively).

The number of urine samples with visible precipitation increased during storage (44% after three months); those samples also tended to have lower pH values ($P > 0.05$), but the presence of precipitates was not clearly correlated with the decrease of albumin content during prolonged storage.

The possibility that storage conditions of urine samples may affect albumin measurement must be taken into account when monitoring or detecting paucialbuminuria. Storing urine samples before determination of albumin concentration is not recommended for periods markedly exceeding two weeks.

Table 1. Urinary Albumin Excretion Determined from Samples Stored at −20 °C for Two Weeks and for Three Months

Treatment[a]	Albumin excretion, mean ± SEM, mg/24 h		
	Day 1	Week 2	Month 3
Uncoated + uncentrifuged	39.69 ± 5.32	40.05 ± 5.63	37.89 ± 5.67
Uncoated + centrifuged	38.62 ± 4.99	44.73 ± 6.87	35.25 ± 5.26
Coated + uncentrifuged	41.37 ± 5.20	40.40 ± 5.32	31.88 ± 4.38
Coated + centrifuged	44.95 ± 5.84	45.42 ± 5.84	36.96 ± 5.27

[a]Samples stored in either coated or uncoated tubes with or without centrifugation before assay.

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Effects of Four Days of Regular, Moderate Consumption of Ethanol on High-Density Lipoprotein Cholesterol Concentrations in Plasma, *L. G. Howes, H. Krum, and P. A. Phillips* (Clin. Pharmacol. and Therapeutics, Austin Hospital, Heidelberg, Victoria, 3084, Australia)

Increases in concentrations of triglycerides and urate in plasma are generally perceived as early biochemical markers of substantial increases in alcohol (ethanol) consumption. In recent years it has become apparent that alcohol consumption is also associated with an increase in high-density lipoprotein (HDL) cholesterol in plasma. However, the time course of the changes in concentrations of HDL cholesterol in plasma in relation to changes in alcohol consumption has not been adequately determined. In a previous study comparing the effects of six weeks of consumption of low-alcohol beer with that of ordinary beer, Masarei et al. (*1*) found that changes in the apoprotein A content of HDL was a more sensitive indicator of change in alcohol consumption than were either triglyceride, uric acid, or gamma-glutamyltransferase (gamma-GT; EC 2.3.2.2) activity. In the present study we have compared the effects of regular alcohol consumption over a four-day period with a similar period of abstaining from alcohol on the concentrations in plasma of lipoproteins, uric acid, and gamma-GT.

Twelve healthy male volunteers, ages 18–65, either consumed alcohol, 1 g/kg of body weight per night, for four nights or abstained from alcohol and consumed an isocaloric glucose substitute for four nights in a randomized cross-over study. At least four days separated each study phase. On the final morning of each study phase, venous blood was collected with an indwelling cannula from the subjects after 15 min of supine rest. There were no significant changes in calorie intake or body weight between the two study phases. The HDL cholesterol increased significantly after this relatively brief period of regular alcohol consumption and accounted for an increase in total cholesterol that almost achieved statistical significance (P = 0.066). In contrast, concentrations of low-density lipoprotein (LDL) cholesterol, triglyceride, uric acid, and gamma-GT remained unaltered. The plasma hematocrit also remained unaltered.

	Mean (SD)	
	Alcohol consumption	Controls
Cholesterol, mmol/L		
Total	5.47 (0.91)	5.23 (0.86)
HDL	1.35 (0.20)[a]	1.23 (0.19)
LDL	3.32 (0.76)	3.26 (0.72)
Triglyceride, mmol/L	1.79 (0.79)	1.66 (0.75)
Uric acid, mol/L	0.36 (0.07)	0.36 (0.05)
Gamma-GT, U/L	20.8 (11.1)	20.2 (10.7)

[a] Significantly different from control values: P = 0.001 (Student's paired t-test).

These data demonstrate that (*a*) the changes in plasma HDL cholesterol that are associated with increased alcohol consumption occur rapidly and (*b*) changes in HDL cholesterol in plasma are a more sensitive marker of short-term alterations in alcohol consumption than are triglyceride, uric acid, or gamma-GT.

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Interpretation of a 2-h Change in the Serum Concentration of Creatine Kinase 2, *J. Valero-Politi, A. Rivera-Coll, and X. Fuentes-Arderiu* (Servei de Bioquím. Clín., Hosp. Prínceps d'Espanya, 08907 L'Hospitalet de Llobregat, Barcelona, Spain)

Measurement of the catalytic concentration of creatine kinase 2 (also known as CK-MB) in serum is one of the main tests for the diagnosis of acute myocardial infarction. When the isoenzyme measurement does not clearly reflect