steps and the requirement for precise test parameters may have contributed to the higher coefficients of variation (less precision) achieved with the digoxin enzyme immunoassay. We expect that with the use of automatic pipettor-dilutors, precision would improve.

Radioimmunoassay III showed intermediate precision between radioimmunoassay II and enzyme immunoassay I. Radioimmunoassay II had slightly better precision in the day-to-day comparison.

Assay II is an equilibrium assay requiring an incubation of 30 minutes at 37 °C, however, the time and temperature did not seem to be critical. Time was varied from 20 minutes to 2 hours, and temperature varied ±5 degrees without harm. The radioimmunoassay II required 1.5 hours to assay approximately 40 tubes. The second radioimmunoassay III averaged 1.5 hours to assay approximately 40 tubes. Counting times were not included.

The enzyme immunoassay gave slightly higher digoxin concentration than did the radioimmunoassays with the control sera and patient sera. For instance, with the 1.3 μg/l control, the enzyme immunoassay result averaged 1.38 μg/l and the radioimmunoassays both averaged 1.23 μg/l. However, the difference was not statistically significant. Therefore, substitution of enzyme immunoassay for radioimmunoassay in the determination of therapeutic concentrations of digoxin would not require any change in the accepted therapeutic or toxic ranges.

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References
blood preserves the glucose in it (3, 8, 10), but, to our knowledge, no quantitative data. Here, we present data that we believe show that cooling with ice effectively preserves glucose in blood samples from both adults and newborns.

**Materials and Methods**

**Collection of Samples and Incubation**

Blood samples from 14 healthy laboratory workers and 14 newborn infants (cord blood) were collected with heparinized syringes and each sample was divided among twelve plain 10 × 75 mm tubes (1 ml blood in each). Two of the tubes were centrifuged within 5 min as zero time duplicates and the plasma was frozen for later analysis. Five tubes were allowed to stand in an ice bath and the other five to stand at room temperature. At intervals of 0.5, 1, 1.5, 2, and 4 h, a tube from each temperature was centrifuged and the plasma frozen. Blood from seven of the 14 newborns was also placed into two NaF-containing tubes (Becton-Dickinson, Rutherford, N. J. 07070; Vacutainer tube No. 32U6 X F18), 5 ml in each, which were centrifuged and analyzed after 0.5 and 4 h. The final NaF concentration was 2.5 g/liter.

**Evaluation of Effect of Cooling**

Several substances were measured in all plasma specimens: glucose by a glucose oxidase procedure (Glucose Analyzer®, Beckman Instruments, Inc., Fullerton, Calif. 92634), Na⁺ and K⁺ by flame photometry (Model 143 Flame Photometer; Instrumentation Laboratory, Lexington, Mass. 02173), chloride by coulometric–amperometric titration with silver (Corning Chloride Meter 920 M; Corning Scientific Instruments, Medfield, Mass. 02052), calcium by fluorometric titration with calcein (Corning Calcium Analyzer 940), and urea by a urease method (Hyland UN test kit; Hyland Laboratories, Costa Mesa, Calif. 92626). Instruments and reagent kit were all used in strict accord with suppliers' instructions.

**Results**

Figure 1 (a and b) shows the decrease in blood glucose as a function of time of storage of the samples from adults and newborns. In samples at room temperature without preservative, plasma glucose decreased 36 mg/liter per hour (0.2 mmol/liter per hour) in the blood from adults and 60 mg/liter per hour (0.33 mmol/liter per hour) in blood from newborn infants. Cooling on ice slowed these rates by about six- to ninefold to 3.9 mg/liter per hour (22 μmol/liter per hour) and 11 μmol/liter per hour (60 μmol/liter/hour). The effectiveness of NaF preservation was studied in seven samples from newborn infants. After 4 h, blood glucose had decreased less than
in samples without preservative but the loss was still two-fold greater than in samples preserved by cooling (Figure 1b).

Of the other substances measured, only potassium concentration was changed significantly by cooling; in cooled specimens from both adults and newborn infants it increased by 0.3 mmol/liter per hour (Figure 2a and b). In samples at room temperature, plasma potassium did not change significantly. Table 1 shows the changes in sodium, calcium, urea and chloride at 4 h. Values for none of these substances had changed significantly by 4 hours in either cooled or room-temperature specimens.

**Discussion**

Our data clearly demonstrate that cooling effectively preserves glucose in blood samples from adults and newborn infants, apparently even more so than preservation with NaF. Furthermore, unlike NaF-treated specimens, calcium and sodium can be measured in the cooled sample. Neither cooling nor NaF interferes with measurement of chloride or urea nitrogen. Apparently the urease concentration in the Hyland reagents is great enough to overcome the inhibitory effect of 2.5 g of NaF per liter. Plasma potassium slowly increased (0.3 mmol/liter per hour in both groups) in the cooled samples. Essentially identical rates of increase were observed by Oliver et al. (10): adults, 0.26 mmol/liter per hour; newborns, 0.4 mmol/liter per hour. The slight increase that occurs in 1 h would not alter the clinical significance of the potassium measurement, but we do not recommend longer storage on ice before separation if potassium is to be measured.

Unusual clinical situations may alter the effectiveness of cooling, NaF, or any other means of preserving glucose. In a normal individual the consumption of glucose is about evenly distributed between erythrocytes and leukocytes (calculated from references 11–13). An increase in leukocytes as great as seven- to ninefold, as may occur in leukemia, would result in a great increase in the rate of glucose consumption in the blood samples. In such a situation, neither cooling nor NaF alone may be effective means for preserving blood glucose (10). Under more common circumstances, however, cooling of whole blood is by itself effective in stabilizing plasma glucose concentration, and this simple procedure may be quite useful in clinical laboratory practice.

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**References**