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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Stabilization of Blood Glucose by Cooling with Ice: An Effective Procedure for Preservation of Samples from Adults and Newborns

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Glycolysis causes a considerable decrease in blood glucose when whole blood is kept at room temperature without preservative. The most commonly used preservative, NaF, makes analysis of other serum constituents such as sodium and calcium and urea difficult or impossible, an especially serious limitation when sample size must be restricted. In samples at room temperature without preservative, plasma glucose decreased 36 mg/liter per hour in blood from adults and 60 mg/liter per hour in blood from newborns. Cooling on ice slowed these rates to 3.9 and 11, respectively. Plasma potassium increased 0.3 mmol/liter per hour in cooled specimens from both adults and newborns. Sodium, calcium, chloride and urea values were unaffected. We conclude that cooling effectively stabilizes plasma glucose for 4 h in samples from both adults and newborns and that potassium may be measured with negligible change for as long as 1 h and other constituents for the entire period.

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Blood glucose decreases considerably and rapidly when whole blood is kept at room temperature (*1–4*). Some precaution must be taken to ensure against a significant change in the blood glucose concentration during the time between obtaining the sample and performing the analysis. Two procedures are commonly used to minimize the error introduced by the metabolism of glucose by blood cells: rapid separation of the cells from plasma or serum, and addition of an inhibitor of glycolysis to the blood sample. Rapid separation is frequently inconvenient and its success depends on a knowledgeable individual making sure that the sample is rapidly delivered to the laboratory. NaF has been the inhibitor most widely used for the preservation of blood samples (*5*). Addition of NaF renders the specimen definitely unusable for assay of calcium or sodium and may interfere with measurement of urea nitrogen by a urease procedure (*6, 7*). In general, a separate blood sample is required exclusively for the analysis of glucose. This limitation is especially serious when sample size must be restricted. A less-common procedure is dilution of the sample at the bedside, either with water or isotonic NaF (*8, 9*). This procedure is effective, but it makes accurate measurement of glucose dependent on the precision of the dilution by the blood drawer at the bedside.

Because these three procedures possess disadvantages, we investigated cooling as an alternative means of preservation. There are conflicting statements as to how effectively cooling

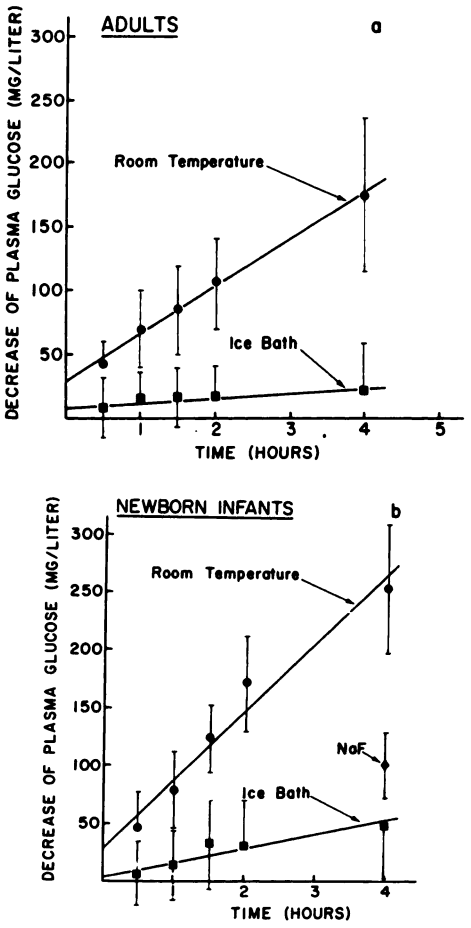

Fig. 1. Decrease in plasma glucose on storage of whole blood from (*a*) adults and (*b*) newborns

Bars are one standard deviation. Rates of decrease (slope, in mg/liter per hour) are: adults, room temperature, 36, ice, 3.9; newborns, room temperature, 60, ice, 11. In blood from newborn infants stored with NaF, the decrease at 4 h was intermediate between that of cooled blood and blood stored at room temperature without preservative

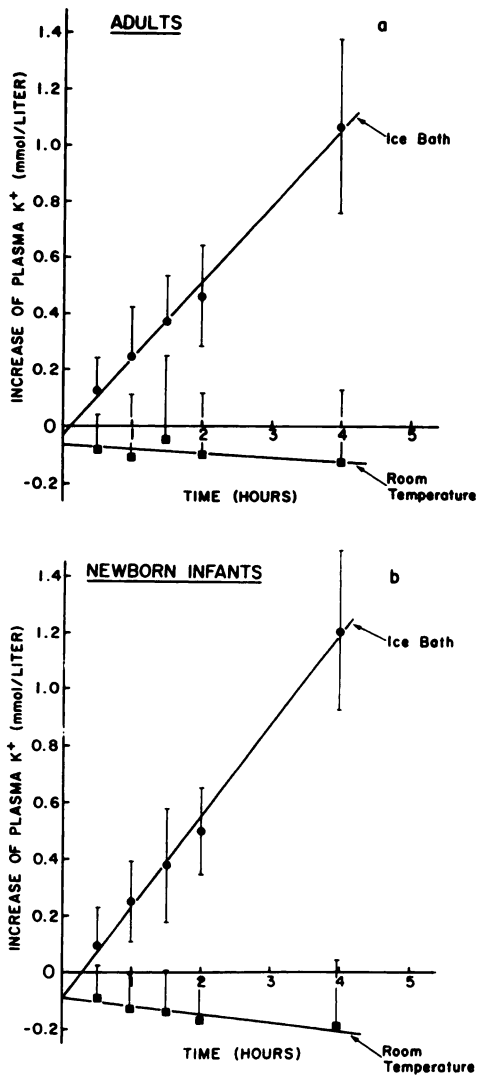

Fig. 2. Increase in plasma potassium on storage of whole blood from (*a*) adults and (*b*) newborns

Bars are one standard deviation. Cooling with ice caused an increase of 0.3 mmol/liter per hour (slope) in samples from both groups. At room temperature there was no change with storage

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 mg/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

Table 1. Changes in Plasma Sodium, Calcium, Urea Nitrogen, and Chloride Concentrations after 4-h Storage at Room Temperature (R.T.) or on Ice[a]

	Sodium		Calcium		Urea N		Chloride	
	R.T.	Ice	R.T.	Ice	R.T.	Ice	R.T.	Ice
Adults								
Mean	−1.6	−1.8	−0.05	−0.08	−0.1	−0.9	−2.1	−2.9
SD[b]	±2.3	±1.2	±0.04	±0.07	±0.3	±0.5	±1.8	±2.9
Newborns								
Mean	−0.5	−1.9	−0.01	−0.06	−0.2	−0.9	−2.0	−1.9
SD[b]	±1.7	±1.6	±0.10	±0.8	±0.5	±0.4	±2.9	±2.0

[a] All values in mmol/liter.
[b] Standard deviation; n = 14.

in samples without preservative but the loss was still two-fold greater than in samples preserved by cooling (Figure 1*b*).

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 2*a* 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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