NBSIR 78-1585

Final Report
FDA Contract No. 74-58(0)

R. Schaffer, R. A. Velapoldi and D. J. Reeder

Center for Analytical Chemistry
National Measurement Laboratory
National Bureau of Standards
Washington, D.C. 20234

October 1978

Report for Period
April 1976 to October 1977
Issued January 1979

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U.S. DEPARTMENT OF COMMERCE, Juanita M. Kreps, *Secretary*

Jordan J. Baruch, *Assistant Secretary for Science and Technology*

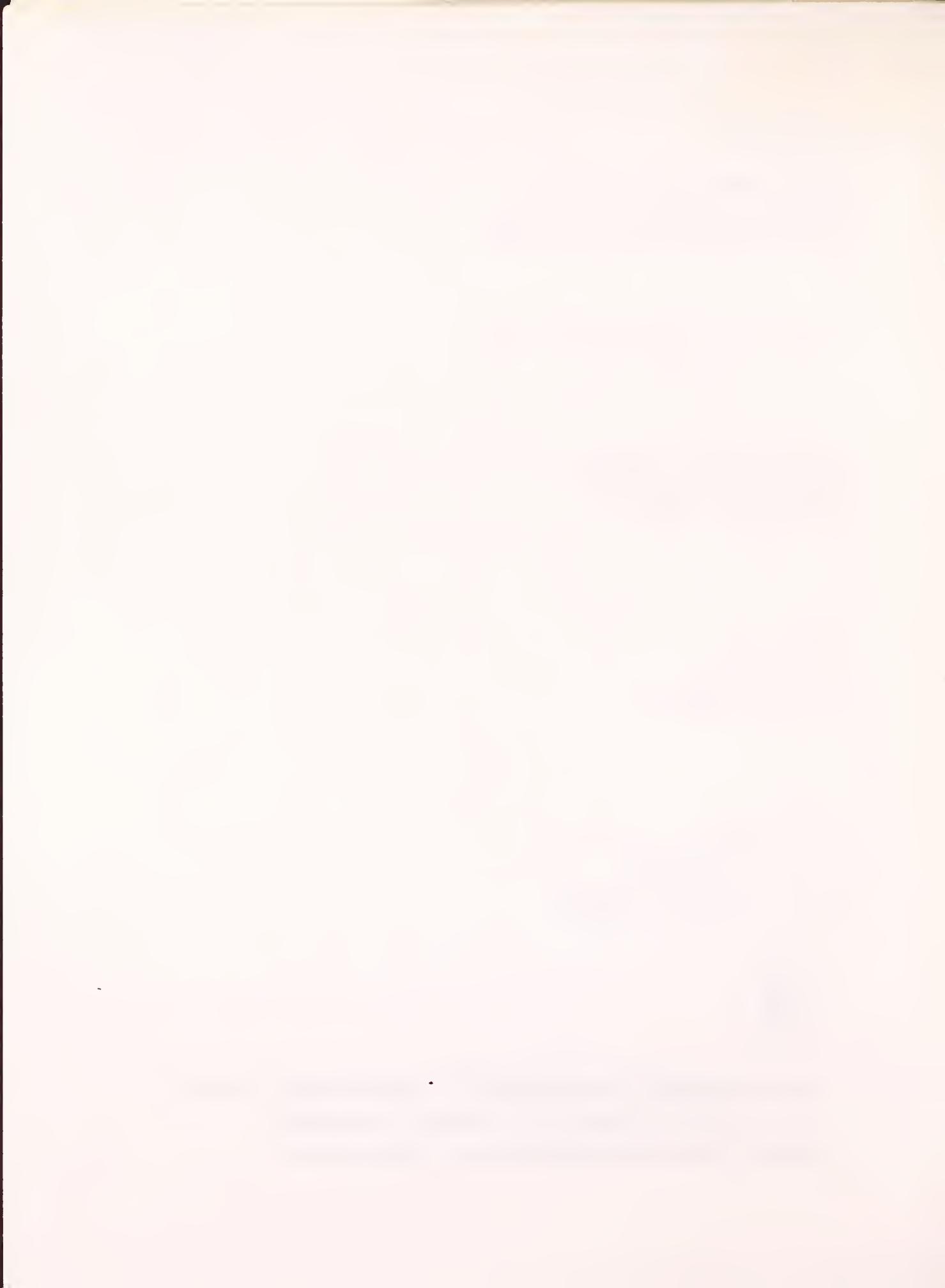**NATIONAL BUREAU OF STANDARDS, Ernest Ambler, *Director***

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INTRODUCTION

This report covers work done at the National Bureau of Standards (NBS) between April 1976 and October 1977 under FDA Contract 74-58(0). The minutes of meetings held during this reporting period in connection with this work have been supplied previously to the Food and Drug Administration (FDA), and therefore are not included here. However, results obtained through September 1978 are included to bring the information up to date. We expect to keep the FDA informed of progress as this work continues.

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Task 1a.   GLUCOSE IN SERUM

A.   Definitive Results and Interlaboratory Test Results
      with the Reference Method

    The statistical analysis of the serum-glucose values that were determined by interlaboratory testing of the reference method and by the ID-MS method at NBS was sent to the Food and Drug Administration in September 1976.   That analysis showed that there was no bias between the methods with serum glucose at a concentration of 0.8 g/L, but that relative to ID-MS the reference method gave a higher result at a concentration of 0.4 g/L and lower results at concentrations greater than 1.3 g/L.

B.   Additional Definitive Method Analyses

    We sought to analyze additional samples from these serum pools to enhance the precision of the ID-MS measurements, but only one more set could be analyzed because the supply of these samples was exhausted.   These additional ID-MS results are shown in Task 1a, Table 1, labeled as Set V.   The table also provides old and new results for Sets III and IV, listing the results shown in Table 19 of the September 1976 statistical report and giving new results for Sets III and IV which were obtained when Set V was being measured.   This was to assure that the results for Set V could be reliably compared with the old results.   The new results for Sets III and IV are remeasurements by GC/MS of the specimens of isolated diacetone glucose that had been originally measured.

Task 1a, Table 1. ID-MS Results for Glucose in Pools 1-5
             (in g/L); Results for Set V and Previous
             and New Results for Sets III and IV

	1	2	3	4	5
Set III	0.4029	0.7765	1.320	1.928	2.929
previous	0.4027	0.7778	1.332	1.927	2.927
	0.4028	0.7745	1.328	1.923	2.927
	0.4034	0.7784	1.327	1.923	2.924
Set III	0.4005	0.7724	1.335	1.934	2.919
new data	0.4025	0.7802	1.328	1.938	2.937
	0.4030	0.7822	1.333	1.929	2.931
	0.4023	0.7782	1.325	1.923	2.928
Set IV	0.3939	0.7632	1.314	1.878	2.883
previous	0.3937	0.7624	1.313	1.891	2.867
	0.3930	0.7626	1.314	1.890	2.883
	0.3940	0.7632	1.314	1.894	2.867
Set IV	0.3935	0.7630	1.309	1.911	2.878
new data	0.3950	0.7632	1.318	1.902	2.879
	0.3944	0.7643	1.308	1.894	2.851
	0.3926	0.7655	1.310	1.892	2.859
Set V	0.3908	0.7662	1.305	1.887	2.853
	0.3955	0.7645	1.302	1.871	2.839
	0.3968	0.7693	1.314	1.892	2.847
	0.3969	0.7727	1.302	1.885	2.849

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Task 1a, Figure 1 illustrates the results obtained on reanalysis of the serum pools over a period of time, and are shown as percent changes from the initially determined ID-MS values. The data used for this figure are the averages of the values listed in Table 19 of the September 1976 report and the averages of the values for Set V, given in Task 1a, Table 1. Note that non-uniform time intervals between the sets of analyses are shown in the figure. The finding of lower results upon later reanalyses of each of the serum pools raised the possibility that the glucose levels in these serum pools were declining. However, the similarity in the magnitudes of the differences in concentration and the imprecision of measurement obscured the evidence for sample instability.

C. Definitive Method Analysis of New Pools

To substantiate the finding of a concentration-dependent bias in the reference method and to obtain additional data on the precision of the ID-MS method, we planned to use the ID-MS method on new serum pools that CDC proposed to prepare (using human serum) and on which CDC would perform the reference method analysis. At the close of the project reporting period, the preparatory wet-chemical work-up for the ID-MS analysis of these new samples had begun, but now at the time of writing this present report, six sets of samples from these pools have been analyzed. Although the results have not been intercompared with reference method results nor examined statistically, a

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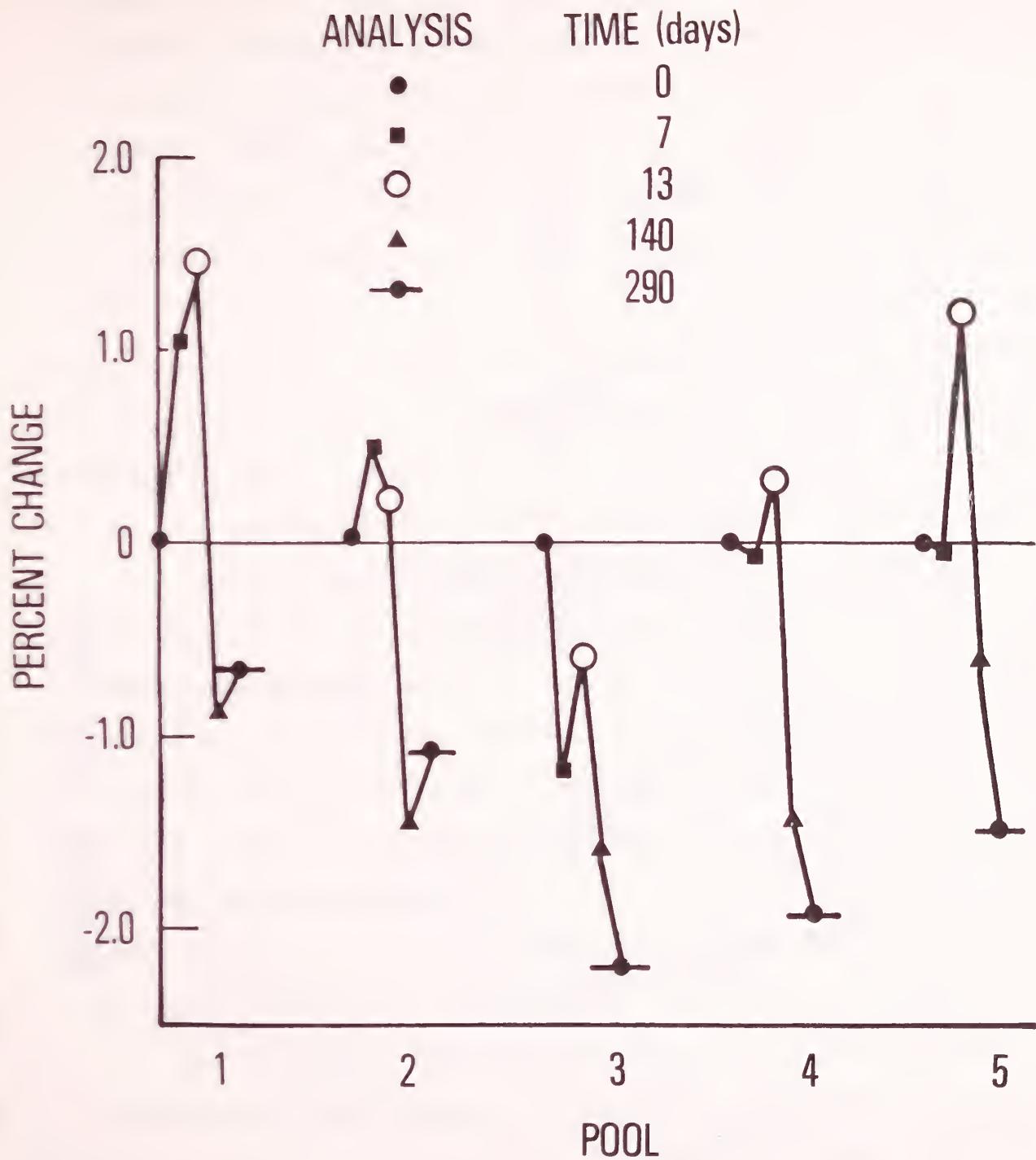
Task 1a, Figure 1. Percent changes in the glucose concentrations found in the five IE-III serum pools, as measured at different times by ID-MS.

graphic representation of the percent changes found on sequential analysis, given in Task 1a, Figure 2, provides a preview of these ID-MS results. Because the first samples taken for the analysis of Pools A and E were lost, the percent changes in determined values for all pools are based on changes from the results found at 20 days. Again as in Task 1a, Figure 1, the time frame in Task 1a, Figure 2 is non-linear. Our ID-MS results on these new pools appear to suggest that loss of glucose occurs with time. The concentrations of glucose in these pools range from 3.5 to 20 mmol/L. The precision of these new analyses is better than was obtained with the previous pools.

D.   Definitive Method Analysis of WHO Reference Serum

During the project reporting period, the ID-MS method was employed for the analysis of the so-called World Health Organization Reference Serum. The analysis was performed essentially as a within-day precision study in that the additions of labeled glucose to eight serum aliquots were made on one day. Possible inter-vial inhomogeneities were eliminated by pooling the contents of several randomly selected vials and taking the eight aliquots for analysis from the mixture. The result, based on 16 measurements (two on each of the eight aliquots), was 5.444 ± 0.0093 mmol/L representing the mean and one standard deviation, respectively.

E.   Preliminary Work on an Alternative Definitive Method

As an independent means for establishing the accuracy of results obtained by the ID-MS method involving diacetone glucose,

Task 1a, Figure 2. Percent changes in the glucose concentrations found in six pools of human serum, as measured at different times by ID-MS.
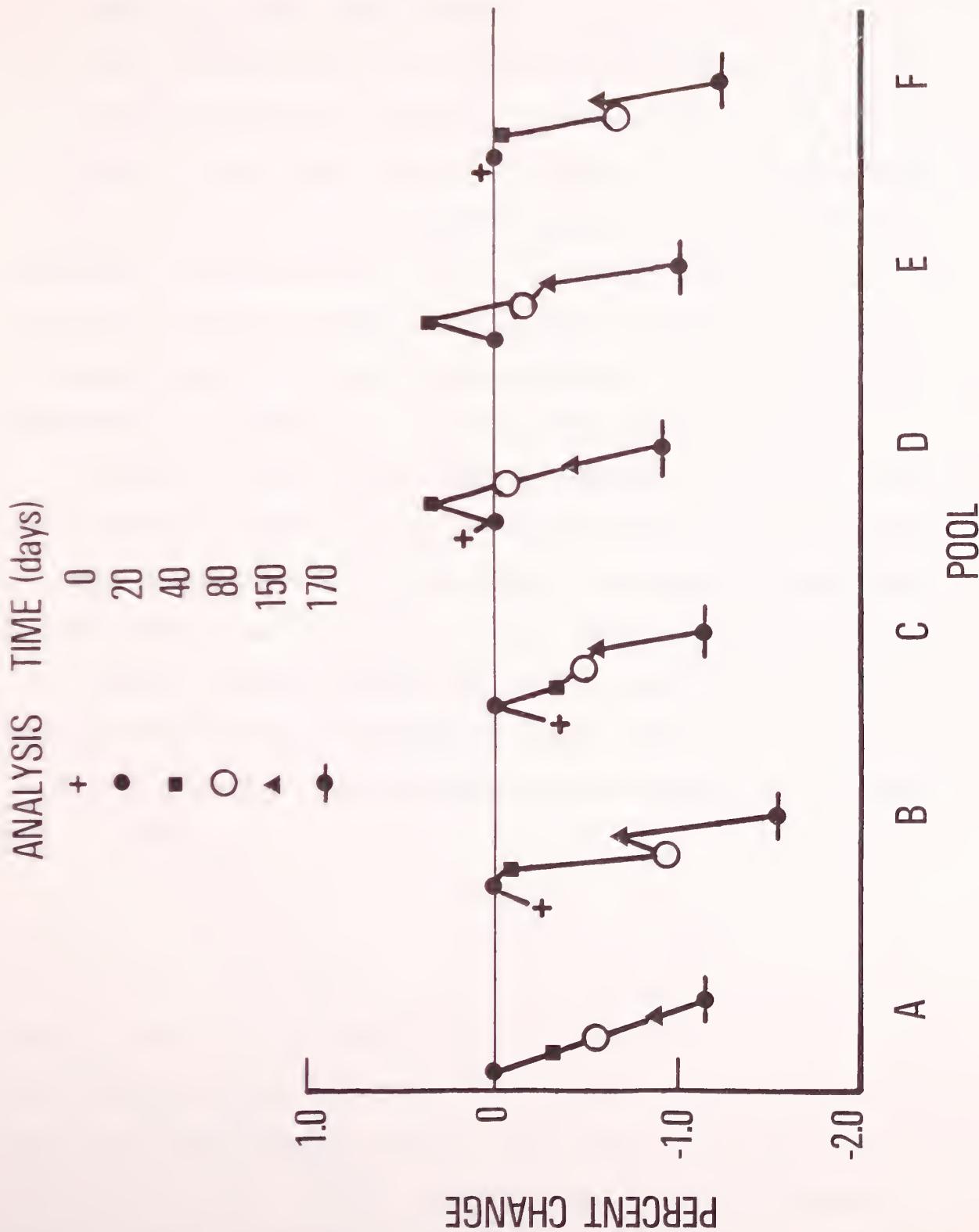
preliminary experiments were begun with an ID-MS method that
employs the bis-butaneboronate acetate derivative of glucose
(J. Wiecko and W.R. Sherman, J. Amer. Chem. Soc., 98, 7631
(1976)). Use of this derivative for ID-MS was reported by
D. M. Bier, W. R. Sherman, W. H. Holland, and D. M. Kipnis,
(Proceedings of the First International Conference on Stable
Isotopes in Chemistry, Biology, and Medicine, May 9-11, 1973,
Argonne, Il., P. Klein, Ed., p. 397). The method they reported
cannot be used as a definitive method because the butaneboronate
acetate derivatives of other hexoses present in serum are not
separated from that for glucose in the GC procedure the authors
describe. (In the diacetone glucose method, TLC is run in
several systems to remove other hexoses that may be present.)
Our preliminary experiments indicate that these other hexose
butaneboronate acetates may be resolved from the glucose deriva-
tive by use of capillary column gas chromatography. Work
beyond this preliminary stage has been held up until the backlog
of samples to be measured by GC/MS has been cleared up.

Task 1b. LITHIUM, MAGNESIUM, SODIUM, POTASSIUM, AND CHLORIDE

Summary: Statistical analyses of the results from the
Interlaboratory Exercises (IE) for Lithium IV, Magnesium II,
Sodium II, Potassium II, and Chloride II were presented and
discussed at the Experts Committee meeting held at NBS on
November 22, 1976. The Committee members, NBS statisticians,
and other NBS participants present agreed that the goals set
originally by the Committee for precision and accuracy for the
Li, Na, K, and Cl reference methods had been achieved. The
goals and statistical results are summarized in Task 1b, Table 1.
The results are discussed in detail for each electrolyte.

A.   Lithium: Statistical analysis of the results from IE-IV
was completed. The exercise consisted of tests on four samples
(provided in separate vials) at each of four different concen-
trations (Pools 1, III, 5, V). Two vials at each concentration
were analyzed on day 1 and the two other vials were analyzed on
day 2. A summary of the results for the manual (M) and semi-
automated (SA) procedures is given in Task 1b, Table 2. The
number immediately after the 'M' or 'SA' is the lab identifica-
tion and the last number (either 1 or 2) denotes the day of
analysis. The values in the columns are averages of the results
from the two vials for that day. Laboratory 7 (M 72X) did not
obtain results by the manual procedure for day 1. The data are
illustrated in Task 1b, Figures 1a and 1b, as percent deviations
from the ID-MS assigned values. The pools are designated in the
figure by 1, III, 5, and V. The same pool-sample sequence is
used for each laboratory's results.

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Task 1b: Table 1. Imprecision and Bias Goals and the Values Obtained by Statistical Analysis from Interlaboratory Exercise Testing for Various Electrolytes.

Ion	Concentration (mmol/L)	IE	Imprecision[a] $\hat{\sigma}_{Total}$ (mmol/L)			Bias[b] $\overline{X}-X_{DM}$ (mmol/L)		
			Goal	Semiautomated	Manual	Goal	Semiautomated	Manual
Li	2.0	IV	0.1	0.017	0.028	0.2	-0.036	-0.030
Na	140.0	II	1.5	0.55	0.58	2.0	-0.4	-0.3
K	2.5	II	0.1	0.063	0.049	0.2	-0.027	0.025
K	6.5	II	0.1	0.063	0.049	0.2	0.042	0.065
Cl	100.0	II	1.5	0.52	1.01	2.0	-0.2	-0.1

[a] Imprecision = $\hat{\sigma}_{Total}$ = $\sqrt{\dfrac{\hat{\sigma}^2 repl}{4} + \dfrac{\hat{\sigma}^2 day}{2} + \hat{\sigma}^2 lab}$

[b] Bias = $\overline{X}-X_{DM}$ where $\overline{X}$ is the average value for all labs and X_{DM} is the definitive method value.

Task 1b, Table 2. Averages of Lithium Concentration.
Cooperating Laboratories' Results for IE-IV.

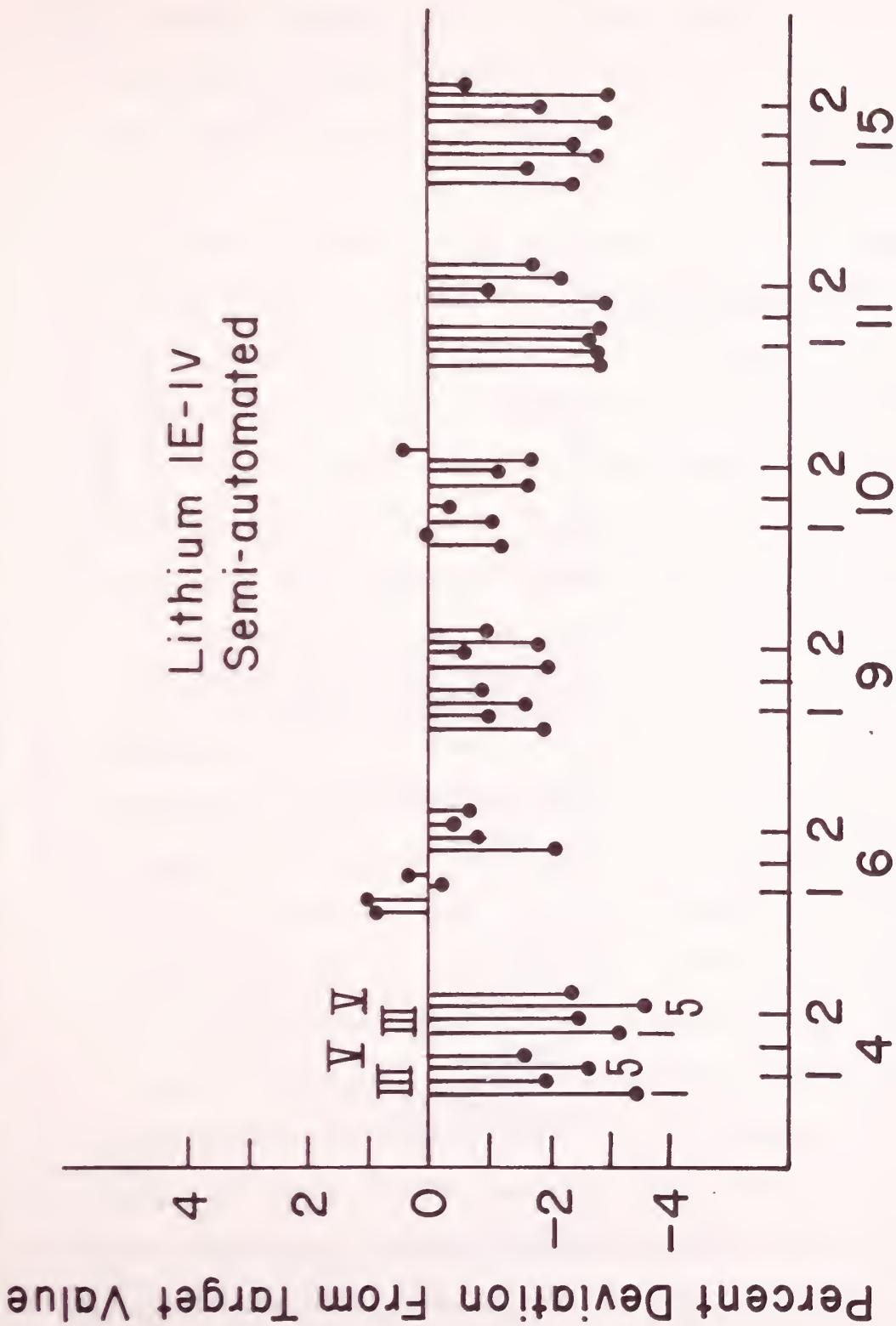| Procedure | | Lithium, mmol/L | | | |
| | | Pool | | | |
Lab	Day	1	III	5	V
M	11	.5430	.9890	1.7580	2.9910
M	12	.5225	.9945	1.7550	2.9080
M	21	.5050	.9910	1.7805	2.9925
M	22	.5030	.9985	1.7670	2.9775
M	41	.5170	.9795	1.7465	2.8695
M	42	.5205	.9890	1.7685	2.9160
M	51	.5185	1.0080	1.7845	2.9460
M	52	.5115	1.0150	1.8060	2.9250
M	72X	.5330	1.0055	1.8090	2.9790
M	81	.5360	1.0315	1.8435	3.0195
M	82	.5360	1.0295	1.8260	3.0515
M	91	.5225	.9940	1.7810	2.9260
M	92	.5230	.9960	1.7725	2.9260
M	101	.5260	1.0060	1.8395	2.9495
M	102	.5265	.9990	1.7690	2.9535
M	131	.5200	.9795	1.7665	2.9145
M	132	.5205	.9840	1.7700	2.9000
M	141	.5205	.9840	1.7630	2.8985
M	142	.5230	.9855	1.7635	2.9020
M	151	.5200	.9790	1.7625	2.8930
M	152	.5210	.9900	1.7605	2.8925
SA	41	.5150	.9835	1.7590	2.9035
SA	42	.5165	.9785	1.7425	2.8810
SA	61	.5390	1.0145	1.8050	2.9645
SA	62	.5225	.9950	1.8015	2.9320
SA	91	.5235	.9935	1.7795	2.9260
SA	92	.5230	.9975	1.7750	2.9240
SA	101	.5275	1.0045	1.7890	2.9420
SA	102	.5250	.9915	1.7780	2.9675
SA	111	.5185	.9750	1.7590	2.8690
SA	112	.5180	.9930	1.7675	2.9010
SA	151	.5210	.9870	1.7580	2.9120
SA	152	.5185	.9855	1.7560	2.9350
ID-MS Values		.534	1.004	1.809	2.954

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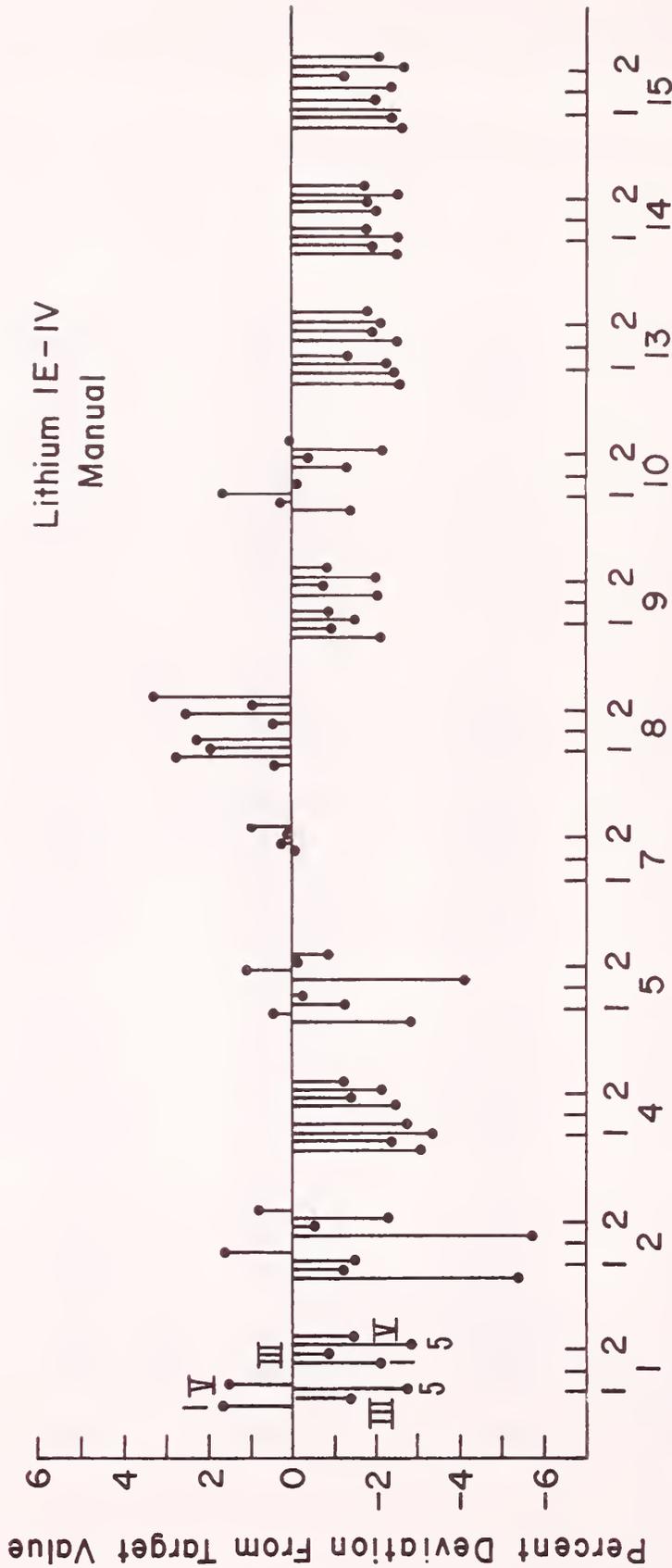
Task 1b, Figure 1a. The percent deviations of the lithium reference method measurements from the ID-MS assigned values, obtained by the manual pipetting procedure for IE-IV. The pools are identified by 1', III, 5, and V near the results from laboratory 1. The same designations are used for the remaining sets of laboratory results.

Lithium IE-IV
Semi-automated

Task 1b, Figure 1b. The percent deviations of the lithium reference method measurements from the ID-MS assigned values, obtained by the semi-automated pipetting procedure for IE-IV. The pools are identified by 1, III, 5, and V near the results from laboratory 4. The remaining results have the same designations.

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Although a bias of approximately two to three percent was observed, the statistical analysis showed that the results from IE-IV were within the imprecision and bias goals; therefore further investigation was not necessary. Work leading to an NBS 260 Special Publication was begun.

B. Magnesium: IE-II for magnesium was completed with nine laboratories performing the manual pipetting protocol and four laboratories performing the semi-automated protocol on a total of 12 serum samples consisting of four samples in separate vials at each of three different concentrations (pools 1, 5, 7). Two vials at each concentration were analyzed on day one and the remaining vials were analyzed on a subsequent day. The results are summarized in Task 1b, Figures 2a and 2b, as percent deviations from the definitive method values. These figures reveal that if we exclude from the manual-pipetting procedure results those obtained on both days in laboratory 8 and those from one day in laboratories 7 and 13, the remaining manual procedure values are generally within three percent of the values determined by ID-MS. Furthermore, all of the results obtained by the semiautomated procedure are well within two percent of the definitive method values. However, further interlaboratory testing for this electrolyte was discontinued until the magnesium gluconate dihydrate SRM is made available, since that is a basic requirement for clinical reference method development. (The new supply of the material has been received. It is undergoing certification. Interlaboratory testing will resume when the certification of the SRM is completed.)

Magnesium IE-II
Manual

Percent Deviation From The Definitive Method Value

Laboratory Number and Day Run
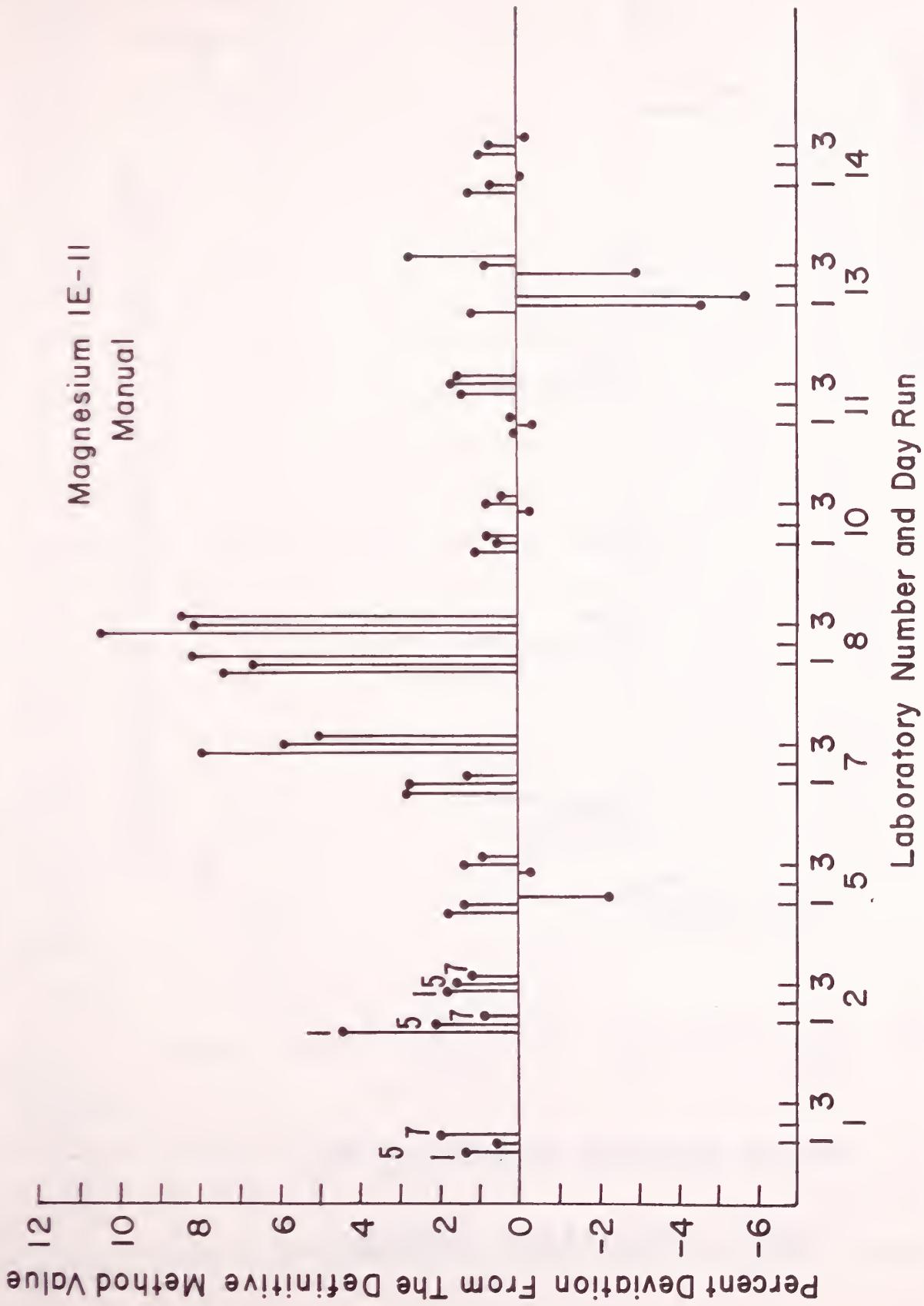
Task 1b, Figure 2a. Percent deviations of the IE-II measurements for magnesium using manual pipetting, from the definitive method values. The analyzed pools are identified by the numbers 1, 5, and 7 near the results from laboratory 1. The designations are similar for the remaining results. The numbers 1 and 3 placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

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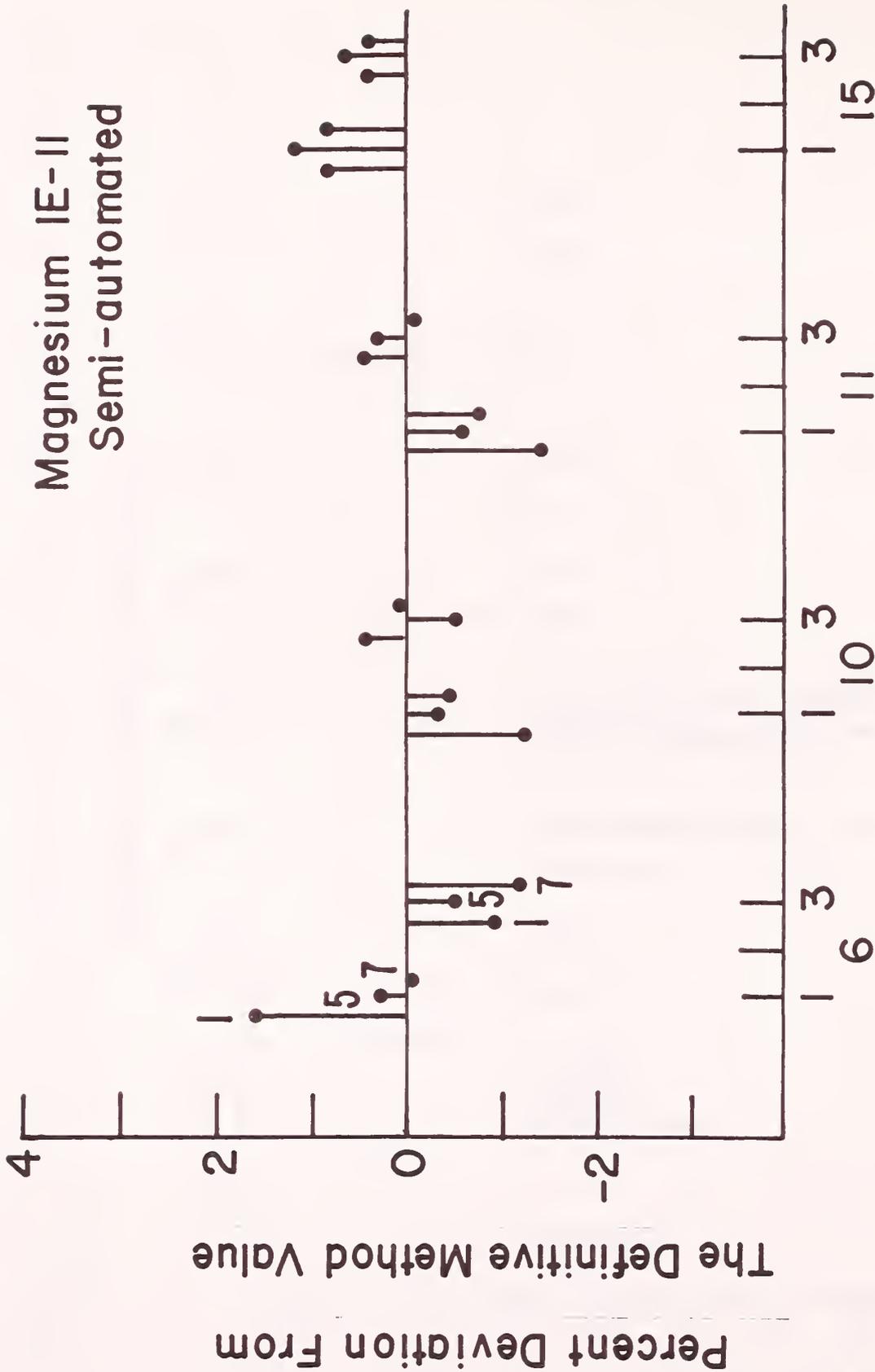
Task 1b, Figure 2b. Percent deviations of the IE-II measurements for magnesium using semi-automated pipetting, from the definitive method values. The analyzed pools are identified by the numbers 1, 5, and 7 near the results from laboratory 6. The designations are similar for the remaining results. The numbers 1 and 3 placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

C.   Sodium: Statistical analysis of the results obtained in IE-II was completed. Four samples were analyzed at each of five concentrations (Pools 1, 3, 4, 5, 7). As usual, two vials of each concentration were analyzed on one day and the remaining pairs were analyzed on another day. A summary of the results is given in Task 1b, Table 3. The designations follow those given in Table 1. These data are graphically depicted in Task 1b, Figures 3a and 3b as percent bias from the values assigned by the ion-exchange/gravimetry technique.

Laboratory 15 experienced blank problems with the manual procedure. This laboratory also performed an extra semi-automated analysis (designated A151X and A152X) in which samples were diluted with 20 parts of diluent rather than with 19 parts of diluent. These data are listed for comparison, but were not used in the statistical analysis.

The statistical analysis of these IE-II results showed that they were within the preset accuracy and precision goals. The final report, NBS Special Publication 260-60, was published after a draft had been sent to the Experts Committee and participating laboratories for comment. A copy of that publication is provided with this report.

D.   Potassium: Statistical analysis of data from IE-II for potassium in serum has been completed. For this exercise, four samples in separate vials were supplied at each of three concentrations (Pools 1, 4, 6). Two vials at each concentration were analyzed on day one and the remaining pairs were analyzed

Task 1b. Table 3.   Averages of Sodium Concentration.
Cooperating Laboratories' Results for IE-II.

| Procedure | | Sodium, mmol/L | | | | |
| | | Pool | | | | |
Lab	Day	1	3	4	5	7
M	41	113.570	129.370	136.350	146.860	157.410
M	42	113.320	129.020	135.470	146.740	157.570
M	51	113.900	129.750	136.350	149.810	157.620
M	52	113.890	129.670	136.430	148.010	157.870
M	71	113.930	128.920	135.860	146.900	157.970
M	72	113.440	129.260	135.680	146.480	157.720
M	81	113.690	129.730	136.360	147.330	158.210
M	82	113.190	129.020	134.660	145.490	157.460
M	91	112.980	128.440	136.180	147.060	158.210
M	92	113.010	128.320	136.700	146.590	156.970
M	111	113.890	129.440	136.340	147.360	157.805
M	112	113.680	129.575	137.000	145.780	157.720
M	131	113.540	130.130	137.685	148.600	158.350
M	132	113.290	129.300	136.500	147.500	158.080
M	151X	118.700	131.445	139.725	149.485	162.220
M	152X	117.950	134.150	141.470	150.445	160.770
SA	11	113.840	129.220	135.980	147.200	157.810
SA	12	113.970	129.980	136.070	147.210	157.930
SA	21	113.705	129.270	135.240	146.165	157.025
SA	22	114.095	130.135	136.855	147.770	158.245
SA	41	113.150	129.310	135.490	146.400	156.760
SA	42	113.210	129.510	136.090	147.310	157.570
SA	91	113.590	128.470	137.710	146.450	157.410
SA	92	113.360	128.700	136.520	147.080	157.530
SA	101	113.710	129.400	135.690	147.290	158.200
SA	102	113.150	128.880	136.100	146.570	157.190
SA	111	115.230	130.475	137.205	148.645	159.495
SA	112	114.030	130.365	137.170	147.720	158.410
SA	151	113.680	129.070	134.745	146.380	157.485
S	152	114.720	129.840	136.080	145.665	156.030
A	151X	113.875	129.065	135.850	146.785	157.455
A	152X	113.360	130.205	135.635	146.065	157.605
Ion-Exchange/ Gravimetry Values		113.2	129.9	136.6	146.3	158.6

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Sodium IE-II
Manual

Percent Deviation From Target Value
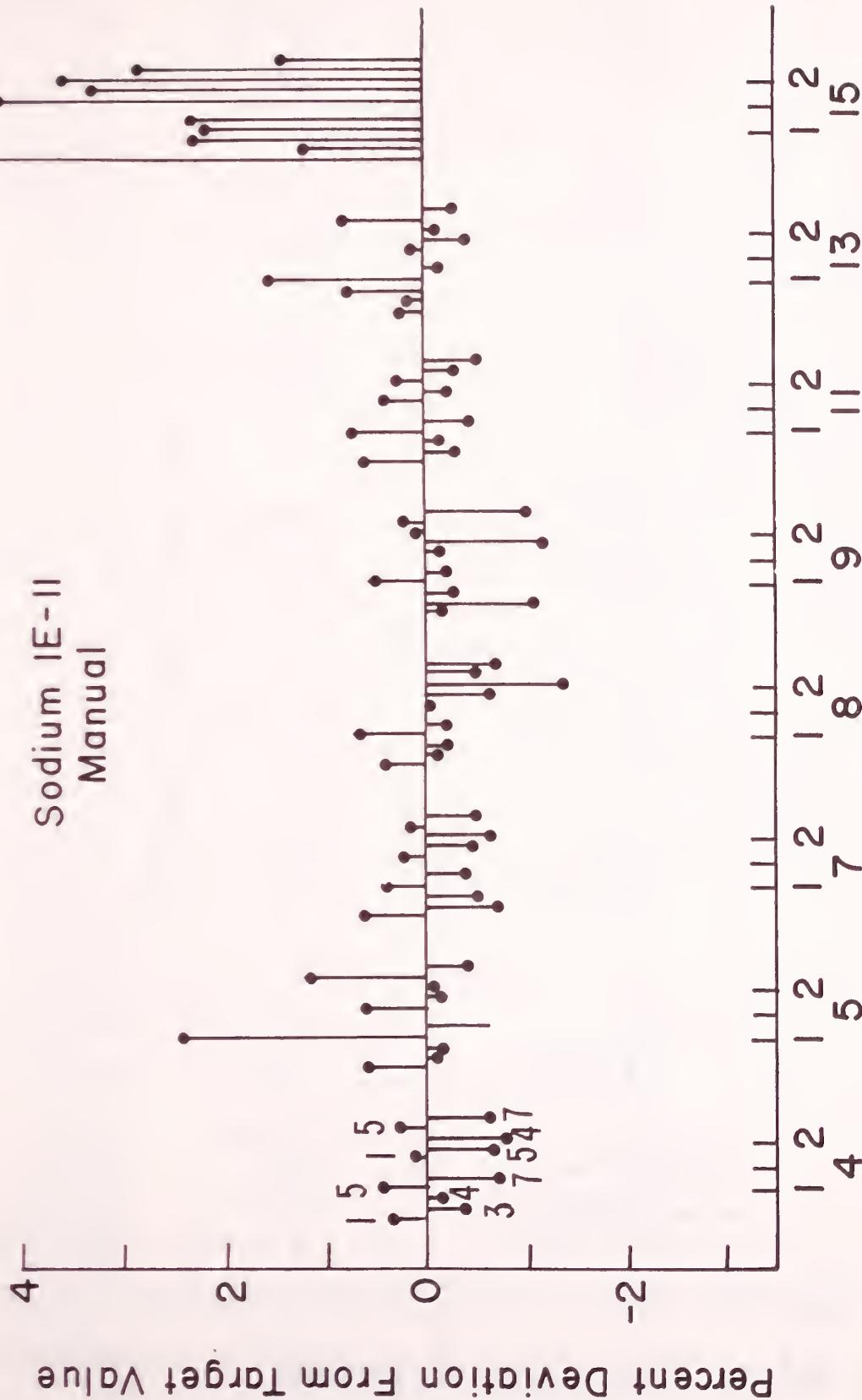
Laboratory Number and Day Run

Task 1b, Figure 3a. The percent deviations of the sodium reference method measurements from the ion-exchange/gravimetry values, obtained by the manual pipetting procedure for IE-II. The pools are identified by the numbers 1, 3, 4, 5, and 7 near the results from laboratory 4 — identification of the pools are the same for the remaining results.

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Sodium IE-II
Semi-automated

Percent Deviation From Target Value

Laboratory Number and Day Run

Task 1b, Figure 3b. The percent deviations of the sodium reference method measurements from the ion-exchange/gravimetry values, obtained by the semi-automated pipetting procedure for IE-II. The pools are identified by the numbers 1, 3, 4, 5, and 7 near the results from laboratory 1 - identification of the pools are the same for the remaining results.

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on day two. The averages of the results for the manual (M) and semi-automated (SA) pipetting procedures are summarized in Task 1b, Table 4. Lab, procedure, day run, etc., designations are used as before. The percent deviations from the ID-MS values are graphically shown in Task 1b, Figures 4a and 4b.

Laboratory 4 repeated the manual and semi-automated procedures (M41X, M42X, SA41X, SA42X) because, due to instrumental problems, the diluted samples and standards had to be stored overnight in a refrigerator. Although shown in the table, these values are not included in the statistical analysis. Results from Laboratory 15 were quite variable. A repeat of the semi-automated pipetting procedure (SA151R, SA152R) provided values with less scatter and, in general, less bias from the ID-MS values.

Statistical analysis of these results showed no intrinsic problem with the reference method and it was decided to proceed with IE-II, and accordingly IE-II samples were sent to the participating laboratories.

IE-II consisted of four samples at each of five different potassium concentrations (Pools 1, 2, 4, 5, and 7). Statistical analysis of the results showed that 0.1 mmol/L imprecision at the 2.5 and 6.5 mmol/L potassium levels (which correspond to coefficients of variation of 4.0 percent and 1.5 percent, respectively) and of 0.2 mmol/L bias at these levels (8.0% and 3.0%, respectively) were reached. These precision and accuracy values were like those obtained in IE-II. The data

Task 1b, Table 4.   Average of Potassium Concentration.
                     Cooperating Laboratories' Results for IE-I.

		Potassium, mmol/L		
		Pool		
Procedure				
Lab	Day	1	4	6
M	41	1.3180	4.3340	6.1110
M	42	1.3100	4.3610	6.1345
M	41X	1.3085	4.3515	6.1625
M	42X	1.3045	4.3080	6.1500
M	51	1.2820	4.3950	6.0680
M	52	1.3410	4.3720	6.1980
M	71	1.4150	4.4190	6.1390
M	72	1.3360	4.3710	6.0710
M	71X	1.7260	4.4190	6.1390
M	81	1.3630	4.6520	6.3970
M	82	1.3720	4.4290	6.1560
M	111	1.3040	4.3460	6.0845
M	112	1.2960	4.3715	6.1630
M	131	1.3425	4.3395	6.1625
M	132	1.3260	4.3400	6.1275
M	151X	1.3645	4.4050	6.0925
M	152X	1.5405	4.5660	6.6250
SA	41	1.3460	4.3015	6.1250
SA	42	1.3200	4.4045	6.2260
SA	41X	1.3320	4.3810	6.0895
SA	42X	1.3020	4.4015	6.1755
SA	101	1.3000	4.2590	6.1840
SA	102	1.3420	4.3520	6.1350
SA	111	1.2640	4.4050	6.3345
SA	112	1.3915	4.5485	6.2560
SA	151X	1.4980	4.5000	6.2645
SA	152X	1.3020	4.3085	6.1540
SA	151R	1.3405	4.3730	6.1590
SA	152R	1.3675	4.3695	6.1470
A	151X	1.3050	4.3330	6.1380
A	152X	1.3425	4.3785	6.1315
ID-MS Values		1.3191	4.3230	6.0921
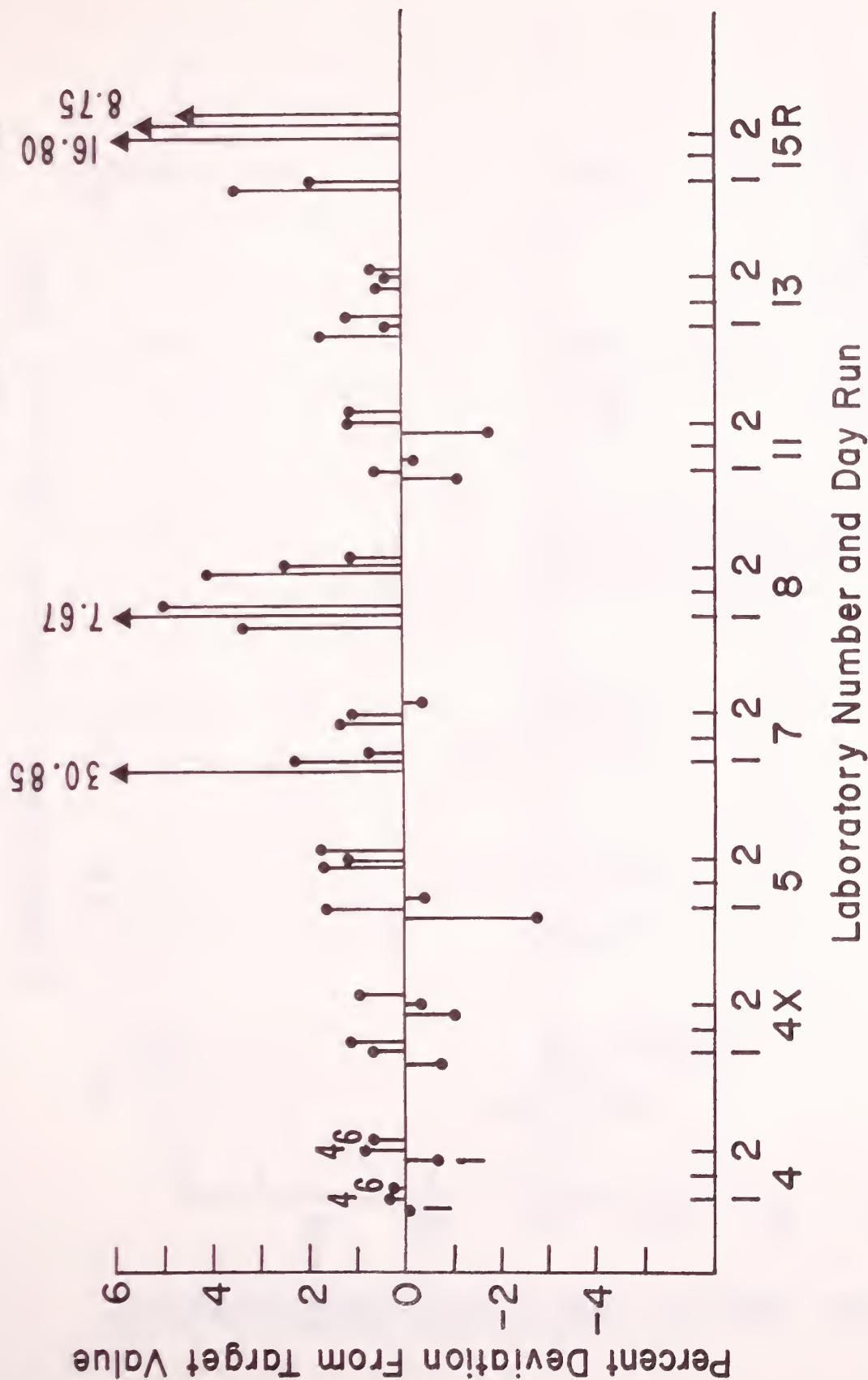
Task 1b, Figure 4a. The percent deviations of the potassium reference method measurements from the ID-MS determined values, obtained by the manual pipetting procedure for IE-I. The pools are identified by the numbers 1, 4, and 6 near the results for laboratory 4 – identification of the pools are the same for the remaining results.
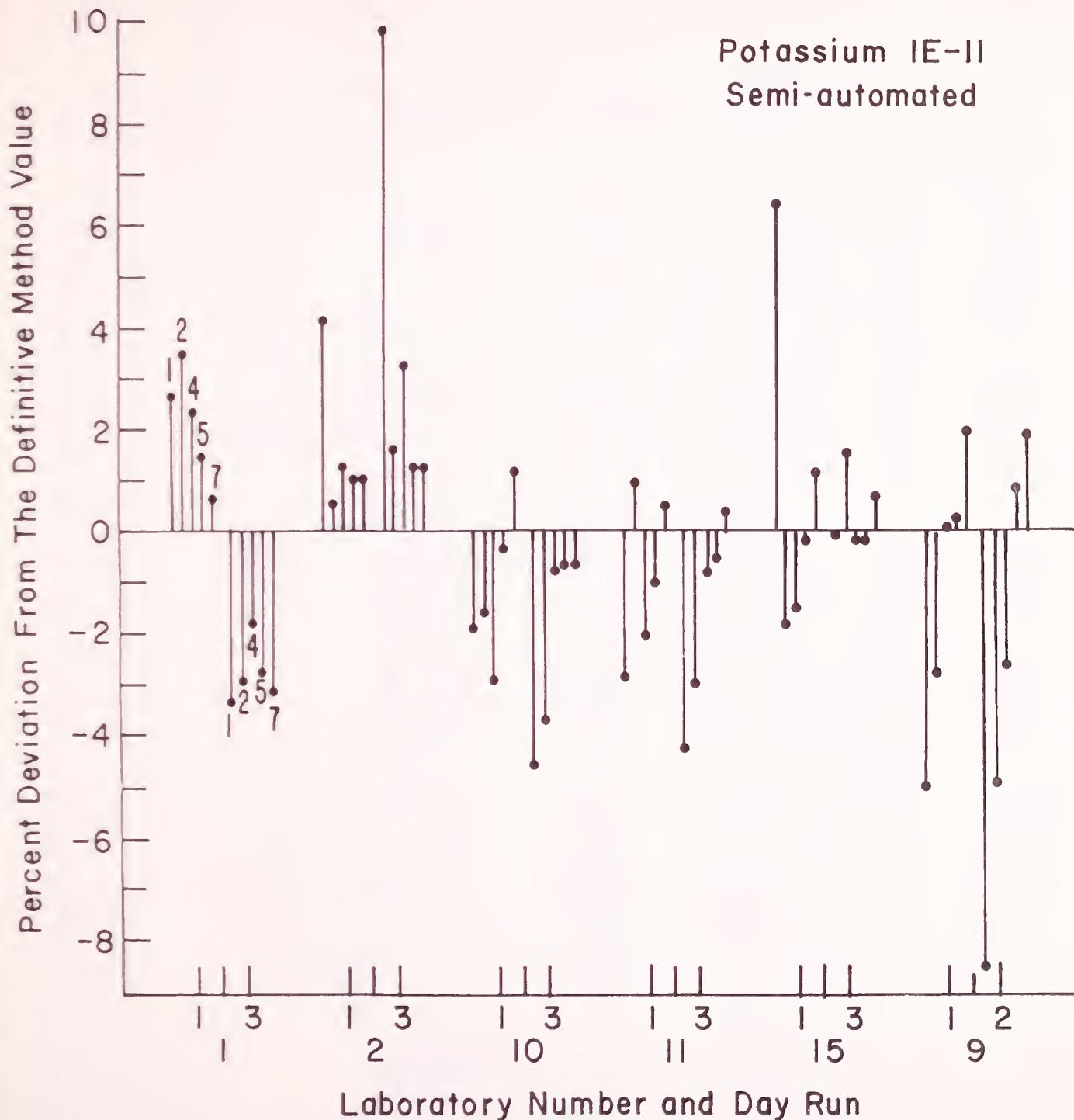
23
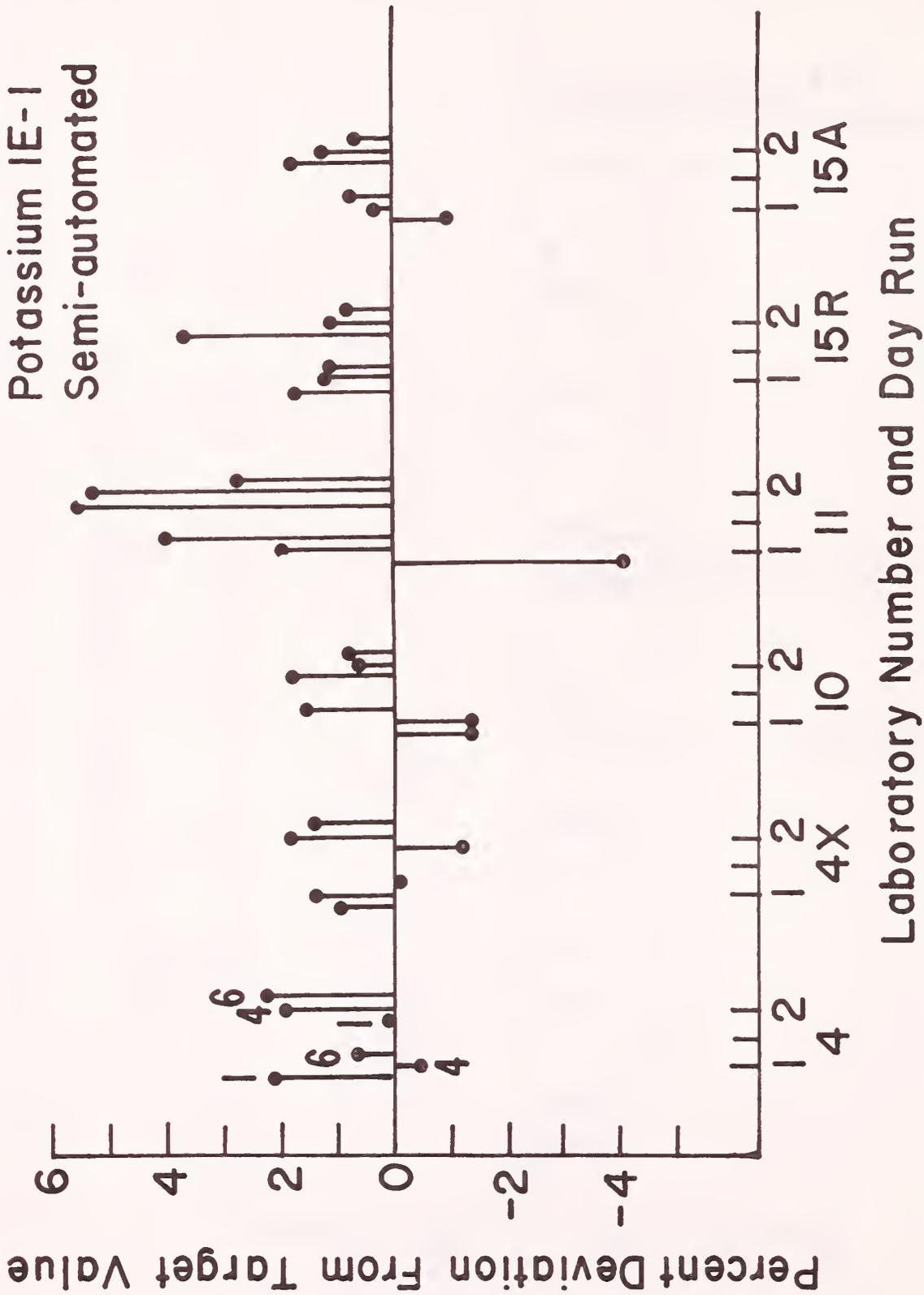
Task 1b, Figure 4b. The percent deviations of the potassium reference method measurements from the ID-MS assigned values, obtained by the semi-automated pipetting procedure for IE-1. The pools are identified by the numbers 1, 4, and 6 near the results for laboratory 4 — identification of the pools are the same for the remaining results.

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for IE-II are presented in Task 1b, Figures 5a and 5b in percent deviations from the definitive method values. After discussion of these results with the Experts Committee and statisticians, a draft of the final report as an NBS 260 Special Publication has been started.

E.   Chloride: Results for IE-II have been statistically analyzed. IE-II involved the analysis of two samples at each of five concentrations (Pools 1, 3, 4, 5, 7) on two different days. The results for the micro (MI) and macro (MA) pipetting procedures are summarized as averages in Task 1b, Table 5. The usual pattern of procedure, lab number, and day-run identifications is used. The results are presented also as percent deviations from the ID-MS values in Task 1b, Figure 6. Statistical analysis showed that their precision and their bias from the ID-MS values were comparable to those obtained in RRI.

    At the Experts Committee Meeting it was decided that the results from IE-II met the required imprecision and bias goals. A draft of a final report on an NBS Special Publication 260 was started.

Task 1b, Figure 5a. Percent deviations of the IE-II measurements using manual pipetting from the definitive method values. The analyzed pools are identified by the numbers 1, 2, 4, 5, and 7 near the results from laboratory 1. The designations are similar for the remaining results. The numbers 1 and 3 placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

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Task 1b, Figure 5b. Percent deviations of the IE-II measurements using semi-automated pipetting from the definitive method values. The analyzed pools are identified by the numbers 1, 2, 4, 5, and 7 near the results from laboratory 1. The designations are similar for the remaining results. The numbers 1 and 3 placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

Task 1b, Table 5.   Averages of Chloride Concentration.
Cooperating Laboratories' Results for IE-II.

Procedure		Chloride, mmol/L				
		Pool				
Lab	Day	1	3	4	5	7
MI	11	79.790	94.735	102.515	108.520	117.475
MI	12	80.230	94.510	102.755	108.035	117.460
MI	21	76.875	91.150	100.650	105.760	115.000
MI	22	77.150	92.380	99.950	105.310	114.600
MI	41	79.660	94.580	101.860	107.820	117.755
MI	42	79.580	95.050	102.290	107.955	118.445
MI	71	78.980	94.890	100.980	107.105	117.585
MI	72	79.905	94.485	101.910	107.440	116.805
MI	81	78.575	94.645	101.660	106.800	116.905
MI	82	79.015	94.200	101.685	107.235	115.975
MI	91	79.850	94.465	101.000	106.880	114.015
MI	92	79.480	93.935	100.570	106.185	116.515
MI	101	78.440	94.265	100.100	106.050	114.800
MI	102	78.950	93.365	100.955	106.715	116.970
MI	131	79.850	94.835	102.350	107.150	116.850
MI	132	79.650	95.000	101.950	106.765	117.100
MI	141	80.850	96.550	104.950	109.750	118.100
MI	142	79.850	94.700	102.050	107.400	116.900
MI	151	79.500	94.675	101.960	107.495	116.905
MI	152	79.450	94.745	101.990	107.315	116.925
MA	51	79.100	94.440	100.670	106.660	115.860
MA	52	79.065	93.805	101.810	106.410	116.670
MA	61	78.435	94.870	101.500	106.335	117.040
MA	62	78.430	93.545	101.485	106.345	116.235
MA	111	79.360	94.310	102.345	107.075	115.165
MA	112	79.855	94.675	101.990	107.820	116.790
ID-MS Values		79.20	94.00	101.8	107.20	116.80

Chloride IE-II

Micro and Macro Samples
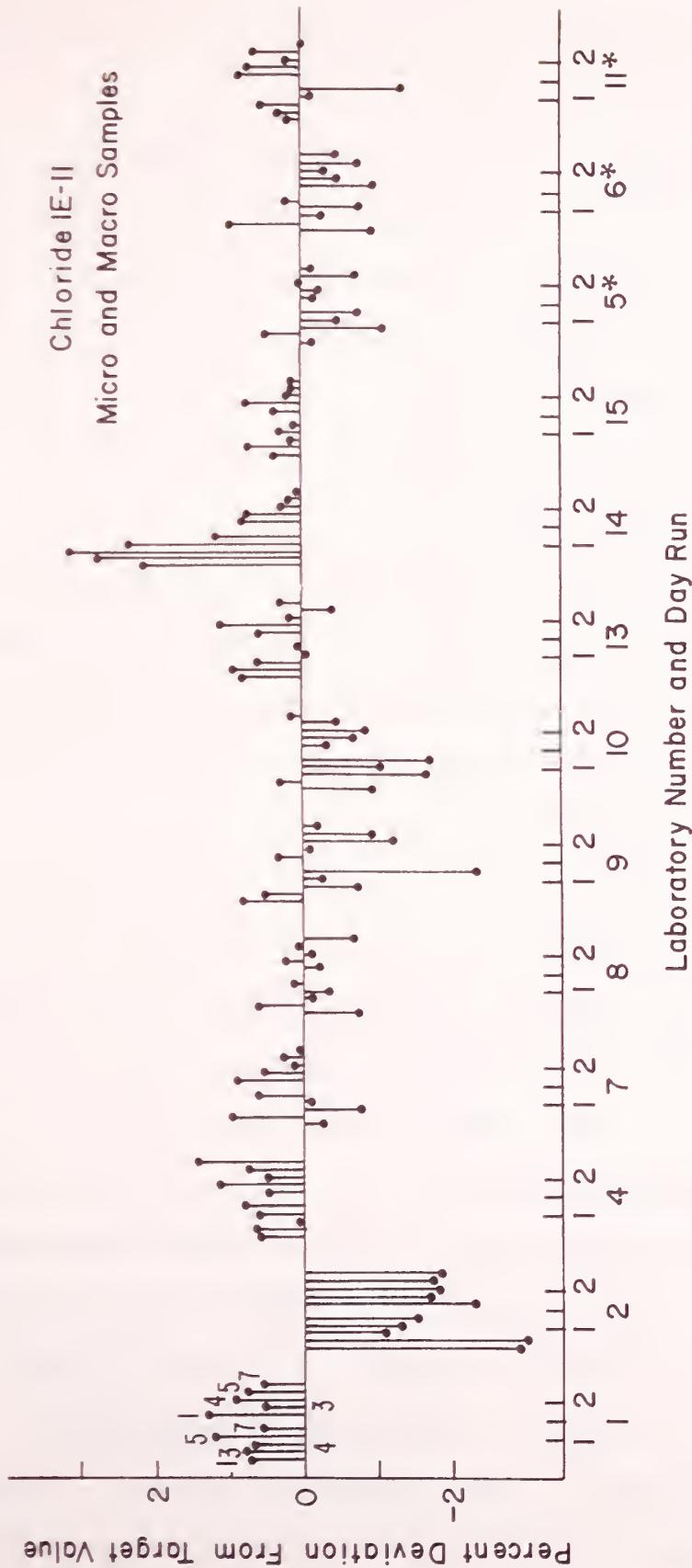
Task 1b, Figure 6. The percent deviations of the chloride reference method measurements from the ID-MS assigned values, obtained in IE-II. The pools are identified by the numbers 1, 3, 4, 5, and 7 near the results from laboratory 1 — identification of the pools are the same for the remaining laboratories' results. The laboratories with the asterisks (5, 6, 11) performed the analyses using 'macro' sampling techniques while the other labs used the 'micro' sampling technique.

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Task 1c. LEAD (Pb) IN BLOOD

A.   Interlaboratory Exercise IV

Based on the fact that two-thirds of the analyses in IE-IV
(June 1975-December 1975) were within 20 percent of the ID-MS
target value, we planned another Interlaboratory Exercise so
that laboratories could use a newly revised protocol. This
revised protocol was sent to the Food and Drug Administration
and all participating laboratories in April 1976.

B.   Interlaboratory Exercise V

Porcine blood, obtained from the USDA, Beltsville, MD,
was processed into three pools for IE-V. In June 1976, each
laboratory received three samples of porcine blood containing
Pb at low, medium, and high concentrations. Results of IE-V
are shown in Task 1c, Tables 1 and 2.

In summary, the results were disappointing, unexpected,
and outside the accepted goal for analytical accuracy and
precision. Statistical analysis of the results of IE-V were
presented and discussed at the Experts Committee Meeting
at NBS on September 17, 1976. Topics discussed were 1) sources
of error and differences between labs, 2) digestion procedures
3) background correction procedures, and, 4) other possible
reference methods. Anodic stripping voltammetry was considered,
but was rejected as a reference method. A graphite furnace
method, developed by T. Rains, at NBS, was discussed as a
potential reference method. Other committee members offered
modifications of other methods that they hoped to try to per-
fect. Minutes of that meeting were sent to FDA.

Task 1c, Table 1. Measurements of Lead in IE-V. Percent
Deviation from Target Value

Lab	Low	Medium	High
A	234	51	74
B	8	15	2
C	4	-12	-5
D	78	41	40
E	38	10	3
F	38	15	13
G	50	22	27
H	27	36	56
Average	60	22	26

Task 1c, Table 2. Measurements of Lead in IE-V. Results in
μg/mL

	Low	Medium	High
ID-MS Target Values	.1738	.4184	.4946
Mean values from laboratories using protocol (±1 SD), n = 8	.32±.21	.54±.14	.62±.11
Mean values from laboratories using "other methods", n = 4	.18±.02	.40±.04	.50±.02

Although additional samples of porcine blood were sent to several laboratories in December 1976, for use in developing better reproducibility and accuracy in their atomic absorption procedures, only two laboratories reported their findings. One laboratory offered improvements in the prior protocol; the other gave instructions for wet digestion of blood.

C.   Preparations for Use of Alternative Procedure

After discussions with committee members, it was decided to explore the use of the graphite furnace procedure that was suggested by Rains as a reference method. Initial committee reluctance to consider the Rains' procedure as a reference method rested on the fact that it would employ an instrument that was available from one manufacturer only. However, several instrument manufacturers now offer suitable equipment for use with the procedure. Several additional laboratories, equipped with graphite furnaces, are to be added to the list of participants and an Interlaboratory Exercise using the Rains' procedure was planned.

A new batch of porcine blood was processed during May and June of 1977 to provide 9 pools of 100 vials of blood. The Pb concentration of the pools ranged from 4 to 90 μg/mL. In addition, a single pool of 500 vials was prepared with a Pb level of 35 μg/mL.

Task 1d.   URIC ACID IN SERUM

A.   Description of the Definitive Method

In our ID-MS method, $[^{15}N_2]$ uric acid is added in a 7- to 10-fold proportion to the natural uric acid in the sample. The size of aliquot of serum used for analysis is adjusted according to its approximate uric acid content so that, after adding the labeled uric acid, a total of about 2 mg of uric acid is present in the sample.

In performing the method, a Li_2CO_3 solution of the labeled uric acid is added to a weighed aliquot of serum; triethylanilinium carbonate is then added. The solution is freeze-dried, and the residue obtained is extracted with methanol. The extract is concentrated and in a sublimation apparatus the residue is heated to 150 °C for five minutes under a pressure of ∿130 N/m^2 (1 mm of Hg on a vacuum gauge) so that the volatile products, which include the tetraethyl uric acid isomers, collect on the cold condenser surface. The two tetraethyl uric acid isomers that predominate in yield (several tetraethyl uric acid isomers form) are isolated from the sublimate by TLC. Although for determining the uric acid content of a serum sample only one of these tetraethyl uric acid isomers needs to be analyzed, the two are measured to enhance the validity of measurement.

For use as standards, mixtures of weighed quantities of SRM uric acid and isotope-labeled uric acid are ethylated under simi-lar conditions, and the same two isomeric products are isolated. Bracketing is used. The corresponding isomeric products from

the serum and the standard mixtures are compared by ID-MS for assigning values. This is necessary because the two isomers obtained on ethylating a standard mixture of uric acid do not have an identical non-labeled to labeled relative molecular ion intensity. (This isotope effect results primarily from the different rates of ethylation of the ^{15}N and ^{14}N atoms located at the same structural positions of the otherwise identical labeled and unlabeled uric acid molecules.)

B.   Initial Results by the Definitive Method

ID-MS uric acid results on five serum pools are shown in Task 1d, Table 1. The values for the two individual isomers isolated from the first sample of pool 1 are labeled as 1A and 1C, from the second sample as 2A and 2C, etc. Task 1d, Table 2 is a summary of the analytical results of ID-MS measurements and early interlaboratory measurements by the candidate refer- ence method. The same samples were analyzed by both methods. The uric acid ID-MS values shown here are somewhat different from the values that appear in the minutes of this Study Group. The data had been submitted to the Study Group in the units of mg/100 g of serum, and were correlated with the preliminary interlaboratory data without correction for the specific gravity of the sera. This error has not been brought to the attention of the Study Group, since it is a relatively minor one. Further, we have discovered another minor source of error that probably affected the results as well. The scaler, a part of the ID-MS data handling instrumentation, was used at the time

Task 1d, Table 1. Uric Acid in Five Serum Pools by ID-MS.
Averages in mg/L

Analysis and Isomer	Pool				
	U1	C1	U2	U3	C2
1A	20.68*	43.41	59.90	80.45	101.03
1C	20.82	43.40	60.34	81.30	101.22
2A	20.82	44.38	59.78	81.02	101.02
2C	20.70	44.27	60.47	81.94	101.67
3A			60.35	81.30	
3C			61.08	81.86	
Average	20.76	43.86	60.32	81.31	101.24

_____

*Each value listed is an average of between 6 and 12 measurements.

Task 1d, Table 2. Summary of Uric Acid Results Obtained by ID-MS and the UV-Uricase Methods (mg/dL)

		Pools				
		C1	C2	U1	U2	U3
ID-MS	$\overline{X}$	4.386	10.124	2.076	6.032	8.131
	S	0.053	0.03	.008	.046	.055
	CV,%	1.21	0.30	0.4	0.76	0.68
UV-Uricase	$\overline{X}$	4.2	9.8	2.1	6.0	8.0
at CDC Lab.	S	0.16	0.29	0.14	0.18	0.23
	CV,%	3.8	2.9	6.7	3.0	2.9
UV-Uricase	$\overline{X}$	4.3	9.9	2.1	6.0	8.0
IE-I	S	0.25	0.25	0.27	0.20	0.20
	CV,%	5.8	2.5	12.8	3.3	2.5
UV-Uricase	$\overline{X}$	4.2	9.5	2.0	5.9	7.9
IE-II	S	0.3	0.4	0.2	0.2	0.3
	CV,%	7.1	4.2	10.0	3.4	3.8

with an improper setting. This would cause an inaccuracy in the absolute ratios observed for ratios of labeled to non-labeled species that are far removed from 1:1, and we had used ratios between 7 to 1 and 10 to 1. However, bracketing would undoubtedly have reduced the error. Hence, we judge the error in our results, due to the defective scaler, to have been small.

C. Study Group Meetings

The Study Group Meeting held on July 23, 1976, dealt with the data from IE-II. Attention was given: (a) to the quality of the interlaboratory data that were obtained on the standard solutions by direct spectrophotometry, and by assay for cali-bration and control and test samples; and, (b) to some of the methodologic details. The review was directed mainly at eval-uating the criteria by which each laboratory can judge itself while performing the reference method.

The June 27, 1977, meeting was a review of IE-III results which were run on the same serum pools as had been previously analyzed by the candidate reference method. The difference for IE-III was in the use of reagents that the laboratories purchased independently and prepared for them-selves. The data showed that interlaboratory precision was not adversely affected. However, data from some of the lab-oratories did not meet all of the criteria set for the method; as a consequence, such data were excluded from the evaluation. IE-IV, the final testing of the candidate reference method, was planned. New serum pools will be involved. Samples of the

pools have been sent to NBS for ID-MS analysis. The prepara-
tory chemical work-up of these samples was completed at NBS
before the close of the project reporting period, but the mass
spectroscopy runs were not. At the time of this writing, the
GC/MS is about to be started.

Task 1e. CHOLESTEROL

A.   Cholesterol-d$_7$ for the Definitive Method

Cholesterol, containing deuterium in place of hydrogen at all positions on carbon atoms 26, 27, and 28, was obtained from Applied Science Laboratories (State College, PA). The preparation received was purified by sublimation and recrystallization. Mass spectrometry then revealed only the presence of a small proportion (~2 percent) of a non-labeled C-25 steroid. (Presumably it is related to the precursor from which the labeled cholesterol was synthesized.) The molecular weight of the impurity was such that it would not interfere with the mass spectrometry. Its presence in the labeled cholesterol remained a source of concern to us only in that it might not be uniformly distributed, and, hence to that extent, could affect the proportions of cholesterol in quantities taken from the supply of the crystalline labeled material. Since removal of this impurity from the labeled cholesterol was not considered feasible, only one crop of this recrystallized, labeled cholesterol was collected for use in ID-MS analyses, with the assumption that in the single crystalline crop the impurity would be homogeneously distributed and therefore without effect on the analyses. Further, the use of bracketing with ID-MS analysis, where a single preparation of labeled cholesterol is employed in both the unknown samples and the standards, would obviate the effects of the presence of an impurity unless the impurity both chromatographs like the analyte and gives ions in the mass spectrometer with the same

mass to charge ratio as are used for measuring the labeled
or unlabeled analyte.

B.   Presentation of the Definitive Method at NBS Symposium

A paper prepared for presentation at NBS on 10 April 1978,
as part of a symposium on trace organic analysis, summarizes
the status of the work on this definitive method as of the
project reporting period. A copy of this paper, "A Candidate
Definitive Method for the Determination of Total Cholesterol
in Serum" was previously submitted to the Food and Drug
Administration.

C.   Comparison of Definitive Method Analyses with ID-MS

Values Obtained at the Karolinska Institute

Since the close of the project reporting period, Schaffer
met with Dr. Ingemar Björkhem of the Karolinska Institute (KI)
in Stockholm and suggested that the cholesterol ID-MS method
in use at the KI laboratory be used to analyze the serum pools
that had been run by the ID-MS method at NBS. That has now
been done. The method used in Stockholm [I. Björkhem,
R. Blomstrand, and I. Svensson, "Determination of Serum
Cholesterol by Mass Fragmentography, Chim. Chim. Acta., 54,
185 (1974)] provides for GC/MS analysis of the isolated
cholesterol directly rather than analysis of the TMS ether
derivative as is done at NBS. The results of the two labora-
tories are compared in Task 1e, Table 1. The results from
the two laboratories differed by from 0.8 to 2.3 percent, and
all the values from the Karolinska Institute were lower than

Task 1e, Table 1.   Comparison of Results from Two Laboratories
doing ID-MS Cholesterol Measurements

	KI	NBS	NBS/KI
Serum Pool	mean ± S.E.M. (mmol/L)	mean (mmol/L)	ratio
I	3.394 ± 0.039	3.430	1.011
II	4.679 ± 0.076	4.719	1.009
III	6.101 ± 0.039	6.149	1.008
IV	7.346 ± 0.047	7.456	1.015
V	8.588 ± 0.066	8.788	1.023

the NBS values. (The Karolinska Institute group found an error
in pipeting aliquots of Serum Pool V and suggested that its
difference from our value should be more like the others.)
Because of the systematic difference between their results and
ours, we investigated their standard reference material, a
cholesterol preparation that they had purified themselves. A
problem with their standard (or even possibly with our choles-
terol, SRM 911a) could account for the more or less regularly
observed differences between the results. By analysis, we
found that their cholesterol standard was only 98.2 percent
pure. We determined this value by the ID-MS method with our
labeled cholesterol used in a series of weighed mixtures with
their standard material and in a similar series with SRM 911a.
Furthermore, we found the impurity in their cholesterol to be
lathosterol, a steroid that has the same mass as cholesterol.

By electron-impact mass spectrometry, lathosterol gives its molecular ion in relatively greater abundance than does cholesterol. There can be little question as to the origin of the systematic error, or that the systematic error occurred in the Karolinska Institute's analyses.

D.   Study Group Meetings

At meetings of the Study Group held 10 November 1975 and 20 July 1976, the several clinical methods for the determination of total cholesterol that had been recognized as potential candidates for the reference method were reviewed. Work done at the CDC on the semi-automated Abell-Kendall method and the cholesterol esterase/cholesterol oxidase enzymatic method was reported. The Study Group recommended a continued study of those methods. The GLC methods in Dr. Kuksis' and Dr. Martin's laboratories were also reported; however, neither laboratory was attempting to upgrade its method to be of reference method quality. The Parekh-Jung method in Dr. Edward's laboratory also continued to provide very good results. The Study Group decided to await the outcome of the studies at CDC, rather than encourage further intensive study of any of these other methods. The remaining problems with the optimization of the enzymatic method and with cholesterol solution standards used with that method were to be studied, as were the causes of small unexplained drifts in the Abell-Kendall method. Also, a comparison of these optimized methods with the ID-MS method was to be made before the next meeting was to take place.

In order to facilitate comparison of the data obtained by the several methods with ID-MS data, NBS at first provided CDC with an estimate of the overall imprecision of the ID-MS results. Thereafter, actually in October 1977, the CDC and NBS exchanged results. It was then seen that a systematic difference of one to two percent existed between the ID-MS values and the Abell-Kendall values. Differences between the ID-MS and the enzymatic methods were even larger. Cooper and Schaffer discussed whether to bring these comparative results before the Study Group at once, but Cooper declined to do this until he had all of his data assembled. Meanwhile, with knowledge of the ID-MS results, Cooper undertook to investigate further sources of bias in the methods being used at the CDC.

Task 1f.   SERUM IRON

A.   Definitive Method

In order to use ID-MS to provide high accuracy iron analysis at the concentration levels present in serum, it was necessary to minimize the possible contamination that could occur during the wet-chemical processing of samples. In the class-100, filtered-air, clean room where the chemical work would be performed, it was necessary to replace all the steel laboratory furniture and the many implements present made with iron. The specially purified reagents used for this ID-MS analysis were meticulously monitored for iron.

It was recognized at the start that the ID-MS method as applied would provide total iron measurements, which includes hemoglobin iron, and not just serum iron (or more specifically, transferrin-bound iron) as the reference method is expected to do. The Study Group thought it would be possible to correct the total iron values by measuring the hemoglobin in the samples. However, the hemoglobin content needed to be measured very accurately in order to apply meaningful corrections. Since total iron can be measured by ID-MS with an estimated inaccuracy of 0.4 percent and 1.0 mg of hemoglobin corresponds to 3.47 µg of iron, a several milligram or larger error in the estimation of hemoglobin would produce a significant loss of attained accuracy in the analysis of a sample with a total iron content of, for example, 1000 µg/L.

Although the direct determination of serum iron by ID-MS has not been attempted, the direct analysis may be a better approach since it obviates the need to correct for the over-estimation of iron. For direct analysis, the serum and the isotope enriched iron would be combined and the mixture would be treated with a complexing agent to liberate all the iron (except the hemoglobin iron) from its binding sites. Then this ionic iron would be reduced to Fe II to ensure equilibration of the isotopic forms. Finally, the protein precipitation would be carried out. The isotope ratio measurements would be performed on the iron isolated from the supernatant liquid.

B. Study Group Meetings

Our attempt to organize an experts committe to develop a reference method for serum iron was merged with a similar effort of the AACC Standards Committee which was being organized at that time. Dr. Eleanor Berman had been charged with the responsibility for organizing a study group for the Standards Committee. Dr. Richard Carter was asked to head the Study Group. Dr. Schaffer arranged to fund the following for participating in this study:

Dr. Eleanor Berman, Cook County Hospital, Chicago, IL

Dr. George N. Bowers, Jr., Hartford Hospital, Hartford, CT

Dr. Thomas J. Giovanniello, Veterans Administration
    Hospital, Boston, MA

Dr. Philip J. Garry, University of New Mexico,
    Albuquerque, NM

Dr. Theodore Peters, The Mary Imogene Bassett Hospital,
    Cooperstown, NY

Dr. Robert Carter, MD, Bowman-Gray School of Medicine,
    Winston-Salem, NC

Other members of the Study Group were:

Dr. Richard Carter, CDC, Atlanta, GA

Dr. I. Lynus Barnes, NBS

Dr. R. Schaffer, NBS

The Study Group met for the first time on 16 September 1976 and again 19 July 1977, during the project reporting period. Subsequent meetings were held 28 October 1977 and 28 May 1978.

Although a considerable body of information already existed on optimum methods for the determination of serum iron (see "Modern Concepts in Hematology", G. Izak and S. M. Lewis, Eds., Academic Press, NY, 1972, pp. 69-129), the Study Group considered it worthwhile to test various methods in common use as well as the method tentatively recommended by the International Committee for the Standardization of Hematology (ICSH), which is given on pages 126-7 of the reference just cited, and to test minor variations of the methods thought to be of possible benefit. The methods were performed using samples prepared at the CDC, some of which (deliberately) contained substances that might interfere with iron analysis. These analyses were to be done with a standard for iron to be supplied by NBS and with newer and possibly better spectrophotometers than previously were available, and thus the results were expected to afford some fresh insights.

NBS would also provide total-iron, ID-MS measurements on the same samples. Hemoglobin would be measured in laboratories other than NBS.

High purity electrolytic iron was judged most appropriate for the iron SRM, and samples of an electrolytic iron preparation of high purity were sent to the members of the Study Group for use with their iron determinations. By the 28 October 1977 meeting, the quality of the results obtained in the inter-laboratory study of the test sera that CDC prepared led the Study Group to decide to concentrate its work on testing a minor modification of a tentatively proposed ICSH reference method. Consequently, the same serum pools were reanalyzed, and at the conclusion of that round of study some minor changes in the ICSH procedure were discussed. Meanwhile, the ICSH published an approved method for the determination of serum iron [Br. J. Hematol. 38, 291 (1978)]. Nevertheless, some of the procedural details still seemed worth further study, and they are still being done.

Task 1g. BILIRUBIN IN SERUM

A. <u>Isotope-labeled Bilirubin for Use in a Definitive Method</u>

An ID-MS method that might be developed for bilirubin was
considered the best available route to a definitive method.
Bilirubin with isotopically tagged atoms attached to the first
two and also the last two of the linearly linked, tetrapyrrole
rings appeared to be the type of isotope-labeled material needed
for our purpose. It would leave open the option of analyzing
for bilirubin by use of dipyrrole fragments or analyzing for the
intact molecule. Such a multiply-labeled bilirubin might be
prepared by biosynthesis (through feeding experiments) with a
$2,2,3,3-d_4-5$-aminolevulinic acid as a precursor. The bilirubin
produced would then have deuterium atoms in the two propionic
acid side chains in positions where the labeled atoms could be
expected to be stable. The labeled precursor (also labeled
bilirubin) was not commercially available. We tried to syn-
thesize it by an alkaline-D_2O treatment of 5-aminolevulinic
acid, but this was unsuccessful. A much more involved synthetic
procedure will be needed to produce this intermediate, but work
on it was deferred.

B. Study Group Meetings

Our interest in a bilirubin reference method complemented
that of an AACC Standards Committee Study Group being set up for
the same purpose. Dr. Basil Doumas (Medical College of Wisconsin,
Milwaukee) was the head of the small group composed of

Dr. R. Carter (CDC), Dr. Robert McComb (Hartford Hospital, Hartford, CT) and Dr. R. Schaffer (NBS).

By use of a carefully prescribed procedure for the Jendrassik-Grof analysis for bilirubin, very high interlaboratory precision was obtained; however, a wide-ranging study was then carried out of potential metal ion interferences, and the results, which revealed a previously unknown but significant absorptivity-enhancing effect of zinc ions, may require some change in the reagents for the Jendrassik-Grof method.

Before working on these changes, the group is awaiting the results of some very important experiments now underway in the laboratory of Dr. Harry Pardue (Purdue University, Lafayette, IN) on the so-called bilirubin glucuronides. (These are esters of bilirubin and properly referred to as glucuronyl bilirubinates.) These experiments are expected to provide previously inaccessible information because these bilirubin esters had not been isolated in sufficient quantity and carefully studied previously. Thus, the molar absorptivity of bilirubin, which is important for the direct measurements of bilirubin and the nature of the products formed in the Jendrassik-Grof assay, may finally become known. The Jendrassik-Grof method is standardized with bilirubin; one therefore assumes that the glucuronic acid residues are cleaved in forming the diazotized dipyrrole reaction products or, if they remain attached, that the products have the same molar absorptivity as the products of the reaction which do not have the groups. These answers will determine the future work

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of the Study Group and may impact on the possibility of developing a definitive method.

Task 1h. UREA

A.   Preliminary Work on a Definitive Method

During our search for a suitable procedure for performing
ID-MS analyses for urea, a paper on this subject appeared in
Clin. Chim. Acta, 71, 199 (1976) by I. Björkhem et al on the
"Determination of Serum Urea by Mass Fragmentometry". In this
method, urea is treated with diallyl-malonic acid diethyl ester
to form 5,5-diallyl-barbituric acid which is then converted
into the dimethyl ester for analysis by ID-MS. The authors
reported a 3.6 percent RSD for the method based on duplicate
analyses of 30 serum samples, and analytical recoveries were
as low as 97 percent. The disturbing fact in this method is
their use of a urea derivative that is a known medicinal with
no provision for correcting for that as a potential source of
error. We did not follow up that method.

In considering the use of a derivative form of urea for
our work, we explored the possible use of the reaction of
urea with diacetyl and with xanthydrol. However, our study
of the reaction of urea with diacetyl and diacetylmonoxime
was not productive. Other diketones were also considered but
these too did not prove useful. Reaction with xanthydrol
[R. Fosse, Compt. rend. 158, 1076, 1588 (1914); ibid, 159,
253 (1914)] showed more promise. The product obtained from
the reaction of two molecules of xanthydrol and one of urea,
N,N´-di-9H-xanthen-9-ylurea, is readily separable and yields
are quite high. But it was difficult to work with this compound

because of its extreme insolubility in a variety of solvents. When we found it possible to isolate urea itself from serum, and use it directly for the ID-MS analysis, further attention to urea derivatives was abandoned.

B.   Description of the Definitive Method

Urea was found to be separable from lyophilized serum by an alcohol extraction. This was followed by a sublimation of the dried extract, and the urea thus separated could be further purified by extraction. The method involves the following steps: addition of stable-isotope labeled urea to a serum sample; lyophilization; extraction of the residue with methanol; concentration of the extract and sublimation; dissolution of the sublimate in water for removal of nonpolar impurities by extraction with $CHCl_3$. Crystalline urea is obtained on evaporation of the water phase. Mass spectrometry is performed after a direct probe insertion of the urea sample into the mass spectrometer.

As a trial of the method, four serum samples were analyzed in duplicate. The bracketing technique with standard mixtures of SRM urea and labeled urea, was used for converting observed intensity ratios for the labeled and unlabeled urea into weight ratios in the sera. The values found by ID-MS as compared to the expected values (i.e., the labeled values given on the vials, supplied by the CDC) in mg/L are 340 vs 346; 470 vs 475; 600 vs 589; and 970 vs 975. Given this degree of agreement, further work with the ID-MS method was deferred until the

serum pools to be used for the round robin studies would be
ready for analysis.

C.   Study Group Meetings

The organization of the reference method work was dove-
tailed with a counterpart effort being set up under the Stan-
dards Committee of the American Association for Clinical
Chemistry. To a large extent the identical clinical laboratory
resources would have been called upon if there were two separate
efforts. Dr. N. Gochman (Veterans Administration Hospital,
San Diego) had been appointed by the Standards Committee of the
AACC to head the effort to develop reference methods for nitrogen-
containing compounds. Schaffer arranged to fund the following
laboratories for participation. From these laboratories the
Study Group was formed. The members are:

Dr. G. N. Bowers, Jr. (Hartford Hospital)

Dr. N. Gochman (VA Hospital, San Diego)

Dr. G. Kessler (The Jewish Hospital, St. Louis)

Dr. R. E. Sterling (U.S.C. Medical Center, Los Angeles)

Dr. R. Vanderlinde (N.Y. State Department of Health)

Dr. C. F. Willis (Cleveland Clinic)

Dr. D. L. Witte (U. of Iowa)

Other participants, not funded:

Dr. C. Burtis (CDC)

Mr. P. Duncan (CDC)

Dr. G. Ertingshausen (Union Carbide)

Dr. F. Ibbott (Bio-Science, Los Angeles)

Mr. W. Ryan (Beckman Inst., Fullerton, CA)

Dr. A. H. Smith (DuPont)

At the first meeting of the Urea Study Group, held on 22 September 1975, some of the desired general characteristics of the reference method were discussed:

1. The reference method would employ high-quality, general purpose equipment for which written specifications could be provided.

2. The method would use reagents that are generally available and for which written specifications could be provided.

3. The method would be applicable to serum.

4. Interferences would be tested by adding potential interferences to serum base.

5. Precision should be 0.25 percent RSD in the normal range.

6. Bias should be within 3 percent of the definitive method.

7. Uncertainty of the definitive method should be within 1 percent of the True Value.

The Study Group agreed that two methods should be studied intensively: manual diacetyl monoxime method and a urease/ glutamate dehydrogenase (GlDH) end-point method. CDC volunteered to work to optimize these two methods, compare them with other methods, and examine them for potential interferences.

Dr. Burtis was asked to serve as Study Group Chairman.

The second meeting of the Study Group occurred on 22 July 1977. Because of other duties assigned to Dr. Burtis, Dr. Witte took over as Chairman.

Dr. E. Sampson (CDC) presented a report on the urease/GlDH method. The optimization of this method appeared to be complete and was accepted as complete by the Study Group. Very little was done with the diacetylmonoxime method for the reason that it seemed unwarrented to devote much energy to a method involving basic chemistry that is poorly understood. Dr. R. Thibert (U. of Windsor, Ontario) attended the meeting to discuss the chemistry of the diacetyl monoxime method, which he is actively studying. The consensus after this discussion was to relegate that method to a secondary position relative to the enzymic method.

Plans were made to have three of the participants set up an automated version of the enzymic method in their labs to screen abnormal serum samples for interferences. Each lab would use its routine method also. Differences in the two results were expected to give evidence for interfering substances.

ACKNOWLEDGEMENTS

4. TITLE AND SUBTITLE

Quarterly Report FDA Contract No. 74-58(O)

7. AUTHOR(S)
R. Schaffer, R. Velapoldi, and D. Reeder

8. Performing Organ. Report No.

9. PERFORMING ORGANIZATION NAME AND ADDRESS

NATIONAL BUREAU OF STANDARDS
DEPARTMENT OF COMMERCE
WASHINGTON, D.C. 20234

10. Project/Task/Work Unit No.

11. Contract/Grant No.

12. Sponsoring Organization Name and Complete Address *(Street, City, State, ZIP)*

13. Type of Report & Period Covered

14. Sponsoring Agency Code

15. SUPPLEMENTARY NOTES

16. ABSTRACT *(A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here.)*

This report covers tasks performed at NBS between April, 1976 and October, 1977, under FDA contract No74-58(O). Definitive and reference methods are discussed for the following organic constituents in serum: glucose, cholesterol, uric acid, urea and bilirubin. Tables include comparisons of analytical results obtained by the definitive and the reference methods.

Clinical reference methods for selected inorganic constituents of serum are reported. Included are statistical analyses of round robin tests completed for lithium, magnesiu, sodium, potassium,chloride, iron, and lead.

17. KEY WORDS *(six to twelve entries; alphabetical order; capitalize only the first letter of the first key word unless a proper name; separated by semicolons)* Definitive methods; reference methods; round robin testing; statistical analyses.

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