

ORNL-TM-3948

GRAVITY ZERO ($G\emptyset$)
ANALYTICAL CLINICAL LABORATORY SYSTEM
PROGRESS REPORT FOR THE PERIOD
APRIL 15 TO JULY 31, 1972



OAK RIDGE NATIONAL LABORATORY
OPERATED BY UNION CARBIDE CORPORATION • FOR THE U.S. ATOMIC ENERGY COMMISSION

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OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37830
operated by
UNION CARBIDE CORPORATION
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U.S. ATOMIC ENERGY COMMISSION

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GRAVITY ZERO (GØ) ANALYTICAL CLINICAL LABORATORY SYSTEM PROGRESS
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SUMMARY

During this period, we completed the evaluation and testing of the Mark I GØ Analyzer and found it to be capable of providing linear, accurate, and precise photometric signals. Several chemical procedures were evaluated with this machine, and the analytical results were found to be consistent with those obtained with the larger, 15-place GeMSAEC Fast Analyzers. The Mark II GØ Analyzer was designed and fabricated, based on knowledge gained from the Mark I as well as the Miniature GeMSAEC Fast Analyzer.

The Mark II GØ Analyzer is similar to the Mark I version in that each has a 17-place rotor that spins through a stationary optical system. In addition, each occupies approximately 1 ft³ of space, weighs 25 lb, and is compatible with the hardware and software of the existing GeMSAEC computer module. The Mark II differs from the Mark I in that it provides automatic monitoring and control of the rotor temperature, and has an automated photomultiplier control circuit that provides a constant signal from the solution contained in cuvet 1. In addition, improvements have been made in the electronics and in the rotor drive and braking components of the Analyzer.

Initial evaluation of the Mark II and its individual components has indicated satisfactory performance. To evaluate the Mark II GØ Analyzer as a ground-based system, a multifunctional 17-place rotor has been designed and fabrication and assembly techniques required to produce a number of these rotors have been investigated. Also, a device to automatically load discrete aliquots of reagents and samples into these rotors and a washing station to clean them between runs have been designed; both are currently being fabricated.

In regard to data acquisition, evaluation of the Mark I Data Printer was completed and two problem areas were discovered. These were solved by (1) replacing the original logarithmic amplifier with a high-performance amplifier, and (2) adding three more sets of absorbance span and maximum

potentiometers to provide analytical flexibility when operating in the multiple-chemistry: single-sample mode. A small, efficient computer data system is being developed and assembled to improve our efficiency in evaluating the various systems and in developing and adapting chemistries. In addition, computer programs for processing data generated by enzymatic substrate analyses have been developed and evaluated.

Several procedures routinely employed in the clinical chemistry laboratory have been adapted for use in the GØ Analyzers, and protocols have been written for them. Wherever possible, commercially available chemical kits have been utilized. During this report period, we began the final development of the more difficult chemistries and their respective evaluation using the Mark I GØ Analyzer. The chemistries being considered include blood urea nitrogen, glucose, calcium, bilirubin, acid phosphatase, and inorganic phosphate.

1. INTRODUCTION

The GeMSAEC Fast Analyzer is a unique photometric system that utilizes the centrifugal field generated by a spinning, multicuvvet rotor to transfer and mix a parallel series of samples and reagents into their respective cuvetts. The rotary motion of the rotor is further utilized to rapidly move the cuvetts through a stationary optical system. These analyzers have been successfully interfaced with small computers, and the computer software necessary for routine and research operation has been developed. In addition, more than 20 clinical chemical procedures have been developed or adapted for use with Fast Analyzers.

The successful development of the GeMSAEC Fast Analyzers for use in the clinical laboratory has led to consideration of the same principle for a miniature clinical analyzer that would be capable of operating under the zero gravity conditions of space flight. The unique centrifugal mode of sample and reagent handling, coupled with rapid, automated data processing, makes this analytical concept particularly adaptable for space applications. Furthermore, the possibilities for miniaturizing the analyzer and using extremely small quantities of sample and prepackaged

reagents are compatible with the weight and space limitations for space flight application. These features are also useful in ground-based operation, especially in the small clinical laboratory, the emergency laboratory, the pediatric laboratory, mobile laboratories, and other special situations. Consequently, a miniature, automated analytical system, based on the GeMSAEC concept, is being developed in a two-year program.

During the first year of this program (i.e., April 1, 1971, to March 31, 1972) a miniature Fast Analyzer [Fig. 1(a)] was designed and fabricated to determine the feasibility of transferring, mixing, and monitoring small volumes of reagents ($< 100 \mu\text{l}$) and samples ($< 10 \mu\text{l}$).^{1,2} Based on knowledge gained from this miniature analyzer, a Mark I GØ Analyzer prototype [Fig. 1(b)] was designed and fabricated.^{1,3} This analyzer has a 17-place rotor that spins through a stationary photometer consisting of a miniature photomultiplier tube, a quartz-iodine lamp, and interference filters. During operation it is possible to monitor the light transmittance of each cuvet every 100 msec. The Analyzer is an integrated, miniature system that occupies approximately 1 ft^3 of space and has been demonstrated to be completely compatible with the hardware and software of the existing GeMSAEC computer system.

Multicuvet rotors that ultimately will be totally enclosed for easy disposal have been designed and fabricated for use with the GØ Analyzers. The unassembled rotors consist of three parts: a central body made of black acrylic plastic, and two cover windows fabricated from ultraviolet-transmitting (UVT) acrylic plastic. The rotors are assembled by gluing the individual components with an epoxy glue.

Rotors developed to date include multifunctional devices in which samples and reagents can be loaded either discretely or dynamically and specialized rotors which are capable of accepting and processing whole blood samples. The final design will result in disposable rotors, probably fabricated from acrylic plastic, that will contain prepackaged reagents.

The Mark I Data Printer was designed and fabricated as an inexpensive and relatively fail-safe backup to the computerized data system. In this device, the absorbance of the solution contained in each cuvet is printed

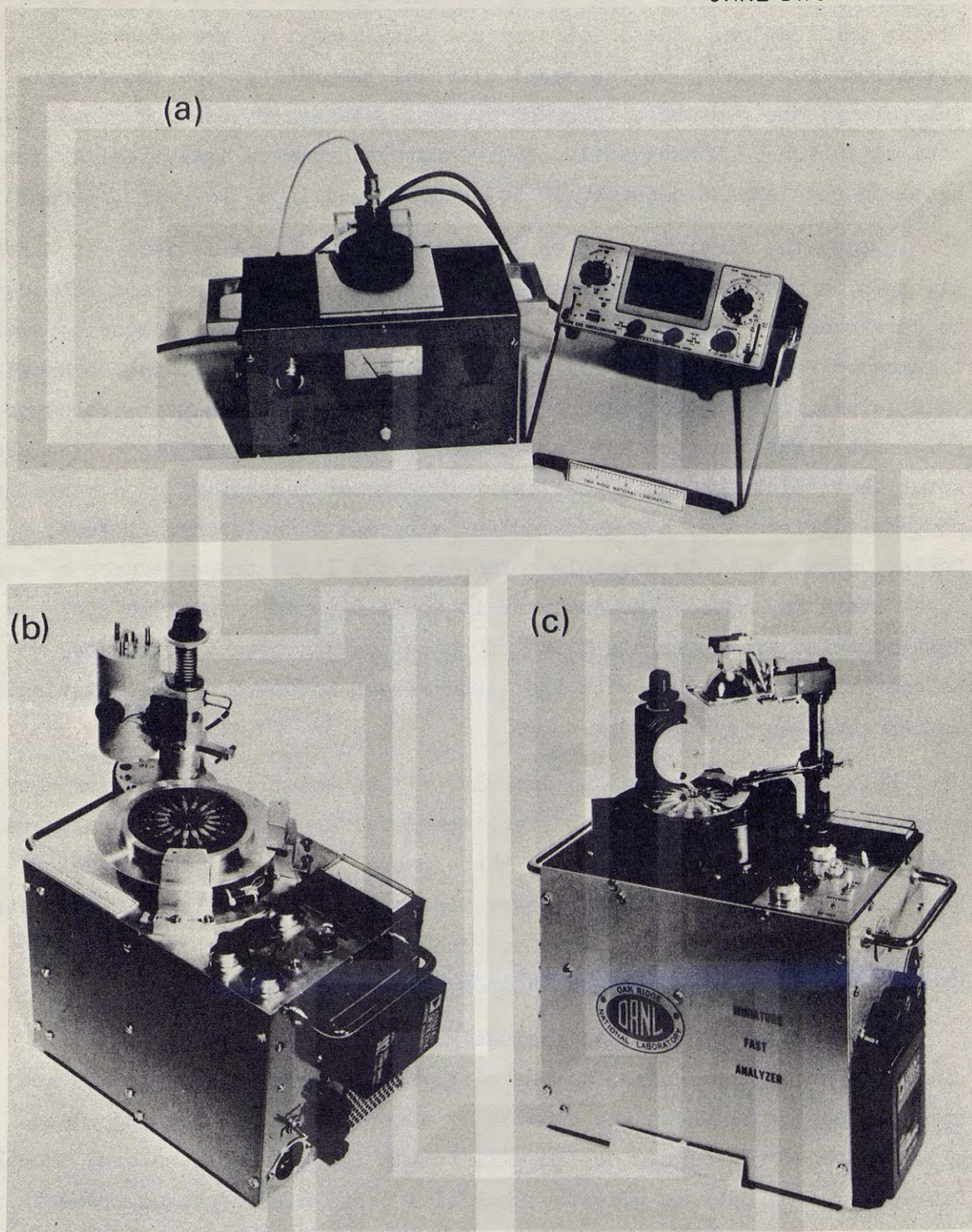


Fig. 1. Composite Photograph Showing Chronological Development of the Three Miniature Fast Analyzer Prototypes. (a) Miniature Fast Analyzer; (b) Mark I GØ Analyzer; (c) Mark II GØ Analyzer.

as a dot on a 4-1/4-by 7-3/8-in. card. By successive scans, a measure of the absorbance change per unit can be obtained; thus, both end-point and kinetic enzyme analyses are possible. Preliminary tests have shown that the device is capable of a linear response over a usable range.

In summary, the progress accomplished in the first year of the program indicated that a miniature analyzer based on the GeMSAEC concept is feasible, and that it is possible to design and fabricate operable, miniaturized systems that will be useful not only in space-flight applications but also in ground-based laboratories. During the second year of the program, the primary objectives will be to design and to fabricate two miniature analyzer systems for evaluation by NASA as ground-based clinical systems. These analyzers will undergo testing in two or more clinical or research laboratories, with the support and consultation of the ORNL development team and NASA personnel. A complete system includes the miniaturized analyzer, several rotors, a portable data printer, an automated sample and reagent loader, and a rotor washing station. The system will also have the capability of on-line computer applications. Accompanying these systems will be several chemical protocols.

During the current report period, the evaluation of the Mark I GØ Analyzer was completed and an advanced system, the Mark II GØ Analyzer, was designed and fabricated. The Mark II GØ Analyzer has provisions for automatic temperature monitoring and control. In addition, studies relative to rotor design and fabrication were continued and the Mark I Data Printer was evaluated and modified. An automated sample and reagent loader and an automated rotor washing station have been designed and are being fabricated, and several chemical procedures have been developed and adapted for use with the GØ Analyzers.

2. ENGINEERING DEVELOPMENT

2.1 Final Testing and Evaluation of the Mark I GØ Analyzer

The Mark I GØ Analyzer^{1,4} was extensively tested and evaluated and found to provide the high degrees of optical performance and analytical accuracy and precision that have been previously demonstrated to be

obtainable with the larger Fast Analyzers.⁵ In addition, the instrument was shown to be capable of performing microchemical analyses that require small volumes of samples (1 to 10 μ l) and reagent (70 to 110 μ l) and of operating in either the multiple-sample: single-chemistry or single-sample: multiple-chemistry analytical mode. As with the larger analyzers, the miniature analyzer can be conveniently interfaced with a computer and/or the Mark I Data Printer. Problem areas associated with the motor drive and braking system and the detector drift have been identified and modifications to solve them, along with a system for automatic monitoring and control of the rotor temperature, are reflected in the design and fabrication of the Mark II G ϕ Analyzer.

2.2 Mark II G ϕ Analyzer

2.2.1 System Description

Based on the information gained from the design, fabrication, and evaluation of other systems, the Mark II Prototype Analyzer [shown in Figs. 1(c) and 2], was designed and fabricated. As with the earlier instruments, the new prototype is compact; it is contained in (and on) a small sheet-metal cabinet (10 x 7 x 8 in.) that occupies only 1 ft³ of space. The entire analyzer weighs only 25 lb and thus may be easily moved and carried by means of the handles located on the side panels of the cabinet. As with the Mark I prototype, the heart of the system is a 17-cuvet plastic rotor, which, when placed in the rotor support housing, is rotated through a stationary optical system at speeds up to 5000 rpm. The rotor, as shown in Fig. 3, is manually placed into the rotor holder and is correctly indexed by mating the two offset pins contained in the rotor holder (Fig. 4) with two matching holes in the rotor. One of these pins contain a thermistor probe (discussed later), which is used to monitor the rotor temperature. Synchronization signals for the computer and analog display scope are provided by moving the synchronization apertures, contained in the rotor housing, through the cuvet and rotor photometric, synchronizing detectors shown in Fig. 2. Synchronization is accomplished by moving each detector along the slide bar until proper adjustment is obtained. At this point, both detectors are physically secured to the top

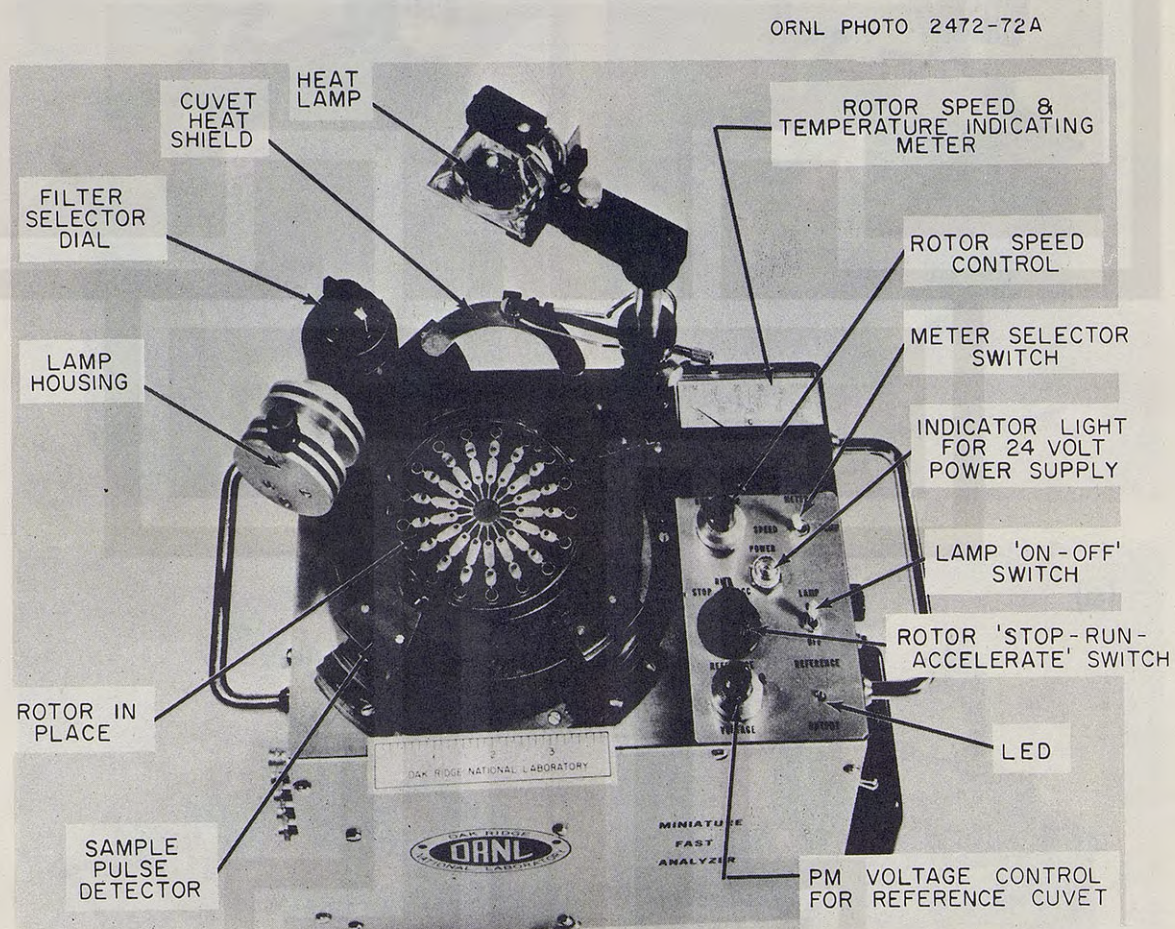


Fig. 2. Top View of Mark II GØ Analyzer.

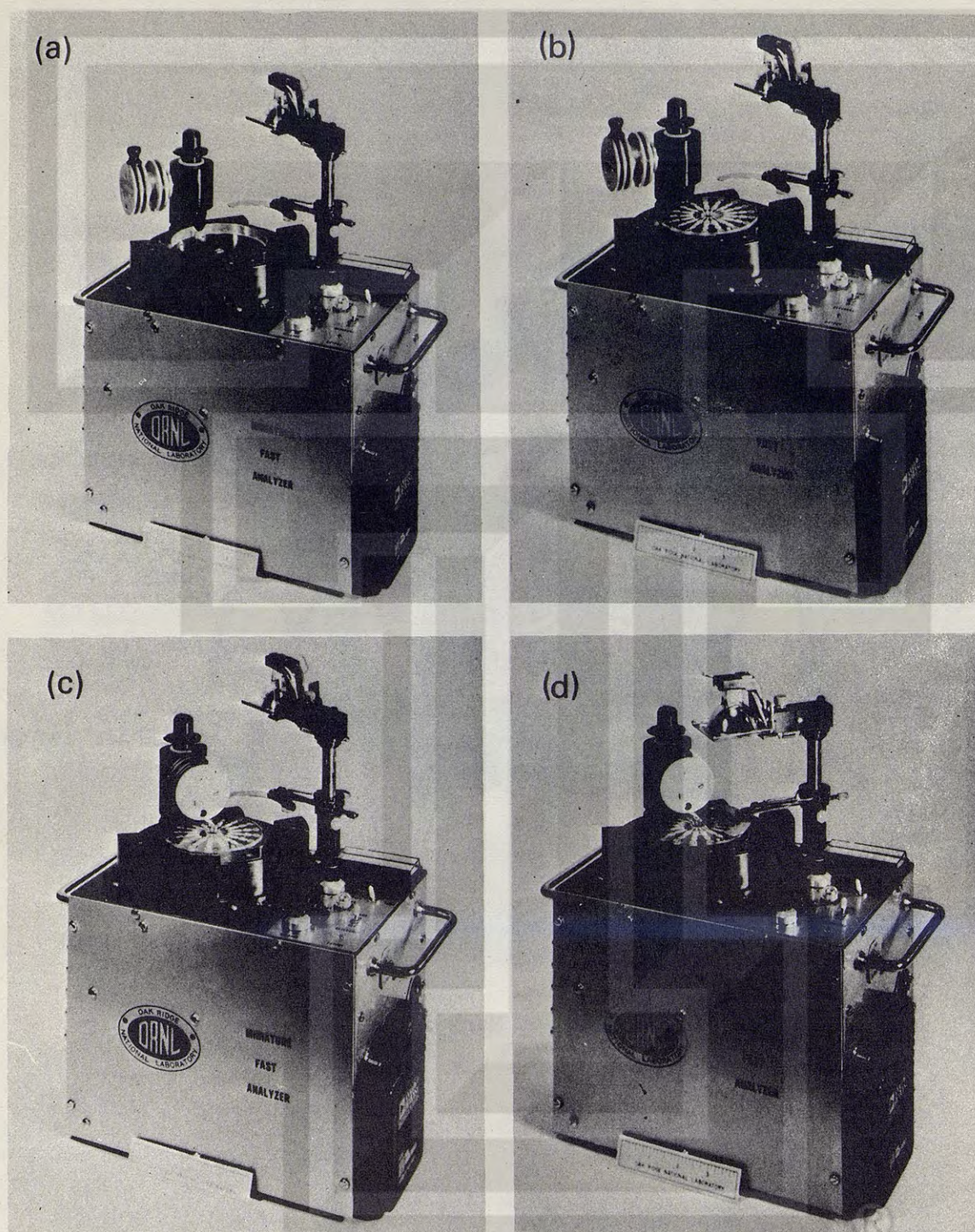


Fig. 3. Placing of Rotor into Mark II Gø Analyzer.

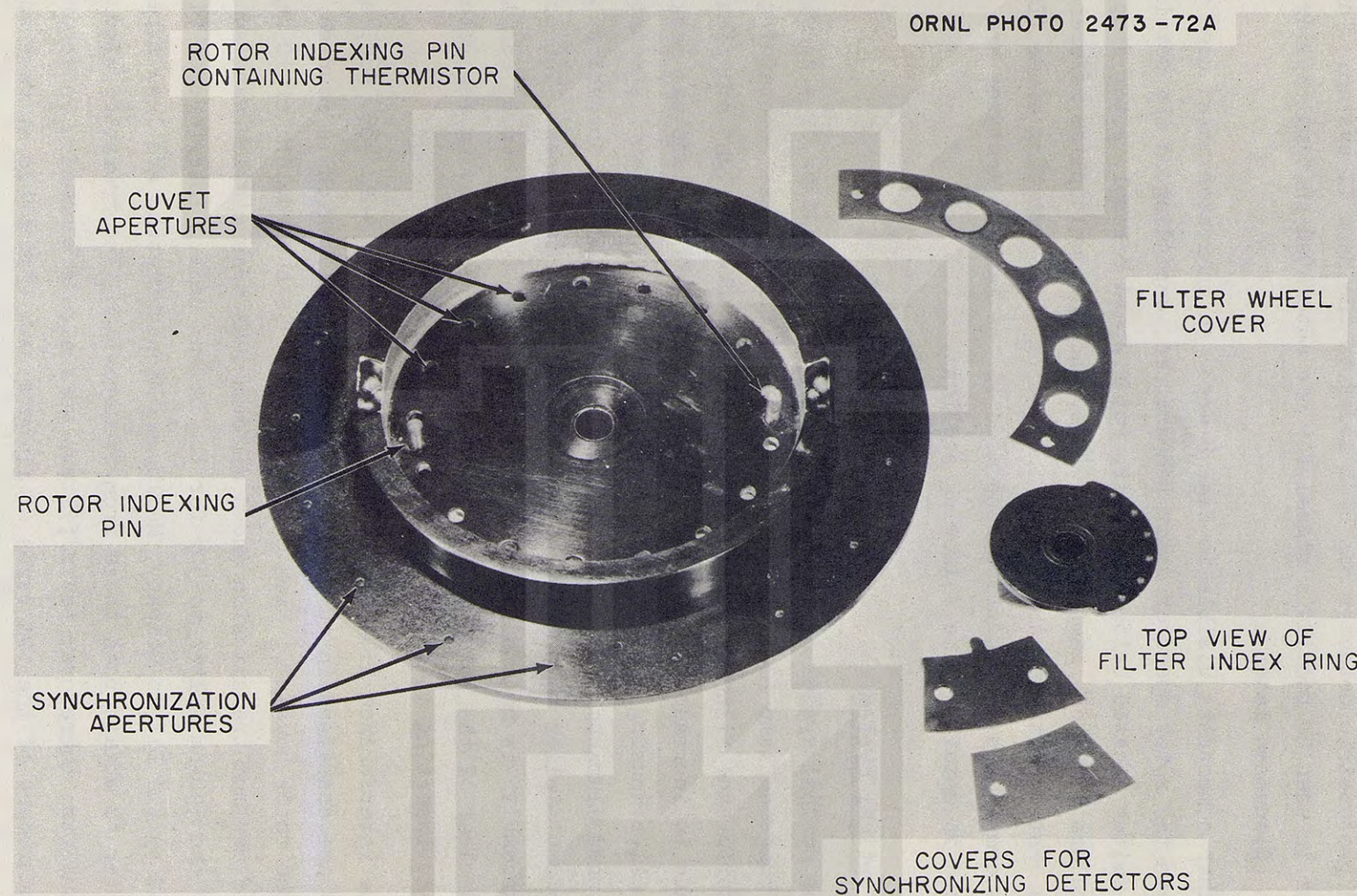


Fig. 4. Top View of Rotor Holder Used in the Mark II $G\phi$ Analyzer.

of the instrument cabinet by means of locking screws. As shown in Fig. 2, a third photometric detector has been added to the Mark II system. This detector is used to provide a signal for the automated photomultiplier circuit (discussed later).

Other components visible in Fig. 2 are: an indicator meter used to designate the rotor speed or rotor temperature, depending on the setting of the switch located below the meter, rotor speed control, rotor stop-run-accelerate switch, lamp on-off switch, and photomultiplier reference voltage control with its associated indicating light-emitting diode (LED). Various components and modifications made to the Mark II Analyzer are discussed in detail in the following sections.

2.2.2 Optical System

The optical system of the Mark II G ϕ Analyzer consists of a quartz-iodine tungsten lamp (G.E. 1974, 20 W, 6 V), a filter wheel containing six interference filters, and a photomultiplier tube. Slight modifications were made to increase the incident light output, and the lamp was packaged in a smaller housing. As shown in Figs. 1(c) and 2, the lamp is mounted in a finned aluminum holder and the light from the lamp is focused onto the photomultiplier tube through two adjustable quartz lenses. The complete assembly is mounted on a vertical rod (Fig. 2) and can be easily rotated clear of the rotor holder for convenience in removing the rotor from (and inserting it into) the holder (Fig. 3).

The interference filters (Spectra-Film 290, 340, 400, 485, 560, and 620 nm) are contained in an adjustable filter wheel, which is mounted just under the rotor housing. Each of the filters has a bandwidth of 10 nm at one-half maximum transmission and a minimum peak transmission ranging from 20% for the 340-nm filter to 50% for the 620-nm unit. Interference filters provided by the Ditric Company, Marlboro, Massachusetts, are less expensive and easier to obtain than those from Spectro-Film, Incorporated, Winchester, Massachusetts; therefore, these filters were also evaluated. In general, the performance of the two types of filters was comparable at wavelengths greater than 400 nm; however, the Spectra-Film filters were found to be

superior at 340 nm. Consequently, the GØ Analyzer prototype systems will probably contain filters obtained from both manufacturers.

The photomultiplier (Hamamatsu R-300) is mounted in a shock-protected, iron holder beneath the baseplate which supports the motor and filter wheel. As with the Mark I Analyzer, the signals generated by the photomultiplier can be conveniently displayed on an oscilloscope. When a 17-cuvet rotor is used, the signals from cuvetts 2 through 17 are compared with the 100% transmittance signal of cuvet 1 to obtain their relative transmittance. The values thus obtained can then be mathematically converted to absorbances and used for calculative purposes.

2.2.3 Automated Photomultiplier Output Control

One of the salient features of the Mark II GØ Analyzer has been the addition of a control circuit that automatically maintains the transmission pulse obtained from cuvet 1 at a constant preset level (i.e., usually from 9 to 9.5 V) by varying the high voltage applied to the photomultiplier tube. A block diagram indicating the function of this control circuit is shown in Fig. 5. One of the three photometric detectors shown in Fig. 2 is used to provide a signal indicating that cuvet 1 is in the optical light path. The automated control circuit then obtains the photomultiplier voltage signal produced by the light transmission peak of the solution contained in cuvet 1 and compares this value to a reference voltage signal, which has been previously preset by the operator via the reference voltage potentiometer located on top of the instrument cabinet (Fig. 2). When this voltage has been correctly set to a value between 9 to 9.5 V, a visual confirmation is obtained by the rapidly flashing LED also found on top of the instrument cabinet. After the voltage of the transmission signal obtained from cuvet 1 has been compared with the preset voltage, the automated circuit will vary the high voltage supplied to the photomultiplier until the two voltages are equal.

It is anticipated that this control circuit will be quite useful when several chemistries are performed at different wavelengths within

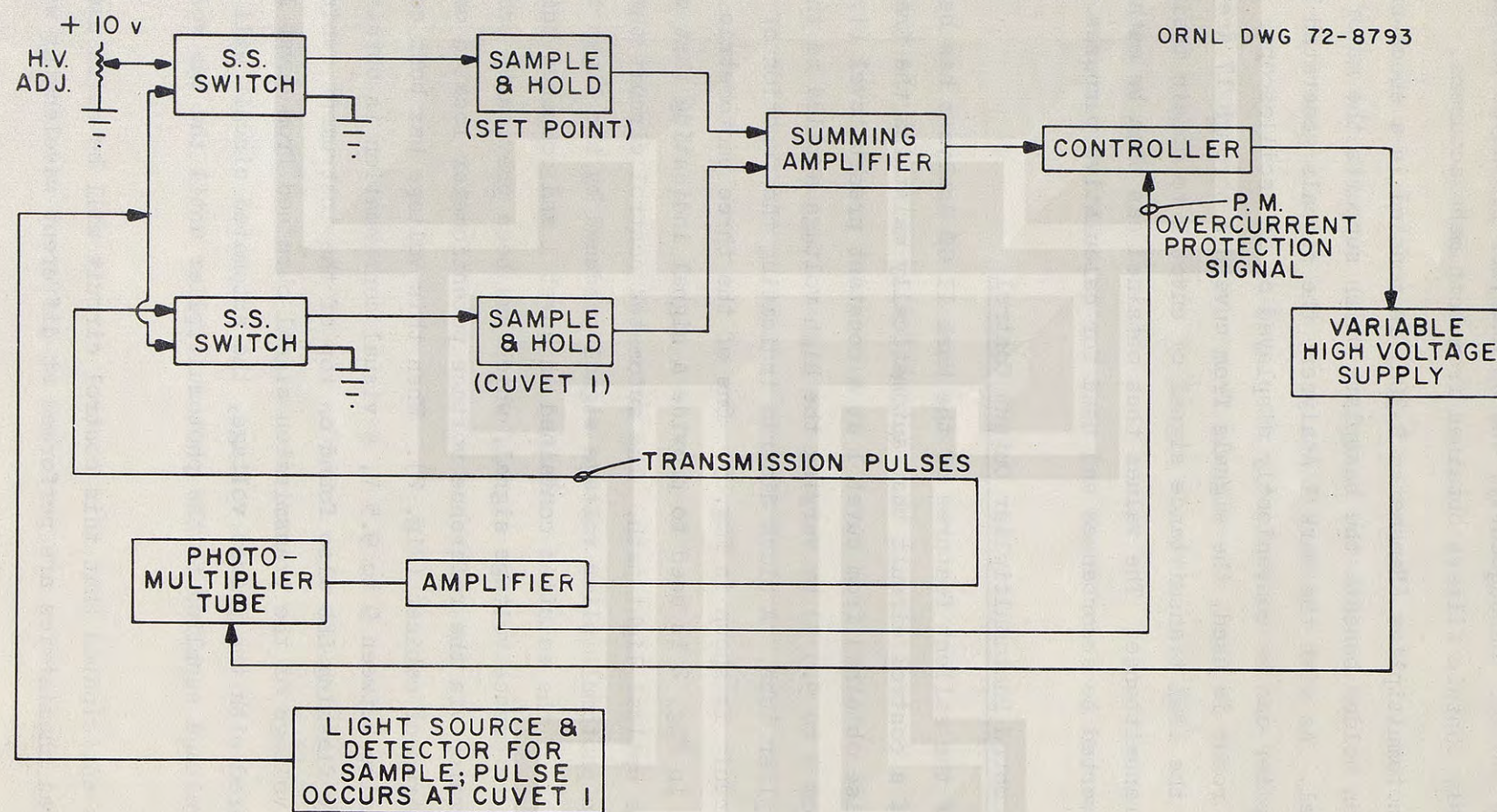


Fig. 5. Operational Diagram of Automated Photomultiplier Control System.

a single rotor since it eliminates the necessity for manually adjusting the high-voltage supply each time a different filter is placed in the optical light path. An additional advantage is that it automatically compensates for electronic drift in the optical system of the Analyzer, thereby improving the performance of the data system interfaced with the Analyzer.

2.2.4 Automated Means for Monitoring and Controlling the Rotor Temperature

The necessity of monitoring and controlling the temperature of the liquid in a cuvet during reaction has led to the development of a system to automatically perform these functions in the Mark II prototype. The temperature of the rotor is measured by a thermistor embedded in a copper-tipped, stainless steel pin (Fig. 4), which is, in turn, mounted on the rotor holder (Fig. 6) so that the measuring section of the pin contacts the center portion of the rotor midway between two cuvetts. The signal from the thermistor is transmitted from the rotor holder through a pair of slip rings (Fig. 6) and brushes to an operational amplifier circuit, which, in turn, provides a meter display of the rotor temperature. The on-board thermistor is calibrated by depressing the calibration button located on one side of the cabinet (Fig. 7) and simultaneously adjusting the potentiometer located on the other side of the instrument (Fig. 8). Correct calibration is obtained when the meter indicator is aligned with the calibration mark on the meter scale (Fig. 2).

The temperature signal is also utilized by an additional operational amplifier circuit, which acts as a proportional controller to maintain the cuvet temperature at a preset level. The output from this controller powers a G.E. ENA-30V-80W lamp, which is mounted above the rotor (Fig. 2) and acts as an infrared source to heat the rotor.

2.2.5 Improved Drive and Braking System

Revisions made to the rotor holder to allow monitoring of the rotor temperature have added some mass to the rotating assembly, which has

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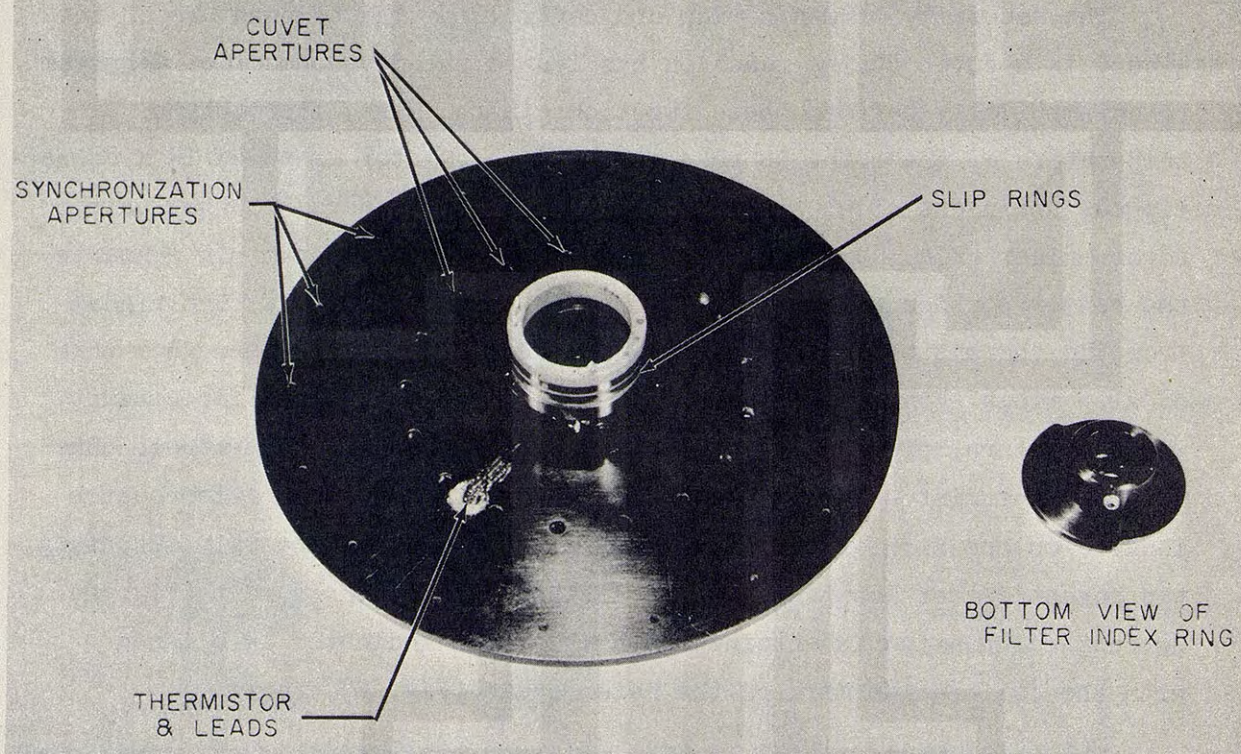


Fig. 6. Bottom View of Rotor Holder Used in the Mark II G ϕ Analyzer.

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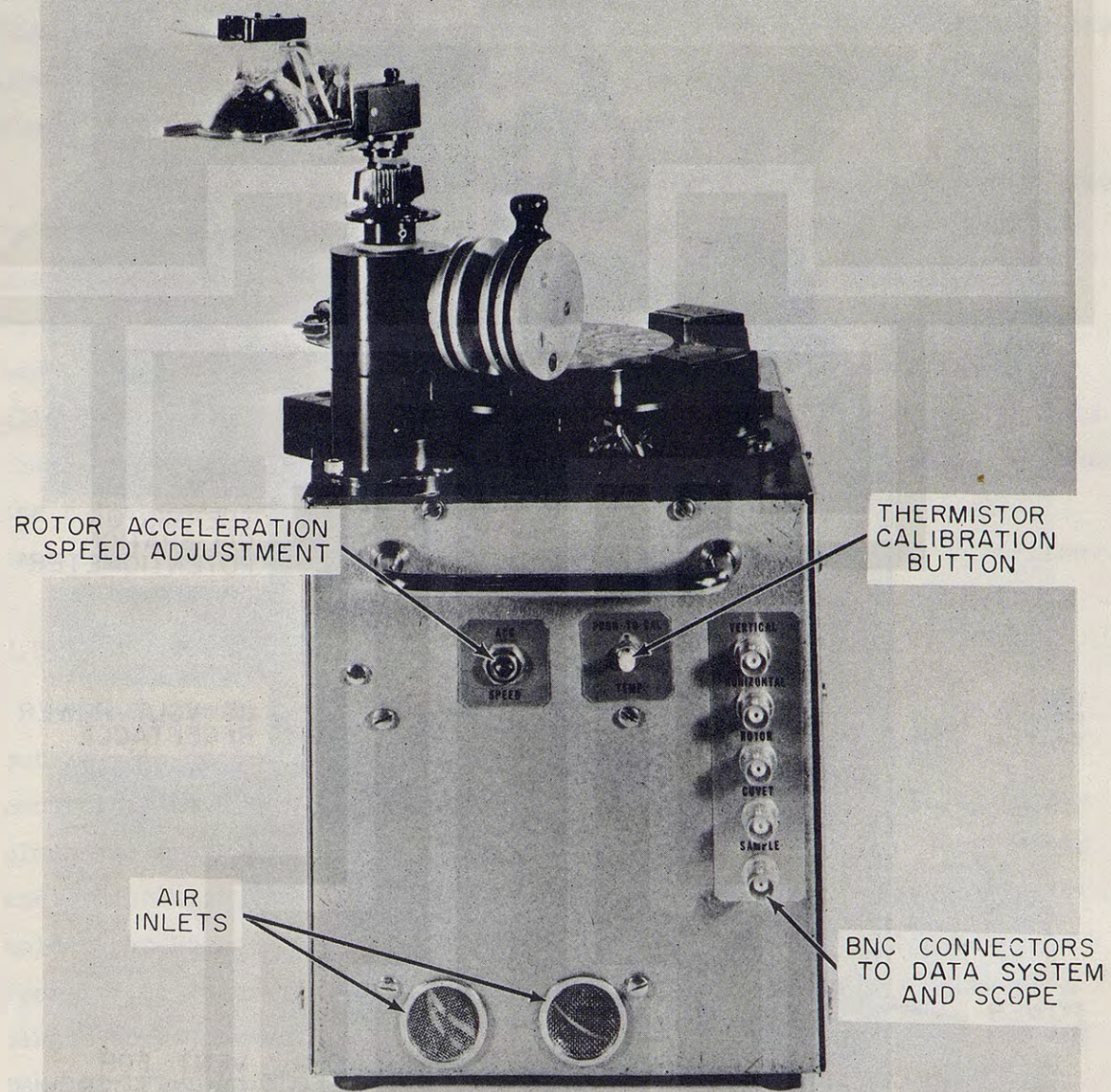


Fig. 7. Side View of Mark II GØ Analyzer.

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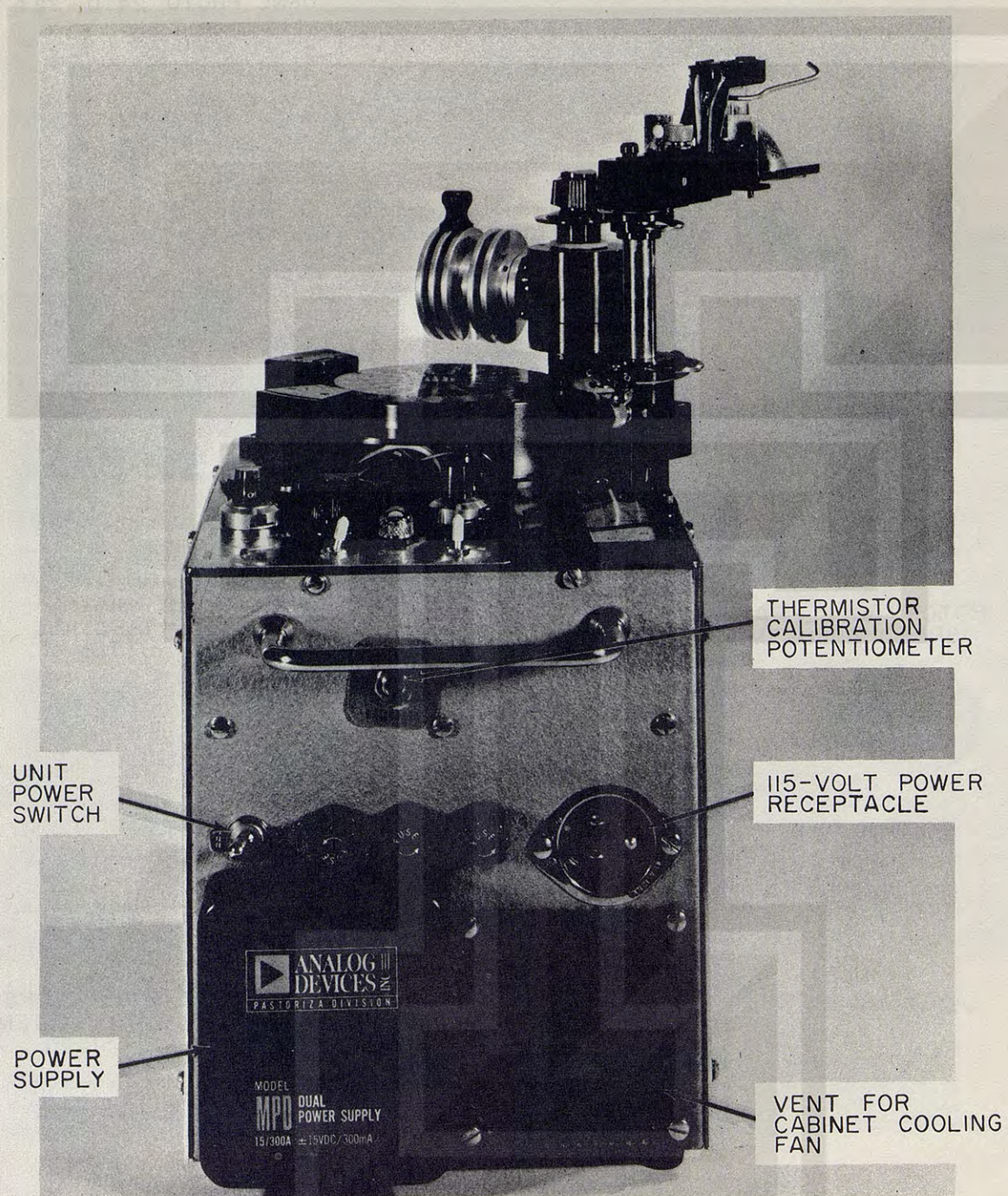


Fig. 8. Side View of Mark II GØ Analyzer. ®

increased its inertia. This, coupled with the desire for rapid acceleration and deceleration of the rotor, has led to replacement of the original drive motor with a Model E-550 motor generator (Electro-Craft Corporation, 1600 Second Street South, Hopkins, Minnesota). This motor has about twice the torque output of the original unit and is contained in a housing that has a slightly larger diameter but still fits easily into the instrument case. The new motor also appears to have better bearings, which should add to the useful life of the Analyzer.

The original braking method (by reversing the polarity of the current to the motor) has been discontinued. The new motor has a double-ended shaft, and an electric brake (Model B-43, manufactured by Simplytrol Products Corporation, 676 Plantation Street, Worchester, Massachusetts) has been attached to the lower shaft extension. This brake, which allows one to completely stop the rotor from a speed of 5000 rpm in less than 2 sec, is rated for many thousands of operations. It should increase the reliability of the system by alleviating the strain on the motor caused by the reverse-current braking method used in the Mark I Analyzer.

2.2.6 Modified Rotor Holder

Since the rotor holder is utilized not only to position the rotor but also to generate the synchronizing pulses for the data system, a convenient way to change the number of cuvetts per rotor would be to replace the rotor holder with one containing the desired number of cuvetts and synchronization apertures. Consequently, an easily replaceable rotor holder has been incorporated into the Mark II Analyzer. It allows the operator to remove the rotor holder from the motor shaft by removing a single screw instead of having to use a set of gear pullers (as was the case with the Mark I).

2.2.7 Electronic Improvements

Some additional minor electrical modifications, other than those mentioned previously, have been made to the Mark II. These modifications have resulted in a machine that has an improved performance over that of

the Mark I prototype. The current output of the power supply section has been increased to provide more effective regulation of the lamp voltage and to provide the additional current demanded when the new larger drive motor is being accelerated. Also, the photomultiplier tube has been placed in an iron mounting rather than in aluminum as in the Mark I. This iron case gives better magnetic shielding and has resulted in a much quieter signal from the photomultiplier. In turn, it has allowed a reduction in the amount of filtering necessary on the photometer amplifiers. This has increased the frequency response of the photometer amplifiers, thus allowing data acquisition at rotor speeds as high as 2000 rpm, and will permit a more rapid data scan rate when the Mark II Analyzer is interfaced with the Mark I Data Printer.

2.2.8 Preliminary Evaluation of the Mark II GØ Analyzer

Optical System at 340 nm. - Since many clinical chemistry determinations are performed by measuring the absorbance of NADH at 340 nm, the optical system of the Analyzer must provide a linear, accurate, and precise absorbance measurement at this wavelength. Consequently, reference solutions of NADH in 0.1 M tris buffer, pH 7.4, were prepared to have calculated absorbances ranging from 0.1 to 2.0 at 340 nm for a 0.5-cm path length. These solutions were loaded into a rotor, from which they were transferred to the cuvetts, and their absorbances were measured. Figure 9, a plot of absorbance vs NADH concentration, indicates that the optical system of the miniature analyzer is linear at 340 nm up to an absorbance of 2.0. As an external check, the absorbances of the NADH solutions were determined, using both a Gilford 300 N spectrophotometer and the Mark I GØ Analyzer, and were found to agree to within 1% of the Mark II GØ Analyzer data. The optical linearity of the Analyzer was also confirmed at other wavelengths by use of various dye solutions.

The absorbances of the NADH solutions were repetitively determined using a tabular absorbance-vs-time computer program. With this program, the absorbance of each solution was determined at 10-sec intervals, for 100 sec, by averaging the data obtained from ten successive revolutions of the rotor (i.e., ten data points averaged per cuvet). The agreement

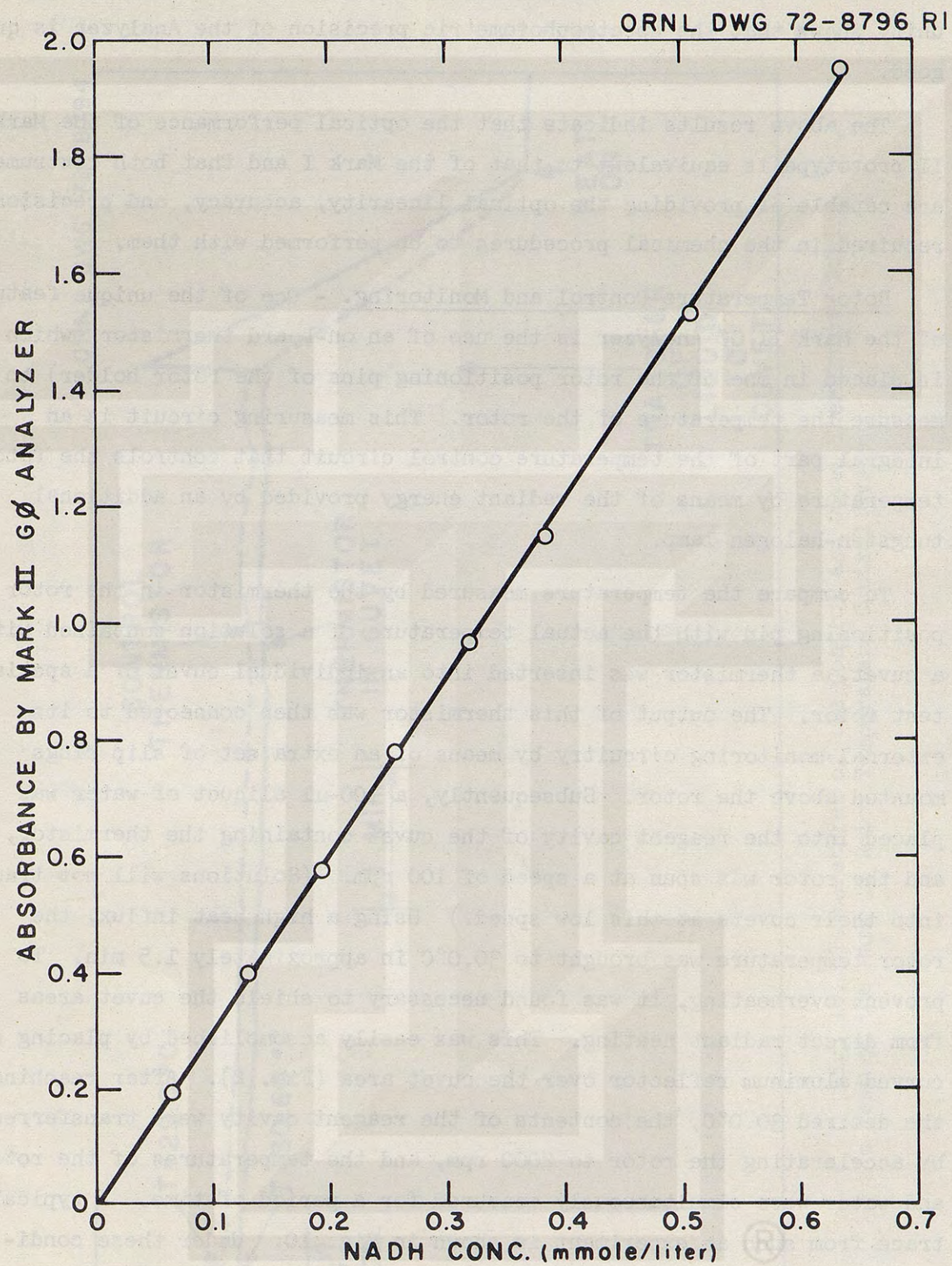


Fig. 9. Optical Linearity of Mark II Gø Analyzer at 340 nm.

of the individual absorbances, to within ± 0.3 - 0.4 of a milliabsorbance unit, shows that the spectrophotometric precision of the Analyzer is quite good.

The above results indicate that the optical performance of the Mark II prototype is equivalent to that of the Mark I and that both instruments are capable of providing the optical linearity, accuracy, and precision required in the chemical procedures to be performed with them.

Rotor Temperature Control and Monitoring. - One of the unique features of the Mark II GØ Analyzer is the use of an on-board thermistor (which is placed in one of the rotor positioning pins of the rotor holder) to measure the temperature of the rotor. This measuring circuit is an integral part of the temperature control circuit that controls the rotor temperature by means of the radiant energy provided by an additional tungsten-halogen lamp.

To compare the temperature measured by the thermistor in the rotor positioning pin with the actual temperature of a solution contained within a cuvet, a thermistor was inserted into an individual cuvet of a special test rotor. The output of this thermistor was then connected to its external monitoring circuitry by means of an extra set of slip rings mounted above the rotor. Subsequently, a 100- μ l aliquot of water was placed into the reagent cavity of the cuvet containing the thermistor, and the rotor was spun at a speed of 100 rpm. (Solutions will not transfer into their cuvetts at this low speed.) Using a high heat influx, the rotor temperature was brought to 30.0°C in approximately 1.5 min. To prevent overheating, it was found necessary to shield the cuvet areas from direct radiant heating. This was easily accomplished by placing a curved aluminum reflector over the cuvet area (Fig. 2). After reaching the desired 30.0°C , the contents of the reagent cavity were transferred by accelerating the rotor to 2000 rpm, and the temperatures of the rotor and water were simultaneously measured for a period of time. A typical trace from such an experiment is shown in Fig. 10. Under these conditions it was demonstrated that a temperature control of $\pm 0.1^{\circ}\text{C}$ can be obtained. As shown in Fig. 10, it is evident that the temperature of

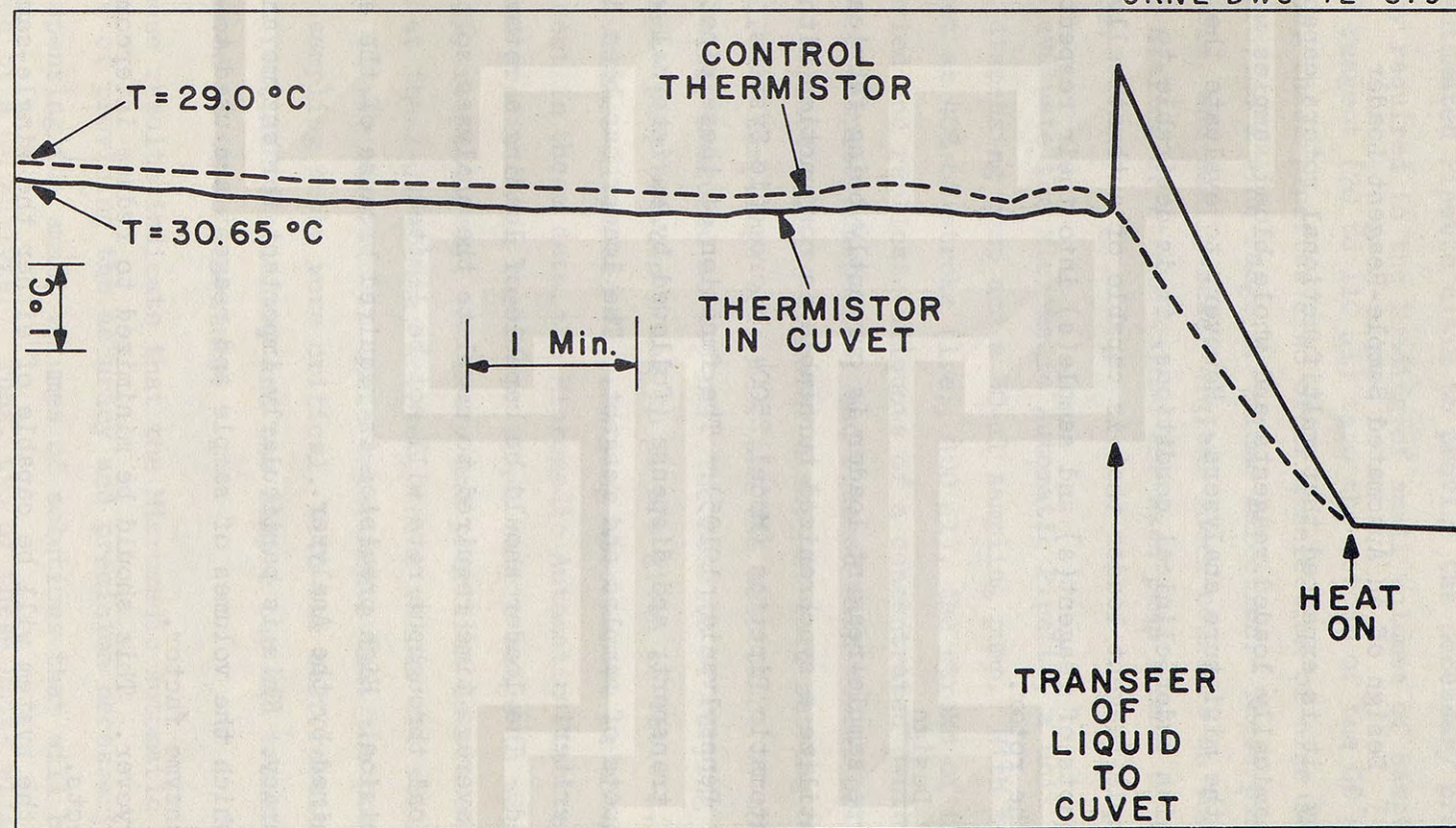


Fig. 10. Temperatures of the Control and Cuvet Thermistors During a Test of the Mark II Rotor Temperature Control System.

the rotor and the temperature of the water in the rotor are slightly different. However, it appears that the rotor thermistor can be calibrated to indicate the temperature of the solution in the cuvet.

2.3 Design of an Automated Sample-Reagent Loader

Ultimately, it is expected that multifunctional rotors capable of accepting dynamically loaded reagents and whole blood samples will be designed for the miniature analyzers. However, to evaluate the first two production units under clinical conditions, it is desirable to have an automated sample-reagent loader that is capable of automatically loading discrete aliquots of reagent(s) and sample(s) into their respective cavities within the rotor.

2.3.1 Loader Design

A prototype sample-reagent loader is currently being fabricated. This device will utilize a synchronized turntable in conjunction with two Micromedic Automatic Pipettes (Model 25004, Micromedic Systems, Inc., Philadelphia, Pennsylvania 19105). The function of these pipettes will be to obtain, transport, and dispense (followed by an internal wash with diluent) aliquots of samples and reagent. The loader has been designed to meet four criteria:

- (1) Speed. The loader should be capable of loading a rotor within the average time required to complete the analyses so that analytical throughput rate will not be limited.
- (2) Precision. High precision is required because of the small volumes required by the Analyzer.
- (3) Accuracy. This is particularly important for enzyme analysis in which the volumes of sample and reagent are used to calculate an enzyme factor.
- (4) Carryover. This should be minimized to reduce intercompartmental effects.

As designed, the system will be capable of either the single-sample:multiple-chemistry or the multiple-sample:single chemistry mode of operation. The integrated loader will be assembled and evaluated during the next quarter.

2.3.2 Evaluation of Micromedic Automatic Pipette

The manufacturer's specifications for the performance of the Micromedic Automated Pipette claim to provide the necessary precision and accuracy required in the loading of small volumes of samples (1 to 10 μ l) and reagent (70 to 110 μ l) into the rotors of the GØ Analyzer. In addition, these pipettes also offer a selection of nine standard pumps, which provide a wide range of volume selection. These pumps are interchangeable, and easily-reset digital index counters on them permit independent dialing of stroke volume for each pump from 0 to 100% of pump capacity.

To evaluate the Micromedic Automatic Pipette, a unit was set up with a 1-ml dispensing pump and a 20- μ l sampling pump. With the dispensing pump set at 40% of stroke (i.e., 400 μ l), the stroke of the sample pump was varied and replicate aliquots of a concentrated p-nitrophenol solution were sampled, dispensed, and flushed with 400 μ l of buffer diluent into a 15-place GeMSAEC transfer disk. At each setting of the sample pump, 14 replicate samplings were made and the absorbance of the diluted dye solution was measured at 405 nm. The results of varying the stroke of the sample pump between 1 and 20 μ l are given in Table 1, and the linearity of the volumes dispensed vs resulting absorbances is shown in Fig. 11. As evident in these data, the Micromedic Automatic Pipette provides accuracy and precision in the 2- to 20- μ l sample range to within $\pm 1-2\%$ and 0.1-0.3%, respectively. Accuracy and precision decreased to $\pm 3.3\%$ and $\pm 0.82\%$ at the 1- μ l volume; however, these values are still within a usable range. It was observed in these experiments that the position and handling of the sampling probe were critical, with the best results being obtained when it remained stationary and the sample cup and transfer disk cavity were moved to it as necessary.

These results indicate that the Micromedic Automatic Pipette is capable of providing the accuracy and precision necessary in sampling and dispensing the small volumes of solutions that will be processed and analyzed by a GØ Analyzer. Thus, two of these units will be integrated in the automated sample-reagent loader; one will be used to obtain, transport, dispense, and dilute aliquots of sample, and the other will be used

Table 1. Evaluation of Micromedic Automatic Pipette

Sample Volume (μ l)	Dilution Ratio	Absorbance		Accuracy (%)	Precision (C.V.)
		Calculated ^a	Measured		
1	1:401	0.0776	0.0802	103.3	0.82
2	1:202	0.1542	0.1585	102.7	0.67
3	1:134	0.2324	0.2353	101.6	0.29
4	1:101	0.3084	0.3132	101.6	0.28
5	1:81	0.3845	0.3903	101.5	0.31
6	1:67	0.4607	0.4653	100.9	0.18
7	1:58	0.5370	0.5389	100.3	0.16
8	1:51	0.6107	0.6129	100.3	0.21
9	1:45	0.6922	0.6856	99.0	0.24
10	1:41	0.7597	0.7572	99.7	0.42
15	1:27	1.1261	1.1084	98.4	0.33
20	1:21	1.4450	1.4289	98.5	0.30

^a A grade A 1:50 dilution of the concentrated p-nitrophenol solution was made, and its absorbance was determined with the Gilford 300 N Spectrophotometer. The expected absorbances at the various dilutions were then calculated.

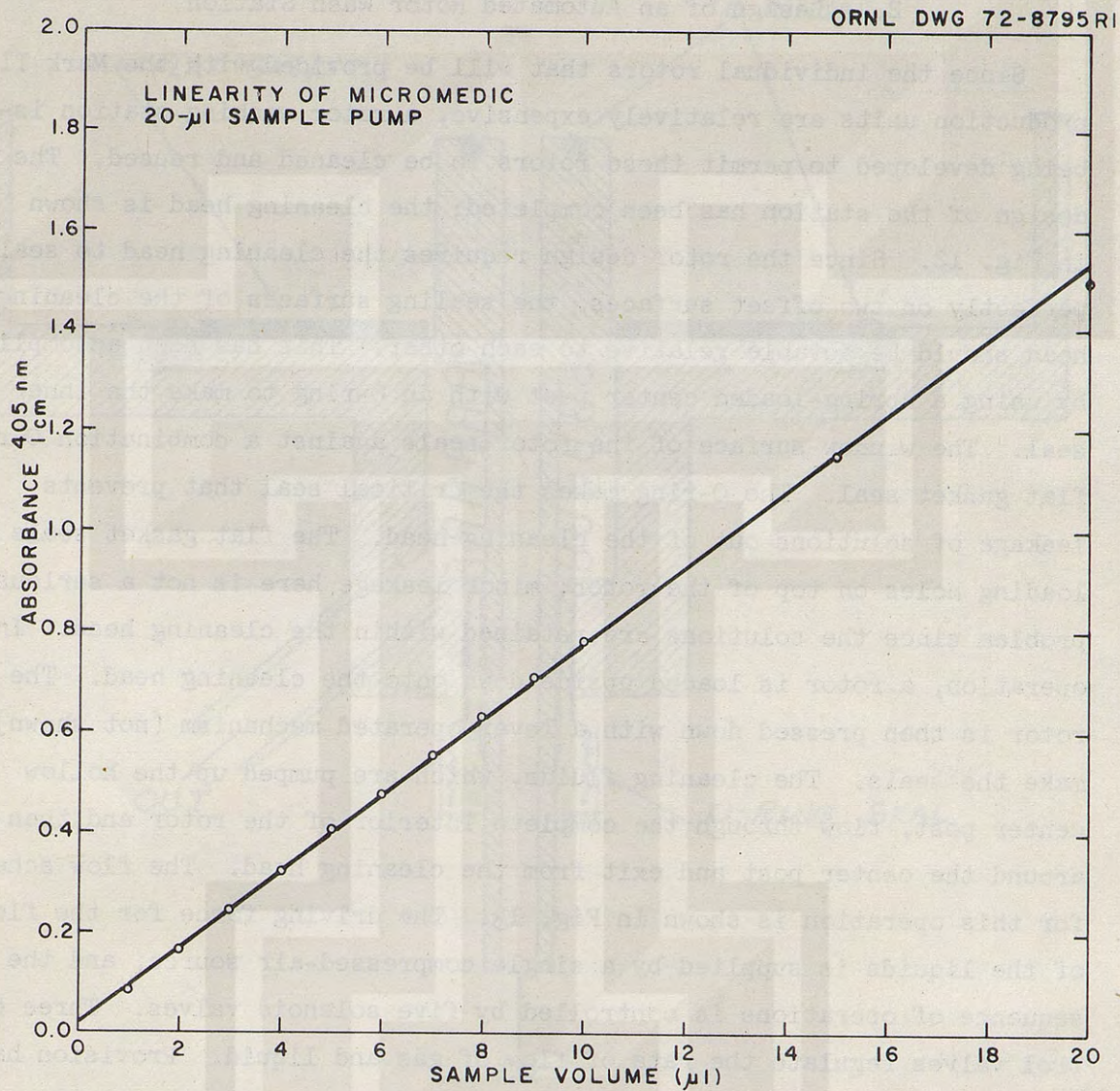


Fig. 11. Linearity of Volume Selection of Micromedic Automatic Pipette.

to obtain, transport, dispense, and dilute aliquots of reagent. When assembled, this loader will be extensively evaluated to determine the extent of intercompartmental carryover.

2.4 Design of an Automated Rotor Wash Station

Since the individual rotors that will be provided with the Mark II production units are relatively expensive, a rotor washing station is being developed to permit these rotors to be cleaned and reused. The design of the station has been completed; the cleaning head is shown in Fig. 12. Since the rotor design requires the cleaning head to seal perfectly on two offset surfaces, the sealing surfaces of the cleaning head should be movable relative to each other. This has been accomplished by using a spring-loaded center post with an O-ring to make the inner seal. The window surface of the rotor seals against a combination O-ring--flat gasket seal. The O-ring makes the critical seal that prevents leakage of solutions out of the cleaning head. The flat gasket seals the loading holes on top of the rotor; minor leakage here is not a serious problem since the solutions are retained within the cleaning head. In operation, a rotor is loaded upside down onto the cleaning head. The rotor is then pressed down with a lever-operated mechanism (not shown) to make the seals. The cleaning fluids, which are pumped up the hollow center post, flow through the complete interior of the rotor and then out around the center post and exit from the cleaning head. The flow schematic for this operation is shown in Fig. 13. The driving force for the flow of the liquids is supplied by a single compressed-air source, and the sequence of operations is controlled by five solenoid valves. Three control valves regulate the rate of flow of gas and liquid. Provision has been made for air to be mixed into the liquid streams to facilitate cleaning by increasing turbulence and by segmenting the liquid streams. The water, methanol, and waste solutions will be housed in a plastic box separate from the cleaning station cabinet.

2.5 Mark I Data Printer

The Mark I Data Printer is a system for acquiring, processing, and printing, without the aid of a computer, the results of analyses carried

ORNL DWG 72-8806 R1

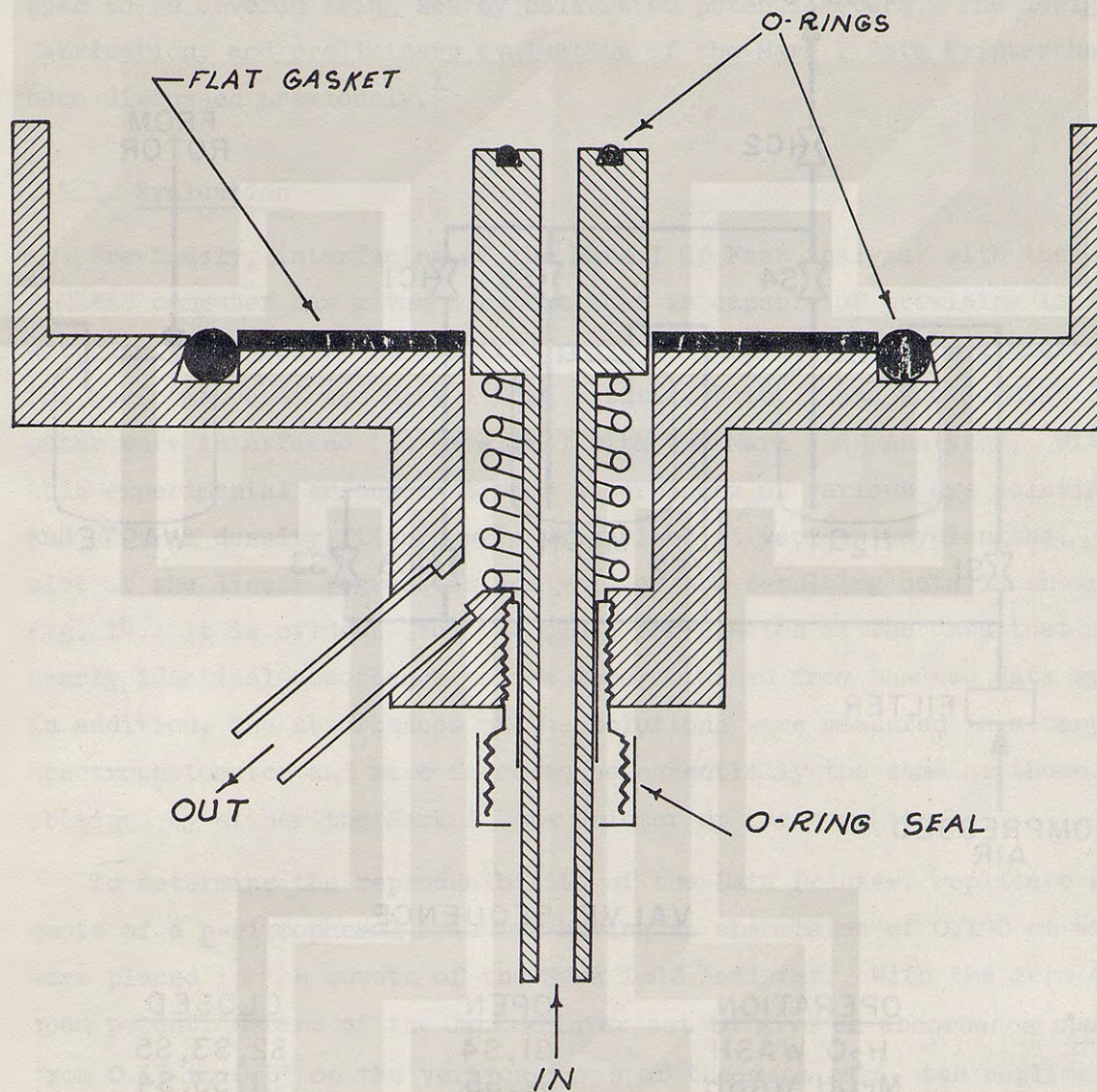
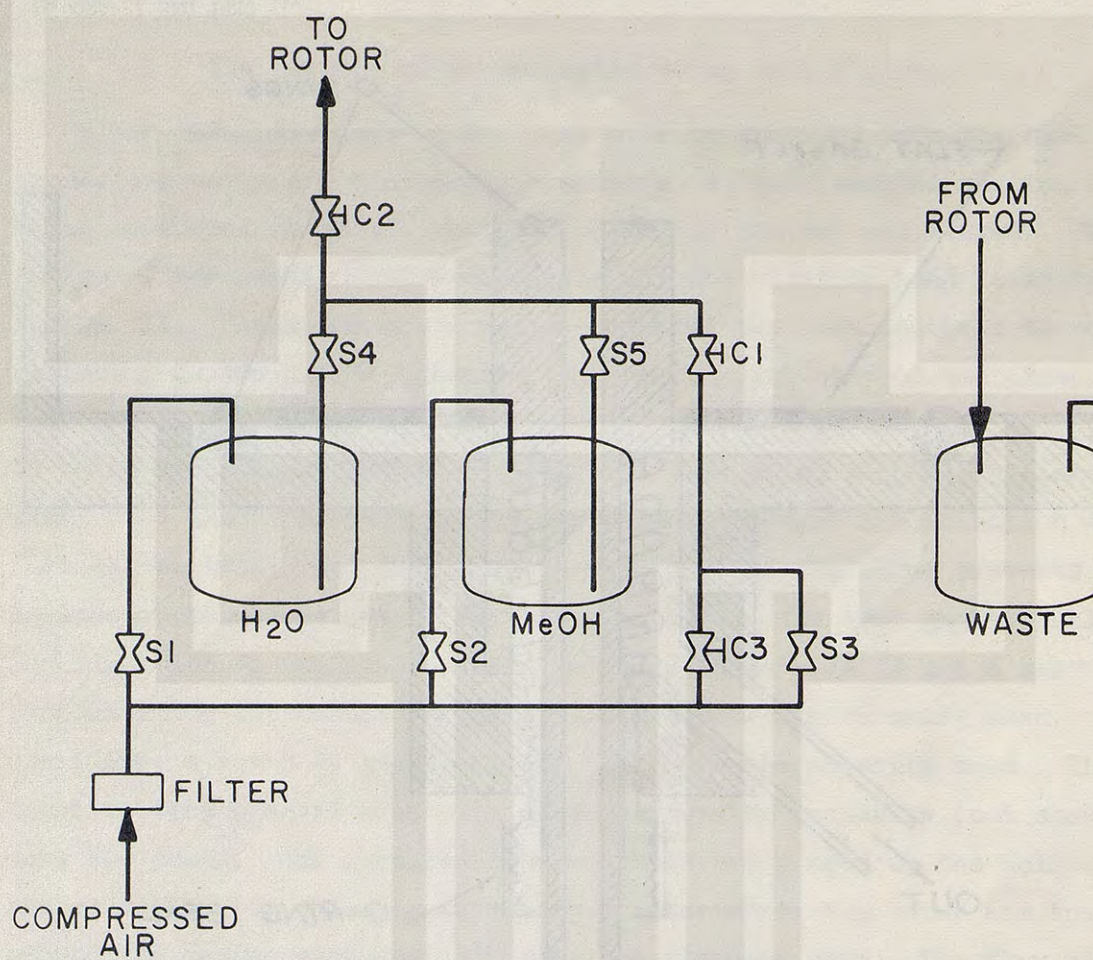


Fig. 12. Cleaning Head for Rotor Washer Station.

ORNL DWG 72-8792

VALVE SEQUENCE

<u>OPERATION</u>	<u>OPEN</u>	<u>CLOSED</u>
H ₂ O WASH	S1, S4	S2, S3, S5
MeOH WASH	S2, S5	S1, S3, S4
AIR DRY	S3	S1, S2, S4, S5

Fig. 13. Fluid Flow Schematic for Rotor Washer Station.

out in Fast and GØ Analyzers. The transmission pulses of the particular analyzer are converted electronically to absorbance values, which are recorded as printed dots on a data card. Vertical height on the card is proportional to absorbance, with the maximum absorbance and the absorbance span to be covered being set by calibrated potentiometers. The design, fabrication, and preliminary evaluation of the Mark I Data Printer have been discussed previously.¹

2.5.1 Evaluation

Previously, interfacing of the Mark I GØ Fast Analyzer with the on-line GeMSAEC computer has given a system that is capable of providing linear, accurate, and precise photometric results. Consequently, to evaluate the performance of the Mark I Data Printer, both it and the GeMSAEC computer were interfaced (in parallel) with the Mark I GØ Analyzer. With this experimental arrangement, the absorbances of various dye solutions and neutral density filters were determined at various wavelengths. A plot of the linear regression analysis of the resulting data is shown in Fig. 14. It is evident from the close correlation of the data that nearly identical absorbance values were obtained from the two data systems. In addition, the absorbances of the solutions were measured in a Cary 14 spectrophotometer and were found to be essentially the same as those obtained by either the Mark I Data Printer or the computer.

To determine the reproducibility of the Data Printer, replicate aliquots of a p-nitrophenol solution having an absorbance of 0.190 at 405 nm were placed in the cuvetts of the Mark I GØ Analyzer. With the zero and span potentiometers of the Data Printer set to give an absorbance span of from 0.15 to 0.25 on the vertical axis of the data card, ten replicate scans were made and the absorbances of the solutions in the individual cuvetts during each scan were measured. By statistically averaging and evaluating the resulting data, reproducibility of the Data Printer was found to correspond to an absorbance of ± 0.0015 . Although this value is higher than the ± 0.0005 obtained with the computer, it is probably acceptable under most analytical conditions.

To determine performance under routine analytical conditions, 16 replicate aliquots of Hyland control sera I or II (Hyland Laboratories,

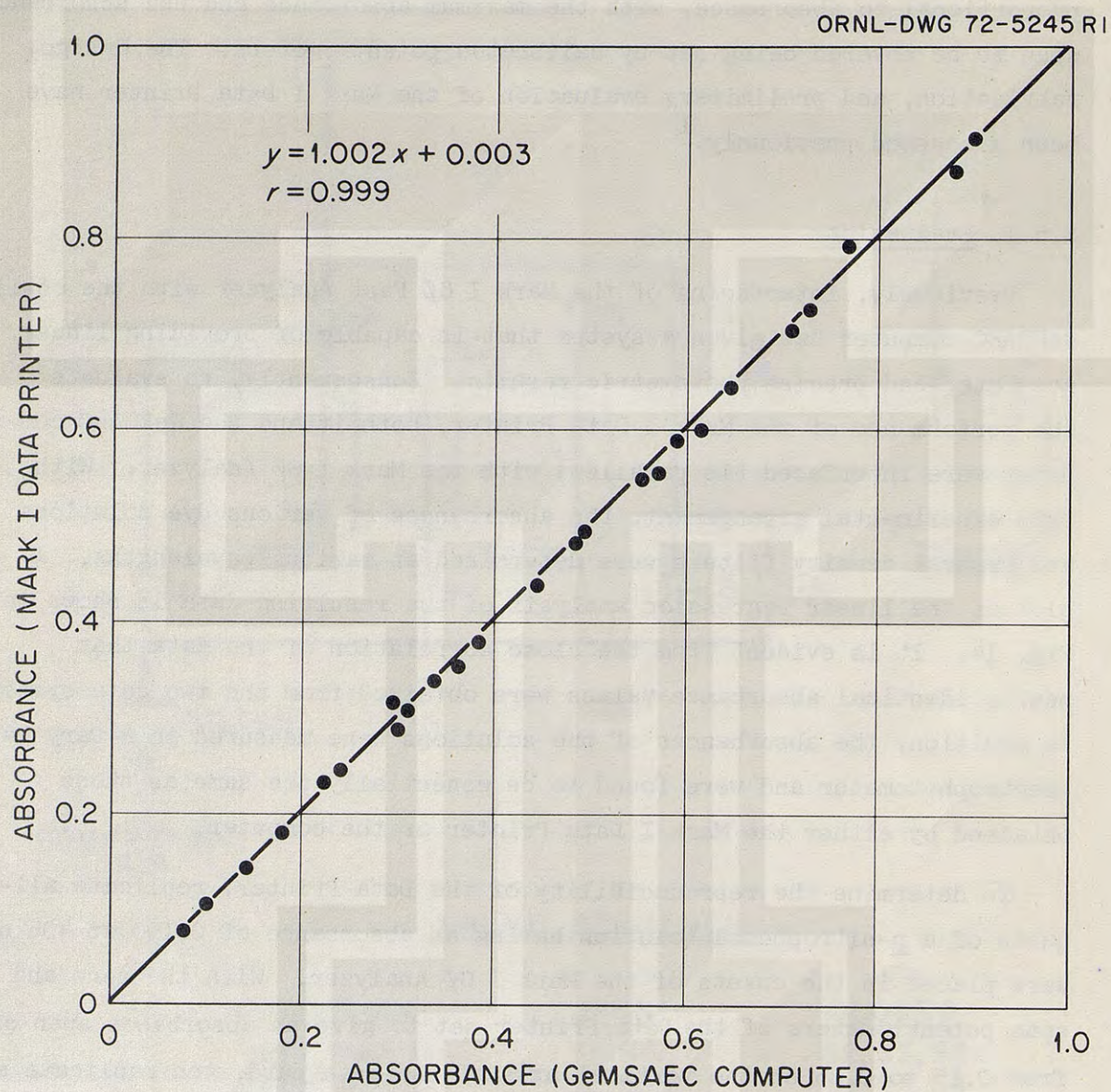


Fig. 14. Correlation of Absorbance Values Obtained Using the GeMSAEC Computer and the Mark I Data Printer.

Costa Mesa, California 92626) were analyzed for LDH-L activity and the results were processed simultaneously by the Data Printer and the computer. The enzyme activities obtained by each data system agreed to within 0.8 to 2.0% (see Table 2). The computer-derived data were observed to be slightly more precise; however, when one considers that the computer performs its calculations on 400 data points per cuvet as compared to only 2 per cuvet for the Data Printer, the performance of either system is quite satisfactory.

A limitation of the Data Printer was observed from the standpoint of routine operation. For example, the unit performed satisfactorily when the analyzer was operated in the multiple-sample: single-chemistry mode. In this mode, it is possible to set an absorbance span and a maximum that will encompass the entire absorbance range that one would expect to encounter during an analytical run, while at the same time allowing the span to be set as small as possible in order to ensure maximum analytical resolution. However, operation in the multiple-chemistry: single-sample mode requires that an absorbance span of 0.5 or greater be set in order to encompass the wide range of absorbances encountered in the various chemical analyses. Depending on the analysis, this may cause poor analytical resolution and a concurrent loss in precision. For example, with an absorbance span of 0.5 or greater, the small absorbance change encountered in a normal SGOT analysis would be difficult to measure precisely. This limitation, in conjunction with an observed drift in the logarithmic amplifier circuit, required modifications to the unit as described below.

2.5.2 Modifications

In the evaluation of the Mark I Data Printer, two problem areas were observed. They were (1) electronic drift in the logarithmic amplifier circuit, and (2) poor analytical resolution when operating in the multiple-chemistry: single-sample analytical mode. The problem involving drift was solved by replacing the original logarithmic amplifier with a No. 4351 Teledyne/Philbrick logarithmic amplifier which, in previous evaluations had been found to be operationally superior to the original amplifier. To solve the second problem, the electronic module of the Mark I Data

Table 2. Comparison of Enzyme Activities Obtained by Means of the Mark I GØ Analyzer, Interfaced with Either a Computer or the Mark I Data Printer

Serum Sample	Data Device	Enzyme Activity ^a	C.V. ^b (%)
Hyland I	Computer ^c	63.3	1.71
	Data Printer ^d	64.5	2.41
Hyland II	Computer ^c	139.8	1.01
	Data Printer ^d	140.9	1.61

^aActivity = I.U. liter⁻¹ min⁻¹ at 30°C.

^bCoefficient of variation (C.V.), % = $\frac{\text{sigma}}{\text{average mean}} \times 100$, N = 16.

^cA PDP 8/I computer was used with the following program constants: delay interval = 20 sec; observation interval = 15 sec; number of data readings averaged per observation = 10; analysis time = 10 min; activity = absorbance change per minute multiplied by 3860.

^dData Printer conditions: scan every min for 10 min; absorbance change between 1 and 10 min used to calculate enzyme activity. Enzyme activity = absorbance change per 9 min multiplied by 926.

Printer was modified by adding three additional sets of absorbance maximum and span potentiometers (Fig. 15). Thus the photometric outputs from cuvetts 2-5, 6-9, 10-13, and 14-17 may each be processed at a different absorbance maximum and span. This modification will allow more flexibility and analytical resolution in operating the GØ Analyzer when it is interfaced with the Data Printer. Additional modifications include:

- (1) The incorporation of an internal clock and circuitry to automatically control the start and run speeds of the rotor.
- (2) Replacement of the original solenoid drivers with state-of-the-art, solid-state switches in order to eliminate the spurious data prints that were frequently encountered with the original Data Printer.
- (3) Replacement of the original scan drive motor with a faster one in order to increase the data rate of the Printer. (Note: This modification was made possible by electronic improvements in the Mark II GØ Analyzer.)

2.5.3 Future Design Consideration

The advent of the automatic photomultiplier control, which allows the Mark II Analyzer to automatically perform the referencing function, and the successful operation of the logarithmic amplifier module have removed many of the factors that previously prevented the use of a simple digital data reduction system with a miniature Fast Analyzer. Such a digital system is currently being designed. It will utilize the most recent MOS LSI memory and logic circuits and will be able to provide concentration or activity units for both end-point and rate reactions.

3. ROTOR DESIGN AND DEVELOPMENT

Ultimately, it is expected that the GØ Analyzer will contain disposable rotors capable of simultaneously performing multiple chemical analyses of a single sample. It is desirable that such rotors be inexpensive, contain prepackaged and preloaded reagents, and be designed to accept a sample of whole blood, separate the plasma from the cellular components,



Fig. 15. Modified Electronic Module of Mark I Data Printer.

and then accurately measure and transfer a volume of plasma to the reaction cuvet.

Thus far, it has been found necessary to design rotors into which aliquots of samples and reagents are discretely dispensed. Such rotors, which are valuable for instrumental evaluation and for the development and adaptation of chemical procedures for use with the Analyzer, will, in general, be the type that will be provided initially for evaluation of the GØ Analyzer production units.

3.1 Rotor Design

The design of the rotors which will be provided with the Mark II production units is shown in Fig. 16. This design, although similar to the older 17-place GØ rotor (3-17-C-1¹), is different in three important aspects. First, the reagent and sample cavities have been machined to contain equal volumes. This will allow more flexibility in selecting the reagent, sample, and diluent volumes when the rotors are loaded via the automated device currently under development. The second modification entails enlarging the connecting port between the cuvet and sample cavities. This will permit a more rapid transfer of solutions into the cuvet and hence allow one to decrease the time required for transfer and mixing. The third modification has involved connection of the two sides of the rotor by drilling an injection port through the center of the rotor. This allows for dynamic introduction of a solution from the top side of the rotor, thus eliminating the necessity to stop the rotor and invert it during an analytical run.

As with the earlier 3-17-C-1 rotor design, this rotor will be capable of three modes of operation:

- (1) Aliquots of samples and reagents can be discretely dispensed by means of the automated loader into their individual cavities and then transferred to their respective cuvetts.
- (2) With the rotor spinning, a reagent can be dynamically loaded, apportioned, and aliquots of it transferred into the cuvetts. The rotor can then be stopped, loaded with discrete aliquots of samples, which are then transferred into their respective

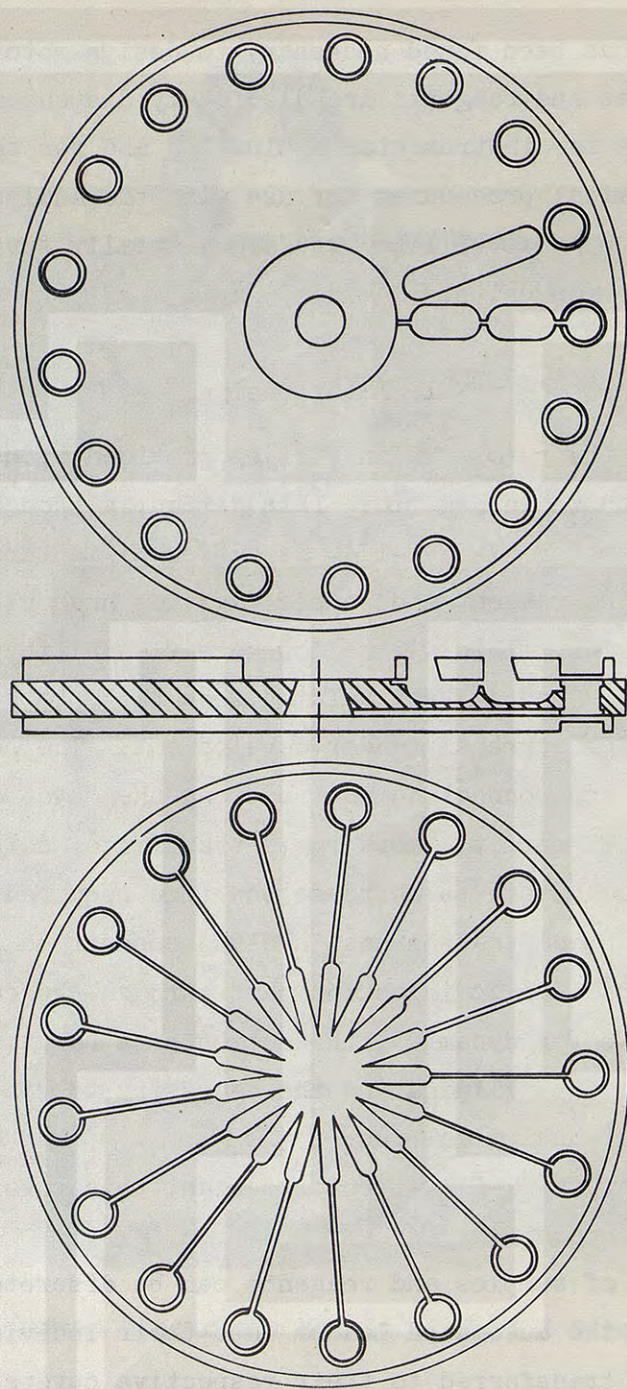


Fig. 16. Design of Rotor That Will Be Provided with the Mark II GØ Analyzer.

cuvets (in this mode, multiple samples are analyzed for a single component).

- (3) The converse of mode 2, in which a sample is dynamically loaded and aliquots of different reagents are discretely loaded (in this mode, a single sample is analyzed for several components).

Thus this rotor is very versatile and, in conjunction with the automated sample-reagent loader, should prove useful in evaluating the Mark II production units.

3.2 Fabrication of Rotor Components

Machined Components. - A GØ rotor is composed of three components: a middle body containing the various cavities, and two cover windows. To date, the components of all the rotors presently being used with the GØ Analyzers have been fabricated by machining bodies from a sheet of black acrylic plastic, and the cover has been fabricated from ultra-violet-transmitting (UVT) acrylic plastic. The black acrylic body serves as an internal light mask for the cuvetts. The use of the UVT acrylic plastic as window material allows the rotor to be usable down to wavelengths as low as 290 nm. The machining operation is a useful fabrication method in instances where only a few rotors (i.e., up to 50) of a particular type are required; however, it has several disadvantages, including the fact that it is time-consuming. Hence the individual rotors prepared in this manner are expensive, and rotor-to-rotor variation would be expected to be greater. This latter disadvantage of variability between rotors can be minimized by use of an automated machining method, which is currently being investigated; however, the individual rotors will still tend to be relatively expensive.

Molded Components. - In instances where many identical rotors are required, a molding technique would be more desirable since it would be more convenient, less variable, and less expensive. Initial studies and design conferences have indicated that injection molding is the technique of choice for fabricating the individual rotor components. With this

technique, the injection molding dies will be either machined or electroformed using a machined master die, the choice depending on which method is most suitable for the rotor body or cover window. The rotor body will be molded from black acrylic resin and the windows from UVT acrylic resin. This technique should provide rotors that are more uniform and, in addition, cost only a few dollars each.

3.3 Rotor Assembly

After the rotor components have been fabricated, they must be assembled by gluing (solvent or catalyzed monomer), heat pressing, or sonic welding. Heat pressing and gluing with epoxy have been used to assemble acceptable rotors. One of the advantages of injection molding for fabrication of the components is that a large number of inexpensive parts can be sacrificed in testing the assembly methods. The most promising methods for assembling large quantities of rotors appear to be solvent gluing and gluing using a catalyzed acrylic monomer. Rotor components should be available for assembly tests by October 1; assembled and tested rotors are projected to be available by November 1.

3.4 Design and Assembly of a Calibrated Absorbance Rotor

To calibrate the Mark I Data Printer, solutions of known absorbance are required. We have utilized solutions of p-nitrophenol (400-415 nm), NADH (340 nm), and Blue Dextran (340-700 nm) for this purpose, but the preparation and standardization of these solutions are laborious and time-consuming. In addition, the absorbances of these solutions may change with time as the result of evaporation and chemical instability. In an attempt to obtain a stable absorbance reference to calibrate the Printer and to periodically check its calibration we have assembled a rotor containing, in its various cuvetts, disks of photographic film that have been exposed for various periods of times to yield disks of increasing absorbance.

The rotor was assembled by sandwiching the film disks between two windows of UVT acrylic plastic. The windows were oriented and held in place by top and bottom aluminum cover plates, which have 17 apertures

to correspond to a 17-cuvet rotor. The result is a 17-place rotor in which cuvet 1 contains no film (reference cuvet) and cuvet 2-16 contain disks of known absorbance (cuvet 17 also contains no film). Since these disks are gray, their absorption spectra are relatively flat between 340 and 700 nm; hence the rotor may be used as an absorbance reference at a variety of wavelengths. This rotor has proved to be quite useful in our development program since it provides us with a series of reference absorbances that require no preparation other than the initial calibration and assembly and, in addition, are stable with time.

4. DATA ACQUISITION

Fast Analyzers yield data in a form and at a rate suitable for direct input into a digital computer. The miniature GeMSAEC Fast Analyzer and the Mark I and II GØ Analyzers have been designed with computer compatibility and have been interfaced with the GeMSAEC computer module. Manual data reduction of Polaroid photographs of the oscilloscope display is feasible; also, mechanical methods of data reduction are being developed.

4.1 Mechanical Data Acquisition

The Mark I Data Printer (see Sect. 2.5) has the advantage of providing a rapid and inexpensive backup capability for the computer system.

4.2 Data Handling By a Computer

The GeMSAEC Fast Analyzer and the Mark I and II GØ Analyzers, which were designed for on-line computer compatibility, have been successfully interfaced with the previously described GeMSAEC computer system. Although this system has proven quite useful for research, as well as routine operation, its additional memory capabilities and peripheral devices for data display have increased the relative cost of the system. Consequently, a low-cost, small, efficient data system is being developed within the NIGMS Fast Analyzer Program. This system will also be compatible with the GØ Analyzers and thus will be used in developing chemistries for use with these analyzers. In addition, computer programs for processing data generated by enzyme substrate analyses have been developed and evaluated.

4.2.1 Development of a Compact Computer System

A more compact on-line computer system has been designed for use with both the GeMSAEC Fast Analyzers and GØ Analyzers. This system is a modified version of the system previously described.^{6,7} It consists of a Digital Equipment Corporation PDP-8/E computer with 8192 words of core memory, a 120-Hz line-frequency-based clock, a Teletype Model ASR-33 teleprinter/keyboard, and the necessary analog and digital interfaces for coupling the Analyzer to the computer hardware.

The system has been designed to acquire data from Fast Analyzers with rotors containing up to 43 cuvetts that operate at speeds from 100 to 2000 rpm. Analog input voltages are converted to digital data by a successive-approximation analog-to-digital converter with an accuracy of $\pm 0.5\%$ of full scale (-10 V).

The high-level language, FOCAL, was modified and extended with appropriate software to accept data under real-time control of the line-frequency clock. Modified subprograms have been added to permit program and data storage on, and retrieval from, the cassette tape unit. Direct access features of the tape transport permit storage of data from many analyses for more extensive correlation and study, as well as reprocessing of the original data with additional data processing techniques.

4.2.2 Computer Software

The programs used with the on-line computer of GeMSAEC Fast Analyzers were developed with the FOCAL programming language (i.e., A Conversational Language developed by the Digital Equipment Corporation). FOCAL programs for rotor calibration, end-point, and enzymatic rate analyses have been previously discussed.¹ Newly developed programs include those for end-point and kinetic enzymatic substrate analyses. (Note: The chemical and stoichiometric bases for these analyses were discussed in an earlier progress report.¹) Basically, all of the FOCAL programs developed for use with Fast and GØ Analyzer programs are similar in that each of them (1) obtains preliminary information concerning the analysis such as date, procedure employed, technician who performed the work, delay time before

initial data acquisition, reaction time, number of readings, concentration of standards (if employed), etc.; (2) uses the preliminary information to set up the computer clock and data-taking functions; and (3) awaits relay contact closure at the initiation of the reaction and then obtains data, in a useful format, on a teletype unit or display scope. The programs for enzymatic substrate analysis are, in essence, subroutines of a basically flexible and versatile GeMSAEC programming format and have been specifically designed for enzymatic substrate analysis in clinical chemistry.

Enzymatic End-Point Analysis. - The most common type of enzymatic substrate analysis is the end-point or equilibrium method, where the reaction is allowed to go to completion. For spectrophotometric analysis, the initial absorbance (A_0) and the final absorbance (A_f) of a coenzyme, substrate, or product are obtained, and the initial concentration of the unknown substrate is related directly to the absorbance change ($A_f - A_0$) either by using a standard curve or by multiplying ΔA by a substrate factor.

A problem associated with conventional enzymatic end-point determinations is the requirement of analyzing a separate sample containing no reagent to determine the initial absorbance (A_0). This problem is minimized with computer-interfaced Fast and GØ Analyzers since, with their use, absorbance readings can be obtained in the first few seconds after reaction initiation and used to extrapolate to zero time to obtain A_0 . This is easily accomplished in these systems by the use of a clock reset subroutine which allows a variable rate of data acquisition. Employing this function enables several absorbance readings to be obtained in the early stages of a reaction; then, with the clock reset, a considerable length of time can pass before the final data points are taken. This option serves to conserve core data storage by obtaining the maximum amount of useful information during the initial reaction, ignoring the intermediate data, and obtaining a final accurate absorbance reading. Figure 17 shows the reaction-progress curve for a hexokinase-glucose determination, illustrating how the initial data could be used, by extrapolation to $T = 0$, to obtain an A_0 .

The FOCAL program that was written to perform enzymatic end-point analysis is shown in Tables 3 and 4. Basically, the program establishes

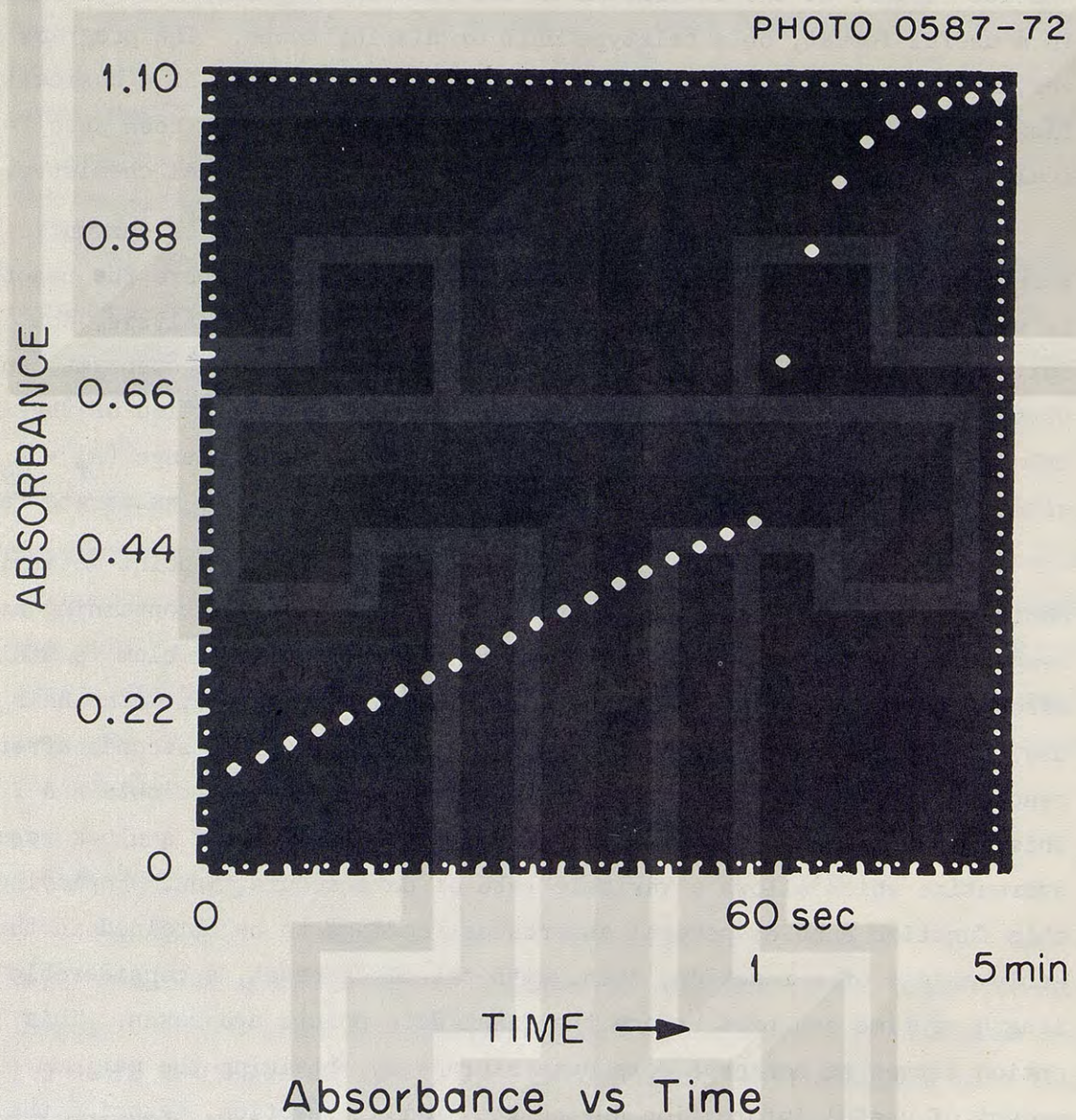


Fig. 17. Variable Rate of Data Acquisition for Glucose (Hexokinase/GDP) Determination.

Table 3. FOCAL Source Program Used for
Enzymatic End-Point Analysis (Part 1)

C-FOCAL 69-GEMSAEC 6/21/71

```

01.01 C - END-POINT ANALYSIS OF SUBSTRATE PROGRAM TOT CAB 11/71
01.02 E
01.08 T !!!
01.10 A "GEMSAEC UNIT?"UN;S NC=FITR(UN);S ST=FITR(100*(10*(UN-NC)-1))
01.12 I (ST) 1.1;I (400-ST) 1.1;I (NC-15) 1.1,1.14;I (NC-42) 1.1,1.14,1.1
01.14 A !!!"PROCEDURE",P
01.16 A !!!"DATE",P
01.18 A !!!"OPERATOR",P
01.20 A !!!"RUN NUMBER",P
01.22 A !!!"IS SUBSTRATE FACTOR KNOWN?",A
01.26 I (A-0YES) 1.28,1.44,1.28
01.28 I (A-0NO) 1.22,1.30,1.22
01.30 T !!!"ENTER PARAMETERS FOR SUBSTRATE FACTOR"
01.32 A !!!"MOLECULAR WEIGHT",MW
01.34 A !!!"SAMPLE VOLUME (ML)",VS
01.36 A !!!"TOTAL VOLUME (ML)",VT
01.38 A !!!"MOLAR ABSORPTIVITY",EX
01.40 A !!!"PATH LENGTH",PL
01.41 S SF=MW*VT*100/(EX*VS*PL)
01.42 T !!!"SUBSTRATE FACTOR =",%8.02,SF
01.43 GOTO 1.46
01.44 A !!!"SUBSTRATE FACTOR",SF
01.46 S DI=12.5;S T1=21;S N1=11;S N2=1;S TT=(T1/N1)*60;S SC=20
01.50 A !!!"REACTION TIME",RT;I (RT-70/60) 1.50,1.50;I (20-RT) 1.50
01.60 S MN=FITR(RT-33.5/60);S CY=(RT-33.5/60-MN)*3600
01.80 D 27;G 4.01
01.90 Q

04.01 C - SET UP AND TAKE DATA
04.06 S D=FCLK(,,,);S D=FTAK(,NC);S D=FTAK(10,N1,NC)
04.11 S N=(NC+1)*N1;T !!!"COMPUTER IS READY!!"
04.16 I (FCON(2))4.16,4.26;Q
04.26 S D=FCLK(0,DI*60,0,TT,1)
04.31 S D=FGET(N);S D=FTAK(SC,N2,NC);S D=FCLK(MN,CY,MN,CY,1)
04.32 S GG=-1
04.36 L G 28,G 29.01
04.41 A !!!"IS ANALYSIS COMPLETE?",A; I (A-0NO) 4.46,1.20,4.46
04.46 Q

27.01 C-DL FOR DETERMINANT & KK & KC
27.06 S X0=N1;S X1=N1*(N1+1)/2;S X2=X1*(2*N1+1)/3
27.11 S X3=X1+2;S X4=X2*(3*N1+2+3*N1-1)/5
27.16 S DL=X0*(X2*X4-X'0)-X1*(X1*X4-X2*X3)+X2*(X1*X3-X2*X2)
27.21 R

```


Table 4. FOCAL Source Program Used for
Enzymatic End-Point Analysis (Part 2)

C-FOCAL 69-GEMSAEC 6/21/71

01.01 C-PART 2 OF END-POINT SUBSTRATE PROGRAM TOT CAB 11/71
01.02 Q

07.01 C - EVALUATE ABS
07.06 S $S3 = (NC+1) * (N-1)$; S $S1 = FGET(S3+1)$; S $S2 = FGET(S3+2) - S1$
07.11 I $(-S2) * 7.31$; T ! "BLANK ERROR"; Q
07.31 S $SN = FGET(S3+J+1) - S1$
07.41 I $(-SN) * 7.51$; S $AB(J) = -999.99$; R
07.51 S $AB(J) = .4343 * FLOG(S2 / SN * FSTR(ST+J))$

15.01 C-CALCULATE ABSORBANCE FROM A0, A1, ANS A2
15.04 S $XP = 1$
15.06 A ! "REACTION TIME IN SECONDS?", X1
15.08 T "CUVET AF AD A1 A2 !!!"
15.11 F $J = 2, NC$; S $Y1 = 0$; S $Y2 = 0$; S $Y3 = 0$; D 29.91; D 28; D 16
15.16 S $XP = 1$; G 15.06
15.18 Q

16.12 S $AZ = AD + 60 * A1 * XT + 60 * A2 * (XT^2)$
16.14 T ! %2, J, " ", %6.04, AZ, " ", AD, " ", A1, " ", A2
16.15 R

27.71 S $N = N1 + N2$; D 7; S $CN = FABS(AB(J) - A0) * SF$; D 29.81; D 29.82

28.01 C-CALCULATE COEFFICIENTS
28.02 I (XP) 28.06, 28.04, 28.06
28.04 D 28.06; S $A0 = AD$; R
28.06 S $AD = [Y1 * (X2 * X4 - X3 * X3) - Y2 * (X1 * X4 - X2 * X3) + Y3 * (X1 * X3 - X2 * X2)] / DL$
28.11 S $A1 = [X0 * (Y2 * X4 - X3 * Y3) - X1 * (Y1 * X4 - X2 * Y3) + X2 * (Y1 * X3 - X2 * Y2)] / (DL * DT)$
28.16 S $A2 = [X0 * (X2 * Y3 - X3 * Y2) - X1 * (X1 * Y3 - X2 * Y2) + Y1 * (X1 * X3 - X2 * X2)] / (DL * DT + 2$
28.18 S $TD = DI - DT$
28.21 S $A0 = AD - A1 * TD + 2 * A2 * (TD^2)$
28.29 R

29.01 C - CALCULATE KBARS & CONCENTRATIONS
29.02 S $GG = 0$
29.26 D 29.76; F $J = 2, NC$; S $Y1 = 0$; S $Y2 = 0$; S $Y3 = 0$; D 29.91; D 28; D 27.71
29.51 T !!!; L G 27, G 4.41
29.76 T ! "CUVET CONCENTRATION(MG/DL) AF A0 AF-A0 AD!!!"
29.81 T ! %2, J, " ", %6.01, CN, " ", %6.03, AB(J), " ",
29.82 T %6.03, A0, " ", AB(J) - A0, " ", AD(J)
29.91 F $N = 1, N1$; D 7; S $Y1 = Y1 + AB(J)$; S $Y2 = Y2 + AB(J) * N$; S $Y3 = Y3 + AB(J) * N + 2$

the necessary analytical information and asks for the substrate factor $[\text{mg dl}^{-1} (\text{absorbance unit})^{-1}]$. If unknown, the computer calculates it from parameters supplied by the technician. The initial absorbance, A_0 , is computed by extrapolation; then, after a time delay based on the reaction time supplied to the computer by the technician, the final absorbance is obtained. Subsequently, the difference in absorbance ($A_f - A_0$) is multiplied by the substrate factor and the substrate concentration is printed out, in units of mg/dl, according to cuvet number. Typical output obtained from the program is shown in Table 5.

To evaluate the performance of this program, glucose (hexokinase), uric acid (uricase), and urea nitrogen (urease) determinations were performed on a 15-place GeMSAEC Fast Analyzer. Reference solutions of urea nitrogen and glucose were prepared as previously described,⁸ and three reference solutions were analyzed using the enzymatic end-point program. The data obtained (Table 6) indicate that use of the Fast Analyzer, coupled with a computer analysis of the data, yields results accurate to within 1 to 2%.

Several replicate samples of normal and supranormal control sera were analyzed to establish the in-run precision of the analyses. These results are given in Table 7. The accuracy and precision of the results shown in Tables 6 and 7 are reasonable. We are currently using the glucose procedure on a routine basis in the ORNL Health Division and find that application of the program is simple. The elimination of calibration standards allows a greater number of samples to be run per disk. (Three controls are routinely included per transfer disk.) The run-to-run variation is of the order of 2 to 4% in the normal range.

The analysis of uric acid, using bacterial uricase and the end-point program, produces an in-run precision of 2 to 3% in the supranormal range and 3 to 4% in the normal range.

Kinetic and Linear Fixed-Time Enzymatic Substrate Analysis. - The kinetic rate program and the fixed-time program have been combined to correlate one against the other under similar reaction conditions. The rationale for this combination is that the rate program will mainly be concerned with initial rate data, where less than 5% of the reaction is

Table 5. Typical Computer Output Obtained from
the Enzymatic End-Point Program

GEMSAEC UNIT?:15.3

PROCEDURE:GLU

DATE:17

OPERATOR:CM

RUN NUMBER:1

IS SUBSTRATE FACTOR KNOWN?:YES

SUBSTRATE FACTOR:580.65

REACTION TIME:5.0

IS SWITCH IN SINGLE POSITION?:YES

COMPUTER IS READY!

CUVET	CONCENTRATION (MG/DL)	AF	A0	AF-A0	AD
N					
2	68.4	0.143	0.025	0.118	0.000
3	192.5	0.360	0.028	0.332	0.000
4	71.2	0.136	0.014	0.123	0.000
5	89.8	0.165	0.011	0.155	0.000
6	82.5	0.150	0.008	0.142	0.000
7	94.6	0.166	0.003	0.163	0.000
8	84.2	0.146	0.001	0.145	0.000
9	80.6	0.144	0.005	0.139	0.000
10	100.1	0.181	0.008	0.172	0.000
11	96.5	0.168	0.002	0.166	0.000
12	95.0	0.173	0.010	0.164	0.000
13	93.5	0.173	0.012	0.161	0.000
14	107.2	0.192	0.007	0.185	0.000
15	102.9	0.178	0.001	0.177	0.000

IS ANALYSIS COMPLETE?:NO

Table 6. Calculated Substrate Concentrations, Using
Enzymatic End-Point Substrate Analysis

Reference Solution Conc. (mg/dl)	Calculated Substrate Conc. ^a (mg/dl)	Relative Error (%)
A. Glucose ^b		
50.0	49.5	1
100.0	99.1	1
150.0	155.3	4
200.0	201.4	0.7
400.0	390.2	2.4
B. Urea Nitrogen ^c		
10.0	10.0	-
20.0	20.4	2
30.0	30.3	1
40.0	40.2	0.5

^aSubstrate concentration = [substrate factor] x ΔA .

^b1:20 dilution of glucose reference solutions.

^c1:50 dilution of NBS urea reference solutions.

completed, and the fixed-time program will monitor longer portions of the reaction since time is not a critical factor (because of all the processes proceeding essentially in parallel). The kinetic rate program asks for and establishes the necessary analytical information, including the concentrations of four reference standards used for calibration. Either the initial rate (i.e., the velocity of the reaction as time approaches zero) or a rate at a given time, t , can be obtained by using an appropriate delay time interval before data acquisition. A calibration factor is calculated by dividing the rate by the concentration of the corresponding standard and obtaining a mean value of the calibration factor. The product of the rate of the unknown and the mean value of the calibration factor is the concentration of the unknown; and this value is printed out by cuvet, in appropriate concentration units, along with the rate.

Table 7. End-Point Analyses Showing the In-Run Precision of Data

Glucose Concentration of Reference Solution (mg/dl)	Sigma	C.V. (%)	N
Glucose Analysis (Hexokinase); reaction time, 4 min			
66.5	1.4	2.0	10
130.0	1.8	1.4	11
189.5	2.1	1.1	5
Urea Nitrogen Analysis; reaction time, 10 min			
15.0	0.2	1.2	7
34.6	0.5	1.2	11

The fixed-time program also requires standard reference compounds for calibration purposes; however, the number of standards can be varied. The first several cuvetts (2 to 6) are used for calibration with reference

standard solutions. For linear fixed-time analysis, the absorbance change for each standard over the fixed-time interval is divided by the corresponding concentration of that standard, and the mean value of $\Delta A /$ concentration is used as a calibration factor. The substrate concentration is obtained by multiplying this factor by the absorbance change over the fixed time of each unknown time interval. Concentrations, in units of mg/dl, are printed out in an acceptable format. The FOCAL program written for this purpose requires two storage files, which are listed in Tables 8 and 9.

To evaluate the programs, urea nitrogen was determined in normal and supranormal control sera using the 15-place GeMSAEC Fast Analyzer. The results of the rate analysis for urea nitrogen in the normal control serum (12.7 ± 2 mg/dl) was 12.9 ± 0.7 mg/dl, with a relative standard deviation of 5.1%; for the rate analysis of the supranormal control (34.3 ± 2 mg/dl), the result was 35.6 ± 1.0 mg/dl, with a relative standard deviation of 2.9%. The result of the fixed-time analysis of urea nitrogen for the normal control serum (12.7 ± 2 mg/dl) was 13.4 ± 0.3 mg/dl, with a relative standard deviation of 0.8%.

Table 10 gives a comparison of the three analytical approaches: end-point, rate, and fixed time. In the case of urea analysis, the fixed-time approach is shown to be the method of choice.

Nonlinear Fixed-Time. - An additional program, which is actually a modified approach to the fixed-time kinetic enzymatic substrate analysis has been written. This program, called the nonlinear fixed-time program, uses a simple numerical analysis method to bracket the absorbance change of the unknown within the absorbance change of two standards such that the absorbance change of the unknown is greater than the absorbance change of one of the standards but less than that of the next-higher-concentrated standard. A linear interpolation is made between these two standards to find the concentration of the unknown. This concentration is then printed out in units of milligrams per deciliter. The calibration standards are chosen to fit a range corresponding to approximately 95% of all samples (for glucose, 0 to 400 mg/dl) to be encountered; absorbance changes for any unknowns greater than the absorbance change of the highest standard are labeled "out of range".

Table 8. FOCAL Source Program Used for Kinetic and Linear
Fixed-Time Enzymatic Substrate Analysis (Part 1)

C-FOCAL 69-GEMSAEC 6/21/71

```

01.01 C - VARIABLE REACTION ORDER RATE PROGRAM  JMJ 09/17/71
01.02 E
01.08 T !!!
01.10 A "GEMSAEC UNIT?"UN;S NC=FITR(UN);S ST=FITR(100*(10*(UN-NC)-1))
01.12 I (ST) 1.1;I (400-ST) 1.1;I (NC-15) 1.1,1.14;I (NC-42) 1.1,1.14,1.1
01.14 A " REACTION ORDER"RE;S NG=48/FITR(NC/15)
01.15 A "IS INITIAL RATE PROGRAM REQUIRED?"P
01.16 I (P-0YES) 1.17,1.20,1.17
01.17 I (P-0NO) 1.15,1.19,1.15
01.19 S IR=1;
01.20 A !!"TIME INTERVALS"!
01.25 A " DELAY TIME INTERVAL"DI;I (DI) 1.2,1.2;I (60-DI) 1.2
01.30 A " INITIAL RATE TIME INTERVAL-"?T1?
01.40 A " FINAL RATE TIME INTERVAL-"?T2?;I (T2) 1.4, 1.4;I (900-T2) 1.4
01.50 A !!"SAMPLES/INTERVAL & SAMPLES/CUVET"!
01.60 A " #IN T1"N1;I (N1-3) 1.6;I (NG-N1) 1.6
01.70 A " #IN T2"N2;I (N2) 1.7,1.7;I (NG-N1-N2) 1.5
01.71 A " #SAMPLES/CUVET IN T2"SC;I (SC-1)1.71;I (20-SC)1.71
01.72 S D1=60*T1/(N1-1);I (D1-15) 1.16;S DT=D1/60
01.74 S T=FITR(60*T2);S D2=FITR(T/N2);I (D2-8*(SC+1)) 1.16
01.76 S Z1=FITR((D2-6)/3600);S Z2=(D2-6)-3600*Z1
01.78 S Z3=FITR(D2/3600);S Z4=D2-3600*Z3
01.80 D 2;D 3;D 27;G 4.01
01.90 Q

02.01 C - GET STANDARDS
02.06 A !!"CONCENTRATIONS OF STDS (MG/100ML)"!
02.11 F J=2,5;D 2.91
02.16 R
02.91 T " CN("%2,J,")";A C0(J);I (C0(J)-1) 2.91;I (500-C0(J)) 2.91;R

03.01 C - GET FIRST ORDER PARAMETERS
03.06 I (RE-1) 3.26, 3.11, 3.26
03.11 A !!"FIXED-TIME INTERVAL"!
03.16 T " T11(",%2,0," TO",N2-1,")";A I1;I (I1) 3.16;I (N2-1-I1) 3.16
03.21 T " T12(",%2,I1+1," TO",N2,")";A I2;I (I2-I1+1) 3.21;I (N2-I2) 3.2
03.25 I (I2-I1) 3.11, 3.11, 3.26
03.26 R

04.01 C - SET UP AND TAKE DATA
04.06 S D=FCLK(,,,);S D=FTAK(,NC);S D=FTAK(10,N1,NC)
04.11 S N=(NC+1)*N1;T !!"COMPUTER IS READY!"!
04.16 I (FCON(3))4.16,4.26;Q
04.26 S D=FCLK(0,DI*60,0,D1,1)
04.31 S D=FGET(N);S D=FTAK(SC,N2,NC);S D=FCLK(Z1,Z2,Z3,Z4,1)
04.32 S GG=-1
04.36 L G 38,G 29.01

05.01 C-SELECT ANOTHER FIXED TIME INTERVAL
05.02 A !!"DO YOU WISH TO SELECT ANOTHER FIXED TIME INTERVAL?"P
05.04 I (P-0YES) 5.06,5.10,5.06
05.06 I (P-0NO) 5.01,5.08,5.01
05.08 Q
05.10 D 3;L G 38,G 29.01
05.12 R

27.01 C-DL FOR DETERMINANT & KK & KC
27.06 S X0=N1;S X1=N1*(N1+1)/2;S X2=X1*(2*N1+1)/3
27.11 S X3=X1+2;S X4=X2*(3*N1+2+3*N1-1)/5
27.16 S DL=X0*(X2*X4-X3*X3)-X1*(X1*X4-X2*X3)+X2*(X1*X3-X2*X2)
27.21 R

```


Table 9. FOCAL Source Program Used for Kinetic and Linear
Fixed-Time Enzymatic Substrate Analysis (Part 2)

C-FOCAL 69-GEMSAEC 6/21/71

```

01.01 C - PART 2 OF VAR REACTION ORDER PGR    MJM 09/17/71
01.02 Q

07.01 C - EVALUATE ABS
07.06 S S3=(NC+1)*(N-1); S S1=FGET(S3+1); S S2=FGET(S3+2)-S1
07.11 I (-S2)7.31; T !"BLANK ERROR";0
07.31 S SN=FGET(S3+J+1)-S1
07.41 I (-SN)7.51; S AB(J)=-999.99;R
07.51 S AB(J)=.4343*FLOG(S2/SN*FSTR(ST+J))

27.51 S KK=A1/[FEXP(RE*FLOG(C0(J)>))] ; S K1=K1+KK; S K2=K2+KK+2
27.71 IF (A1/KB) 27.72,27.72; S CN=FEXP(FLOG(A1/KB)/RE)
27.72 S CN=-99.99
27.81 S DA=DA-AB(J); S KC=DA/C0(J); S K1=K1+KC; S K2=K2+KC+2; D 29.71
27.91 S DA=DA-AB(J); S CN=DA/KB; D 29.66

28.01 C-CALCULATE COEFFICIENTS
28.06 S A0=[Y1*(X2*X4-X3*X3)-Y2*(X1*X4-X2*X3)+Y3*(X1*X3-X2*X2)]/DL
28.11 S A1=[X0*(Y2*X4-X3*Y3)-X1*(Y1*X4-X2*Y3)+X2*(Y1*X3-X2*Y2)]/(DL*DT)
28.16 S A2=[X0*(X2*Y3-X3*Y2)-X1*(X1*Y3-X2*Y2)+Y1*(X1*X3-X2*X2)]/(DL*DT+2
28.21 R

29.01 C - CALCULATE KBARS & CONCENTRATIONS
29.02 S GG=0
29.03 IF (IR-1) 29.06,29.30,29.06
29.06 S K1=0; S K2=0; D 29.86
29.11 F J=2,5; S Y1=0; S Y2=0; S Y3=0; D 29.91; D 28; D 27.51; D 29.96
29.16 S KB=K1/4; S KS=FSQT(FABS(4*K2-K1*K1)/12)
29.21 T !"K BAR ",%8.03,KB," STD. DEV. OF K BAR ",%8.06,KS,!!
29.26 D 29.76; F J=6,NC; S Y1=0; S Y2=0; S Y3=0; D 29.91; D 28; D 27.71; D 29.81
29.30 IF (RE-1) 29.55, 29.31, 29.55
29.31 S K1=0; S K2=0; T !!!!!"CUVET KC=[A!TI2 - A!TI1]/C0"!!
29.36 F J=2,5; S N=N1+I1; D 7; S DA=AB(J); S N=N1+I2; D 7; D 27.81
29.41 D 29.16; D 29.21
29.46 D 29.76; F J=6,NC; S N=N1+I1; D 7; S DA=AB(J); S N=N1+I2; D 7; D 27.91
29.55 T !;;L G 37,G 5.01
29.66 T !%2,J," ",%6.01,CN," "%6.03,DA
29.71 T !%2,J,%6.03,? KC?
29.76 T !"CUVET CONCENTRATION[MG/100ML] A2-A1"!!
29.81 T !%2,J," ",%6.01,CN," "%6.03,A0," "%7.05
29.86 T !!"CUVET ABS=A0+A1*T+A2*T+2 KK=[(DA/DT!T=0)/C0+N?
29.91 F N=1,N1; D 7; S Y1=Y1+AB(J); S Y2=Y2+AB(J)*N; S Y3=Y3+AB(J)*N+2
29.96 T !%2,J,%6.03,? A0, ?%,?A1, A2, KK?

```


Table 10. Comparison of Results Obtained with Three Approaches to the Analysis of Urea Nitrogen

	End-Point	Kinetic	
		Fixed-Time	Rate
Analysis time, min	15-20	2	1.5
Calibration	Substrate factor	Reference stds.	Reference stds.
Results:			
<u>Sample A^a</u>			
$\bar{X}$, mg/dl	11.7	13.3	12.9
Sigma, mg/dl	0.2	0.3	0.7
C.V., %	1.5	2.0	5.0
<u>Sample B^b</u>			
$\bar{X}$, mg/dl	34.6	34.3	35.6
Sigma, mg/dl	0.5	0.3	1.0
C.V., %	1.2	0.8	2.9

^a1:20 dilution of normal control serum (12.7 ± 2 mg/dl).

^b1:20 dilution of abnormal control serum (34.3 ± 2 mg/dl).

5. CHEMISTRY

Several procedures routinely employed in the clinical chemistry laboratory have been adapted for use in the GØ Analyzers, and protocols have been written for them. Wherever possible, commercially available chemical kits have been utilized. The general chemical development scheme has been to develop the protocols by, first, using them with the 15-place GeMSAEC Fast Analyzer and, then, scaling them down for evaluation with the GØ Analyzer. For example, protocols have been developed for serum albumin, ammonia, bilirubin, blood urea nitrogen (BUN), calcium, glucose, inorganic phosphate, total protein, uric acid, alkaline phosphatase, CPK, LDH, SGOT, and SGPT.¹ The protocols for the enzymes were evaluated using the Mark I GØ Analyzer.¹ During this report period, we began the final development of the more difficult chemistries and their evaluation using the Mark I GØ Analyzer. Those being considered include blood urea nitrogen, glucose, calcium, bilirubin, acid phosphatase, and inorganic phosphate.

5.1 Blood Urea Nitrogen

The urease--glutamate dehydrogenase procedure for urea nitrogen has been evaluated on the Mark I Analyzer by analyzing aqueous urea reference solutions and commercial control sera at two concentrations. The samples were diluted 1:50 with saline, and a sample volume of 25 µl was used. The reagent volume was 100 µl.

The reagent was a commercially prepared (Calbiochem) vial of urea nitrogen Stat-Pack prepared at 200% of the recommended concentration. This reduces the analysis time by at least one-third and increases the range of the analysis. The sample was dispensed using a 25-µl Eppendorf pipette (24.9 ± 0.3 µl), and the reagent was dispensed using a Micromedic pipette set to deliver 100 µl (98.8 ± 0.4 µl).

Multiple samples of Hyland Control Sera I and II were run on separate rotors (11 samples per disk) to establish the in-run variation. Included in each run were duplicate samples of aqueous 25-mg/100 ml and 50-mg/100 ml standards to establish the accuracy of the analysis. Three runs were

performed using Hyland Control Sera I to establish the disk-to-disk variation. The analyses were carried out over a 10-min period. Initial absorbance and final absorbance were obtained using the enzymatic end-point substrate program (see Sect. 4.2.2), and the concentration was obtained by multiplying ΔA ($A_{\text{final}} - A_{\text{initial}}$) by a substrate factor. The data were obtained and calculations were made on-line. The use of the substrate factor eliminates the requirement of calibration standards. The data are shown in Tables 11 and 12.

The results indicate that this method is capable of providing accurate and precise analytical results, using the miniature analyzer. The results further indicate that single-sample urea nitrogen determinations can be performed with reasonable accuracy and precision, without the requirement of calibration reference standards.

5.2 Glucose

The problems associated with the use of hexokinase glucose-6-phosphate dehydrogenase assay procedures for the determination of glucose have been previously discussed.⁸ These problems arise chiefly from the rapid nature of the hexokinase reaction, coupled with the requirement for absorbance readings early in the reaction in order to obtain an extrapolated initial sample absorbance. Therefore, we have investigated a variety of reaction conditions, using commercial kits; the results are shown in Table 13. The objectives were to find the proper reagent dilution such that a moderate range of glucose values could be measured without encountering substrate depletion and to determine conditions that would enable the reaction to be completed in 5 min, yet allow the reaction to proceed slow enough initially to obtain a representative initial absorbance reading corresponding to a feasible time in which a reading could be made on the GØ Analyzer. Conditions listed under E in Table 13 best fit these requirements. Using these conditions, values were obtained using 1:25, 1:20, 1:15 and 1:10 dilutions of commercial control serum (manufacturer's stated glucose concentration, 80 mg/dl). Sixteen samples were run for each dilution on the Mark I, and the results are shown in Table 14.

Table 11. Analytical Results for Blood Urea Nitrogen^a

Sample	Expected Value (mg/dl)	Determined Value ^b (mg/dl)
Control Serum I (Hyland I)	15.0 $\pm$ 2	15.5
Control Serum II (Hyland II)	49.0 $\pm$ 2	48.9
Aqueous Std. I	25.0	25.9
Aqueous Std. II	50.0	49.2

^a Enzymatic end-point substrate analysis program (reaction time = 10.0 min).

^b Values obtained by multiplying the absorbance change by a substrate factor (F), which was calculated using the following expression:

$$F \text{ (mg dl}^{-1} \text{ A}_{340}^{-1}) = \frac{(\text{mol. wt. of NH}_3) \quad (\text{total volume}) \quad (100)}{(\text{molar absorp. coeff.}) \quad (\text{sample volume}) \quad (\text{pathlength})}$$

Table 12. Precision of Blood Urea Nitrogen Analysis

Sample	Average Concentration (mg/dl)	Standard Deviation	Coefficient of Variation (%)
<u>Within-Rotor</u> Variation (11 Samples)			
Hyland I (15.0 $\pm$ 2 mg/dl)	15.5	0.34	2.18
Hyland II (49.0 $\pm$ 2 mg/dl)	48.9	0.70	1.45
<u>Rotor-to-Rotor</u> Variation (3 runs; 11 samples/run)			
Hyland I	15.6	± 0.42	2.71

Table 13. Effect of Assay Conditions on the Analytical Results Obtained from the Hexokinase-Glucose Analysis Using the Mark I GØ Analyzer

	Run					
	A	B	C	D	E	F
Substrate factor	1940	1540	710	1540	1540	1540
Reaction volumes, μ l						
Sample ^a	10	10	10	10	10	10
Reagent	60	60	60	120	60	120
Diluent	60	60	60	0	60	0
Reagent:sample ratio	6:1	6:1	3:1	12:1	6:1	12:1
Reaction time (min) ^b	10	10	10	10	5	5
Analytical results (mg glucose/dl):						
<u>Expected</u>	<u>Measured</u>					
64		68.0	67.0	66.0	68.8	57.2
80	84.2	87.8	84.6	85.0	87.2	74.0
106	113.7	114.8	112.5	114.2	116.8	100.8
159		173.3	161.9	172.8	173.8	151.0
50	48.7	52.0	50.1	50.5	50.4	43.0
100	99.5	100.0	97.8	98.9	100.8	87.0
150	154.3	153.0	145.6	149.5	153.9	134.0
200	197.8	204.9	185.0	198.3	199.7	179.0

^aSample volume = volume of sample previously diluted 1:20 with distilled water.

^bReaction time = total reaction time following an initial delay of 8 sec.

Table 14. Precision and Accuracy of the Hexokinase-Glucose Procedure Using the Mark I GØ Analyzer

Stated Glucose Concentration (mg/dl) ^a	Measured Mean Concentration (mg/dl) ^b	Sigma	C.V. (%)
64	65.2	0.7	1.1
80	81.4	1.6	2.0
106	106.5	1.4	1.2
159	163.1	1.4	0.8

^aReaction conditions: sample volume = 10 μ l, respectively, of 1:25, 1:20, 1:15, and 1:10 dilutions of a commercial control serum; diluent volume = 70 μ l; reagent volume = 50 μ l (120% concentrated Calbiochem Glucose Reagent); delay interval = 8 sec; reaction time = 5 min.

^bN = 16.

These data indicate that the hexokinase-glucose method can be run on the Mark I instrument with a high degree of accuracy and precision without the necessity of employing calibration standards. This is an important concept for single-sample:multiple-chemistry determinations.

5.3 Calcium

A kit for the determination of serum calcium was obtained (Calcium Rapid Stat, Pierce Chemical Co., Rockford, Illinois) for evaluation. This kit is based on a dye binding method utilizing methyl thymol blue as the dye. This dye will bind serum calcium, with the resultant absorbance change at 612 nm being proportional to the calcium concentration. To evaluate this procedure, aqueous calcium standards were prepared and analyzed using the Pierce Kit in conjunction with the 15-place GeMSAEC Fast Analyzer. Using a 500- μ l reagent volume and a 50- μ l sample volume (actually 50 μ l of a 1:5 dilution of the calcium standards), the kit was found to be linear over a 6- to 12-mg/dl range of calcium concentrations. The precision with aqueous samples and serum controls was 2 to 4% in the 8- to 10-mg/dl range. This method is currently being scaled down for use in the miniature analyzer.

5.4 Bilirubin

Two general bilirubin methods are available for adaptation and scaledown for use with the G ϕ Analyzers. These are: (1) diazo-coupled procedures, and (2) direct spectrophotometric determination. The diazo procedures are subject to hemoglobin interference and are, therefore, of limited value when hemolyzed samples are encountered. We have investigated the effect of hemolysis on the diazosulfanilic acid assay procedure for determining bilirubin. Using a 15-place GeMSAEC Fast Analyzer as a scanning spectrophotometer, we have compared these results with those obtained from a direct, spectrophotometric bilirubin assay procedure. As shown in Table 15, hemoglobin strongly interferes with the diazosulfanilic acid procedure, with a resulting loss in measured bilirubin concentration. With the direct spectrophotometric procedure, however, hemoglobin interference can be minimized by determining the absorbance at 455 and 535 nm

Table 15. Hemoglobin Interference of the Reaction of Bilirubin with a Stabilized Diazosulfanilic Acid Salt

Sample	$\bar{A}_{550}$
(1) 100 μ l of bilirubin standard (1.5 mg/dl); 500 μ l of diazosulfanilic acid reagent	0.155 ^a
(2) 100 μ l of bilirubin standard (4.5 mg/dl); 500 μ l of diazosulfanilic acid reagent	0.468 ^b
(3) 100 μ l of bilirubin standard (1.5 mg/dl); 500 μ l of diazosulfanilic acid reagent; 5 μ l of hemoglobin solution ^d	0.413 ^{b,c}
(4) 100 μ l of bilirubin standard (4.5 mg/dl); 500 μ l of diazosulfanilic acid reagent; 5 μ l of hemoglobin solution	0.645 ^e
(5) 100 μ l of H ₂ O; ^f 500 μ l of diazosulfanilic acid reagent; 5 μ l of hemoglobin solution	0.302

^aAll samples were scanned simultaneously from 400 nm to 600 nm using a GeMSAEC Fast Analyzer and monochromator. (PM voltage was adjusted to 9.0 V each time the monochromator was incremented 5 nm in wavelength.)

^bMean value of analyses run in triplicate.

^cPercent decrease in absorbance = $\frac{0.155 - (0.413 - 0.303)}{0.155} \times 100 = 38\%$.

^dHemoglobin concentration in reaction is 93 mg/dl or $\approx$ 600 mg/dl per 100- μ l sample.

^ePercent decrease in absorbance = $\frac{0.468 - (0.645 - 0.303)}{0.468} \times 100 = 27\%$.

^fBlank.

and using the difference between the two to calculate bilirubin concentration (Table 16). Thus, due to the multiple filter selection, coupled with the automated PM control feature of the Mark II GØ Analyzer, the direct, spectrophotometric bilirubin assay method may be easily performed on the Mark II Analyzer. An additional advantage is that, by knowing the molar absorptivity of bilirubin (68,000), the direct absorbance measurement can be related directly to concentration, thus eliminating the need for on-board calibration standards. Sensitivity of the method is adequate due to the high molar absorptivity of bilirubin. Consequently, a direct, spectrophotometric bilirubin assay procedure is being developed and adapted for use with the Mark II GØ Analyzer.

5.5 Acid Phosphatase

A kinetic acid phosphatase kit was obtained from Union Carbide Corporation for evaluation. The chemical method involves using 1-naphthyl phosphate, which is hydrolyzed by acid phosphatase to 1-naphthol and phosphate, as the substrate. The 1-naphthol, as released, is coupled with diazo-2-amino-5-chlorotoluene naphthalene-1,5-disulfonate (Fast Red), and the formation of the colored adduct is monitored kinetically at 405 nm. Hyland II control sera was assayed for acid phosphatase activity, using a 15-place GeMSAEC. The amount of tartrate-inhibitable acid phosphatase found, 89%, was in good agreement with the stated value of 90%. Comparable results were obtained when the analysis was scaled down and run on the Mark I GØ Analyzer. One disadvantage of the method is that, because of a long lag time, the analysis requires up to 18 min per rotor. A similar acid phosphatase kit, available from Smith Kline Instruments, reportedly has the advantage of a short lag time. Consequently, a sample kit will be obtained from this manufacturer and evaluated in the near future.

5.6 Future Development

Reagent kits for inorganic phosphate, magnesium, and triglycerides have been obtained and are being evaluated. The calcium, bilirubin, and acid phosphatase methods will be scaled down and evaluated with the Mark

Table 16. Effect of Hemoglobin Interference in the Direct Spectrophotometric Bilirubin Assay

Sample	A_{455}^a	A_{535}^a	$\Delta A_{455} - \Delta A_{535}$
50 μ l of bilirubin standard (7.5 mg/dl) in 1.0 ml of acidic ethylene glycol ^b	0.486	0.083	0.403
50 μ l of bilirubin standard (7.5 mg/dl) in 1.0 ml of acidic ethylene glycol ^b ; 1:100 dilution of hemoglobin standard (11.2 g/dl) ^c	0.970	0.586	0.384
1:100 dilution of hemoglobin standard (11.2 g/dl) ^c in 1.0 ml of acidic ethylene glycol ^b	0.514	0.502	0.012

^aHemoglobin in acidic solution is converted to hematin and globin, and is followed with a concomitant wavelength shift. For this reason, the absorbance is monitored at 535 nm instead of the customary 540 nm for neutral solutions of hemoglobin.

^bAcidic ethylene glycol = 10 ml of 0.25 N HCl + 50 ml of ethylene glycol.

^cHemoglobin concentration would correspond to approximately 600 mg/dl if a 100- μ l sample were diluted to 600 μ l for analysis. The hemoglobin concentration is 93 mg/dl in the sample without considering dilution factors.

II GØ Analyzer. After extensive evaluation of the rotor temperature monitor and control system, the serum enzyme procedures will be reevaluated specifically with regard to day-to-day precision and accuracy. Finally, an effort will be made to collate all of the chemical assays into one run to determine the feasibility and problems associated with multiple chemistry: single-sample analyses.

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