Z. Anal. Chem. 261, 257—271 (1972)

PRIZE LECTURE

Preis Biochemische Analytik awarded in Munich, April 25, 1972, for the development of the GeMSAEC Fast Analyzer System. Prize (10,000 D.M.) given by Boehringer Mannheim GmbH. The award is for the most significant advance in analytical or clinical chemistry worldwide over a two-year period.

Compliments of the MAN Program

The Development of Fast Analyzers

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Received June 24, 1972

Summary. New instrumentation has been developed at ORNL for analyzing physiologic specimens. The system is designed for use in clinical laboratories, hospitals, and emergency rooms and for use by researchers. Miniaturized versions may also be used in prolonged space flights.

After consideration of ease of automation, flexibility, and rapid startup, multiple analyses performed in parallel was chosen as the desired approach. Concepts employed in developing the system are discussed, including:

(1) dynamic mixing of samples and reagents through centrifugal transfer from transfer disks into cuvets; (2) rotor design allowing emptying, flushing, and drying of cuvets; (3) precise temperature control; (4) spectrophotometric reaction monitoring; (5) data reduction, with all data stored for possible recall; (6) feedback control for error correction and time saving. Possible future improvements are indicated.

Entwicklung schneller Analysatoren. Im Oak Ridge National Laboratory wurde ein neues Analysatorsystem entwickelt, das für die Analyse physiologischer Proben bestimmt ist und in klinischen Laboratorien, Krankenhäusern, Unfallstationen, Forschungsinstituten sowie in einer verkleinerten Version auch in der Raumfahrt zum Einsatz gelangen kann.

Das System zeichnet sich aus durch leichte Automatisierbarkeit, Flexibilität, rasche Inbetriebnahme und eignet sich für parallel durchgeführte Serienanalysen. Die folgenden Charakteristica des Systems werden diskutiert: 1. Dynamisches Mischen von Proben und Reagentien durch zentrifugale Übertragung in die Küvetten, 2. Entleeren, Spülen und Trocknen der Küvetten durch Rotation, 3. Genaue Temperaturkontrolle, 4. Spektrophotometrische Reaktionsüberwachung, 5. Datenberechnung mit Speicherung aller Werte für möglichen Abruf, 6. Feedback-Kontrolle zur Fehlerkorrektur und Zeiteinsparung. Mögliche zukünftige Verbesserungen werden erwähnt.

Analysatoren: Entwicklung eines schnellen Analysators für physiologische Proben.

Research in biochemistry has given, and will continue to provide at an increasing rate, new tests and new analytical procedures which we would like to see done on patients. Many tests are required for decision making in the emergency room or in the intensive care unit and hence should be done rapidly. Social pressures are increasing to extend new and improved clinical care (which also will increase the number of tests done) to all patients in more countries [20, 21, 25]. Preventive medicine is exploring the use of clinical chemistry to detect illness at an early stage so that preventive measures may be evaluated, and where useful, employed. Modern separation methods provide the medical research scientist with an increased number of fractions from tissue, blood, and urine to analyze. As more becomes known about subcellular fractions, the number of marker substances increases and so does the number of different assays the subcellular biochemist wishes done on all of his fractions. We must add to this the desires of the kineticist who suggests improvements in our analytical methods with more attention directed toward precise measurement of reaction rates under carefully controlled conditions.

In previous studies on zonal centrifuge development [1] and high-resolution chromatography [9, 24, 26] we had repeatedly experienced the problem of large analytical loads. However, until we became aware of the work load problems in clinical chemistry, we had not attacked this problem directly. At the

* The Molecular Anatomy Program is sponsored by the National Institute of General Medical Sciences, the National Cancer Institute, The National Institute of Allergy and Infectious Diseases, and the U.S. Atomic Energy Commission.

** Operated for the U.S. Atomic Energy Commission by the Nuclear Division of Union Carbide Corporation.

request of Dr. Robert Melville of the National Institute of General Medical Sciences I began to examine alternative strategies and approaches with the intention of chosing one for optimization (I use the word in the engineering sense). Parenthetically it should be noted that several very promising approaches remain to be adequately explored or exploited.

The choices which faced us initially were between discrete sample and continuous-flow analysis on the one hand, and sequential and multiple- or batch-parallel analysis on the other.

Three considerations were paramount in making this choice:

The first concerns true automation or feedback control[1]. If feedback control is to be introduced, results must be available during a reaction, i.e., the data must be reduced in real time using an on-line computer. This allows results to affect a process, which is the essence of true automation. If computers are to be used efficiently for analyses done in sequence, then either the rate of the reaction must be very considerably increased or the interval between sample readings must be shortened so that the interval is only a few seconds. Many reactions, especially those usually carried to completion, are difficult to accelerate, thus many knotty problems remain to be solved if sequential analyses are to be speeded up by an order of magnitude using either discrete or continuous-flow systems. With continuous flow, carry-over between samples becomes the major rate-limiting factor, while with discrete analysis reagent and sample measurement and movement at high speed is difficult to arrange.

A second consideration was our desire to develop systems having maximum flexibility which could be rapidly shifted from one analytical procedure to another, which did not use large amounts of reagents during the warmup and calibration period, and which could be used efficiently with one sample or with many.

Finally, startup time should also be minimal so that emergency samples can be handled quickly.

These considerations appeared to limit us to multiple analyses performed in parallel. How can a large number of reactions be arranged to all start at the same instant (actually during a time interval which is very short relative to the reaction time), and to be then all read at uniform intervals? In settling this question one also has to ask whether measurement should be part of the reaction initiation process, as is the case when a sample is delivered into a reaction vessel by blowing out a measuring pipet. Measurement and mixing have often been separated before; an early example is the Thunberg tube used for enzyme assays, where the sample was measured into a side arm and mixed with the reagent after the tube was evacuated. This principle was applied by Otto Warburg to multiple parallel analyses using his familiar flasks and manometers. Here a dexterous operator could add reagents from side arms over a relatively short interval. The same principle of keeping measured samples and reagents separated at rest and then mixing them by tipping the containers and using gravity to bring them together has recently been exploited by Burger for bacterial identification [12].

We are here seeking practical solutions to real problems, quite contrary to much of our previous training in basic research, which has been aimed at

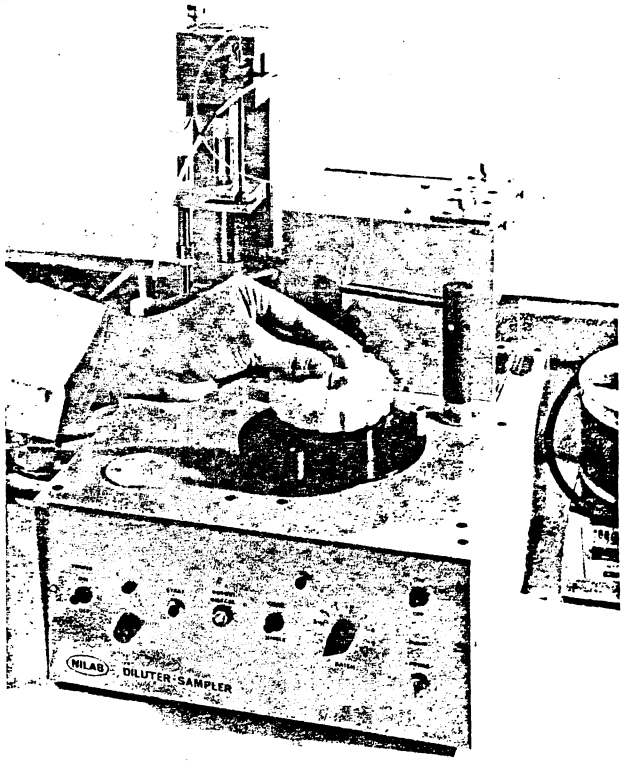

Fig. 1. Teflon transfer disk being placed on automatic loading system

1 The term automation (as distinguished from mechanization) appears to have been introduced independently in 1947 by D. S. Harder of the Ford Motor Company and by J. Diebold. For some time I attempted to get this word properly used in clinical chemistry since *NO* analytical system of which I am aware is truly automated in the proper sense. I must now admit this is a hopeless task even with close associates. We therefore require a new word, such as *Cybernetization*.

discovering basic principles in the belief that someone somewhere will quickly apply them. Implicit in this belief is the assumption that applied or structured research is easier and less original than so-called basic or unstructured research. *The unfortunate result of this view is that much applicable research does not and cannot now reach the average patient.* In music the vast majority of compositions fit a preset form and the problem has been to be original within that structure. I would tend to the view that it is more difficult to compose within the tight confines of a prescribed design or set of requirements than to produce a fantasie. In the former one must provide solutions that solve simultaneously several problems. The same is true in the development of instrument systems, where single solutions to multiple problems have an inherent beauty all their own.

Centrifugal Transfer into Cuvet Rotors

Samples and reagents can be premeasured into Teflon transfer disks, such as are shown in Fig. 1, and remain unmixed at rest [3].

Since a number of automatic sample and reagent loaders may be filling several transfer disks at the same time, this is not inherently a rate-limiting step.

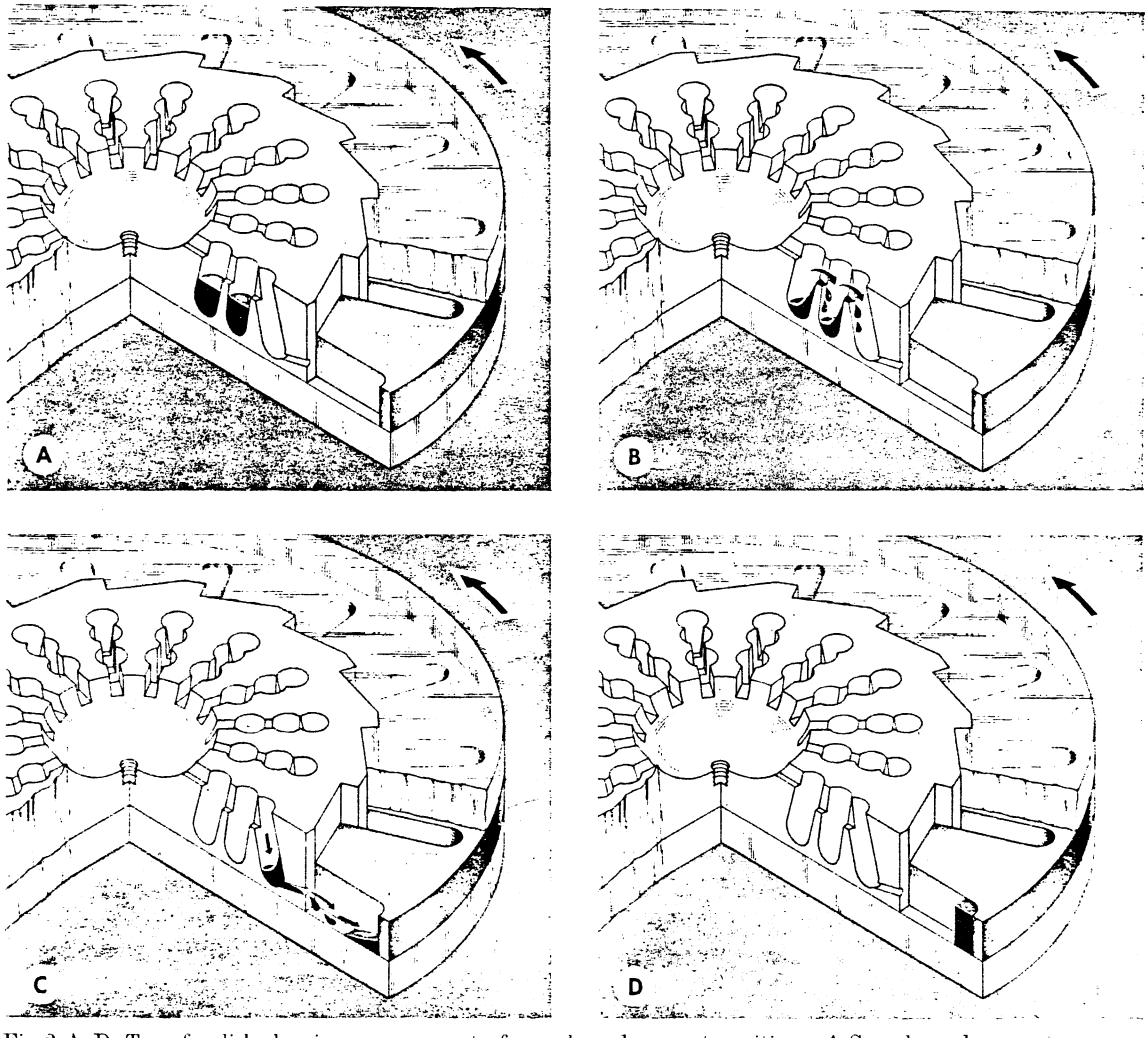

Fig. 2 A–D. Transfer disk showing arrangement of sample and reagent cavities. A Sample and reagent are measured into chambers where they remain separate and unmixed at rest and during rotation at low speed. B As the speed is increased, both liquids begin to move radially into outer chamber of transfer disk. C Liquids flow rapidly out of transfer disk and into cuvets in the cuvet rotor. Note that the transfer disk and cuvet rotate as one unit. D As the speed increases, the mixing liquids are held against the outer walls of the cuvets, and all air bubbles rapidly float out of the mixture. Optical measurements may now begin (from a paper by C. A. Burtis *et al.* [14])

When a transfer disk is spun about its axis, the sample and reagent(s) climb the walls of their compartments and flow together out peripheral holes, with a separate hole provided for each sample and reagent set. The mixture flows immediately out into a cuvet, where it is held against the peripheral wall by centrifugal force. This eliminates completely the problem of bubbles in the optical path.

All reactions start at essentially the same time, i.e., when a rotational speed sufficient to move all fluids out into the cuvets has been achieved, as shown in Fig. 2.

The sample and reagents may have slightly different specific gravities, resulting in a tendency for liquids to stratify and not mix in the cuvet. Countering this is the fact that force must be applied to liquids flowing radially to accelerate them to the tangential velocity obtaining at any given radius. The result is that liquid flows along the trailing edge of the cuvet and, as it comes into contact with liquid already in the cuvet, causes it to spin, thus promoting mixing. In addition the transfer disk may be designed so that reagent fluid mixes with the sample during transfer out of the transfer disk and washes out the sample chamber, as is particularly true of one commercial version of the system.

A second means for rapid mixing is provided, however. Small syphons are provided which connect to the sloping outer edge (or bottom) of the cuvets (Fig. 3) [4]. A burst of air or other gas may be drawn back through these syphons if the rotor is closed, or may be blown through using a special rotating seal on the bottom of the rotor. The net effect is to mix the contents of all cuvets very rapidly with a sudden burst of bubbles. Note that the mixing problem diminishes rapidly in importance as the total reaction volume is decreased; this provides an incentive to build systems having much smaller reaction volumes.

In practice, the cuvet rotor is accelerated initially at a rate which does not cause splashing of samples and reagents out of the transfer disk chambers. As the transfer speed is approached, rapid acceleration begins, and a speed is quickly attained which ensures quantitative transfer. This speed depends on the diameter of the transfer disk and the slope of the sides of the sample and reagent cavities; it is in the range of 1000—2000 rpm for a 15-place rotor. The rotor is immediately braked down to reading speed (usually 600 rpm) and a short burst of air forced through all cuvets. All reactions are thus initiated in parallel. (As discussed subsequently, much faster

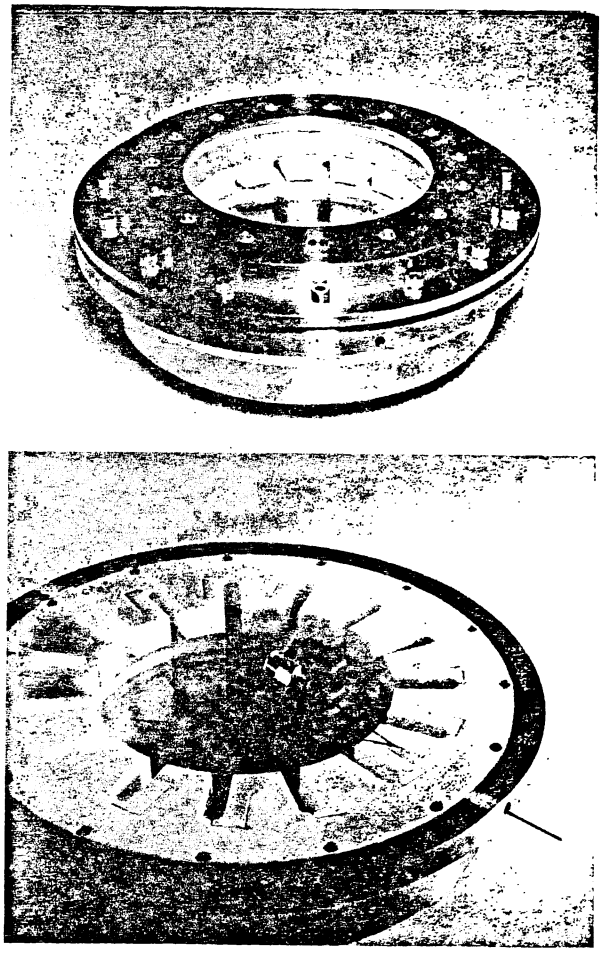

Fig. 3. Partially disassembled cuvet rotor showing cuvet syphons which allow 1. air to be drawn back through the reaction mixture to produce rapid mixing, 2. rapid expulsion of the reaction mixture from all cuvets during rotation, and 3. fast washing and drying of all cuvets in preparation for another group of analyses (reproduced with permission from [4])

methods for initiating reactions are now being explored.) Most of the work at Oak Ridge has been done with 15-place [5—7, 17, 23] and 42-place cuvet rotors [13].

Spectrophotometry

All reactions are monitored using a single stationary light beam and photomultiplier, as shown in Fig. 4. Wavelength control may be obtained with either a monochromator or with interference filters which may be mounted on a filter wheel and controlled either manually or by a computer.

The single beam and spinning rotor do in reverse what is done in a double-beam spectrophotometer, but without using moving or vibrating mirrors. The

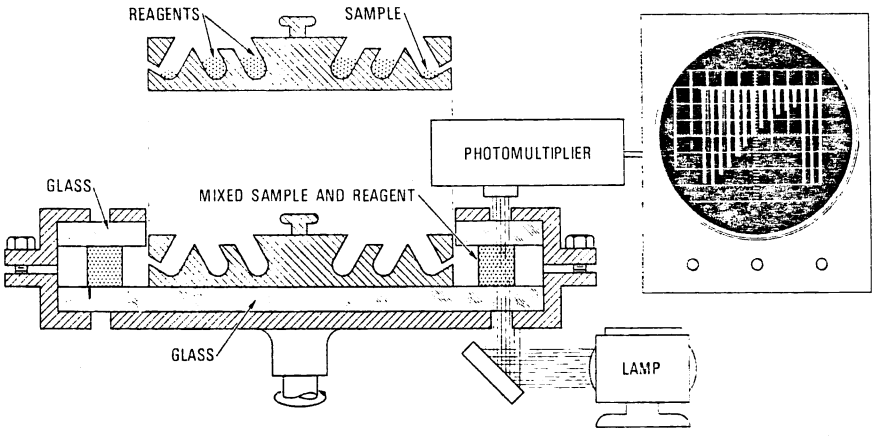

Fig. 4. Diagrammatic view of transfer method and of optical system. Cuvets rotate rapidly past stationary light beam. Photomultiplier output is continuously displayed in synchrony with rotation on an oscilloscope, giving a continuous visual display of events in the rotor (reproduced by permission from [5])

system allows a value for the dark current, and for all cuvets to be obtained once every revolution ($\sim$ 100 msec). This is best illustrated by looking at the signal obtained directly from the photomultiplier using an oscilloscope (Fig. 5). Since the metal between the cuvets blocks out the light between cuvets, the trace returns to zero transmittance (infinite absorbance) between peaks. The apparent base line (zero transmittance) is therefore along the top of the display, and the values are those represented by the bottom peak tips. The tip of the first peak represents the 100% T or water blank level which is at the base line level of continuous-flow charts. In addition to the photomultiplier signal, two additional signals are provided from the rotor using photoelectric pickups. The first is a signal just before the first cuvet moves into the beam. This signal resets the oscilloscope beam to a left-hand start position, and tells the computer that cuvet No. 1 is coming, and to sample the photomultiplier signal to obtain a value for the dark current. A second photoelectric signal series is provided which indicates when each cuvet is in the beam. (A special ramp generator circuit is provided to keep the width of the display constant regardless of rotor speed.) The times when data are taken during one sweep (one revolution) are indicated by arrows in Fig. 6.

To obtain a sample of the peak voltage for digitalization using a sample-and-hold circuit only a few microseconds (millionths of a second) are required. Data may be taken at the tip of a peak in three ways, all of which have been used [19].

In the first method data are taken when the cuvet synchronization pulse is received; this is set to occur 2/3 of the way through the flat portion of the peak, which is approximately 650 μsec long with a 15-place rotor.

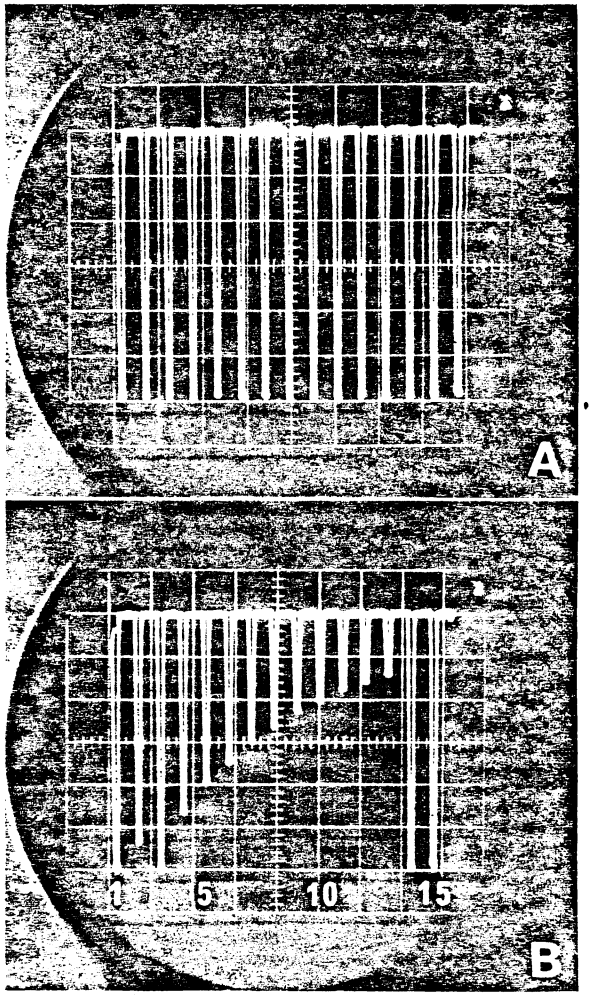

Fig. 5 A and B. Oscilloscope display of 15-place rotor during rotation. A Water in all cuvets. B Standards for biuret protein determination with reagent blank in cuvet 2, and water in cuvets 1, 3, 14 and 15 (reproduced with permission from [3])

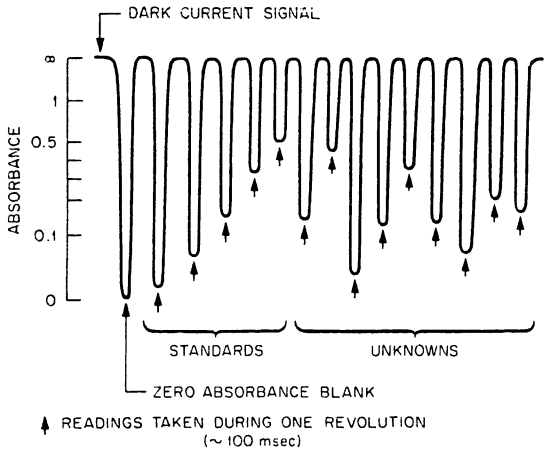

Fig. 6. Drawing showing oscilloscope display and times when readings are taken during one revolution. Note that both the dark current signal and the zero absorbance blank are determined anew during each rotation during which data are collected, and that all other data points during that rotation are referred to these two values. Since this all occurs during ~ 100 msec, the effect of electronic drift is minimized

In the second method a peak follower tracks the transmittance pulse and holds the maximum peak value. When commanded by the cuvet synchronization pulse (which occurs *after* a peak), the peak value is digitalized by a 12-bit analog-to-digital converter. When digitalization is complete, the peak follower is reset to permit tracking the next peak. It is possible to construct a system that detects, follows, and quantitates peaks with no synchronization pulse. However if one cuvet contains an opaque solution, no peak will be seen, and the cuvet count will be off.

The third method, developed by Myron T. Kelley at Oak Ridge, makes full use of the speed of modern computers [19]. Reading of a peak is initiated by a short pulse occurring near the beginning of the peak plateau. A time constant for the signal amplifier is chosen to introduce a little distortion into the signal and yields a sloping plateau area. The sync signal initiates a series of 16 28-μsec-long "read" cycles which are averaged. In this way 16 measurements are made in a little more than half a millisecond!

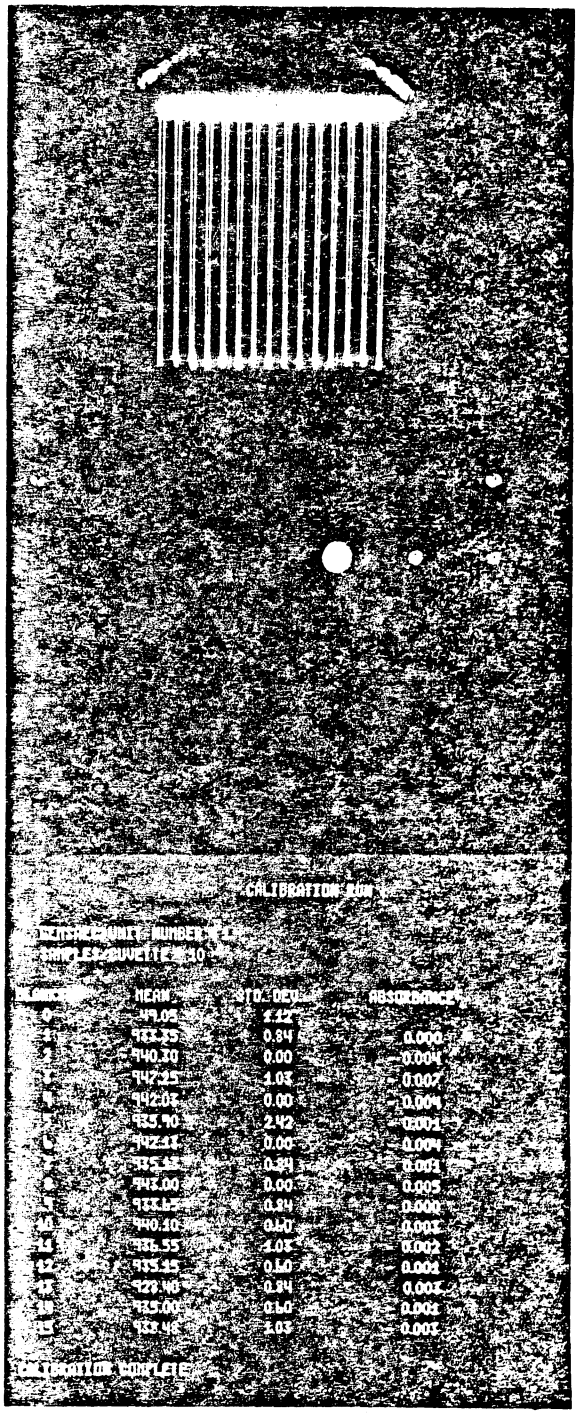

Fig. 7. Data on precision of replicate readings at 340 nm using water blanks. At the top is shown the oscilloscope display of the photomultiplier output. The data displayed on the storage scope (bottom) represent the results from ten consecutive rotations during which data were taken and averaged. First column: Cuvet number. The dark current reading is listed as number zero. Second column: Averaged digital output from analog-to-digital converter which corresponds to photomultiplier voltages digitalized. Third column: Standard deviation of voltages listed in column two in same units used in column two. The average standard deviation is 0.71, which corresponds to 0.07% of the signal and is less than 0.0005 absorbance units in the low end of the absorbancy range. Fourth column: Blank absorbancies. If repeated, one or two cuvets may show a change of 0.001. These blank values are stored and subtracted from subsequently measured values for the same cuvets

With all three methods data from 2—100 revolutions may be averaged to further increase precision. It is this capability which distinguishes fast analyzers.

Note that each signal train may be treated separately. The dark current value is subtracted from all readings, which are then converted first to percent of the 100% transmittance (blank or cuvet No. 1) value, and then to absorbance. Since the measuring time is short, the results from many signal trains (individual rotations) may be averaged. The results indicate that this system is remarkably free from drift, and that the errors observed are essentially those to be expected from the analog-to-digital converter used [22] and from the pipetting procedures employed. In the range between 0—0.1 A, the standard deviation of a set of readings is usually about $\pm$ 0.0005. The error is linear with percent transmission and hence when expressed as absorbance increases as infinite absorbance is approached. Higher precision may be obtained by using better analog-to-digital converters.

The raw results of a series of averaged analog-to-digital computer readings are shown in the first column of Fig. 7, while the standard deviation is shown in the second column. These are for cuvets filled with water. The absorbance is given in the third column; on replicate readings it varies by one in the third place in two or three cuvets. We thus have an almost drift-free system of remarkable stability and precision, with these qualities inherent in the design and not the results of special power supplies, lamps, or circuits. The requirements that the transfer and mixing times and reading intervals be small relative to the reaction time appear to have been met.

Cuvet Rotor Design

Cuvet rotors have generally been made with the side walls of the cuvets defined by a Teflon annulus, and with the upper and lower windows made from a glass or quartz annulus and disk respectively [3, 4, 13, 23]. The components of a 42-place rotor are shown in Fig. 8. When all reagents are mixed at one time, the reaction followed (or an end-point measurement made), and the reaction mixture then drained through the syphons, the rotor is kept spinning during the entire procedure. However, in some instances different reagents must be added at intervals during the course of a reaction. If this is to be done by stopping the rotor and inserting a new transfer disk, then it is essential that liquid not run out of the cuvets at rest. In the 42-place rotor,

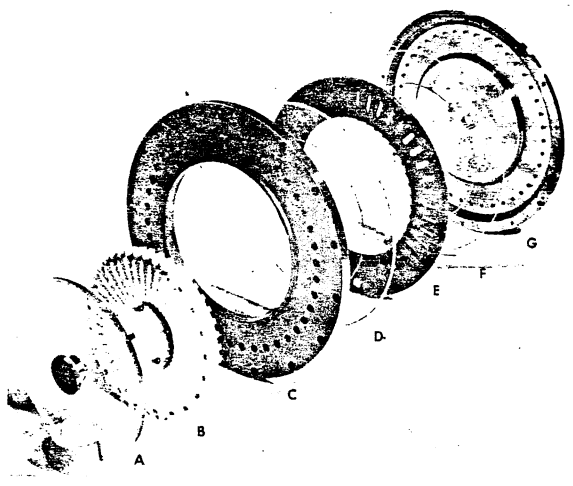

Fig. 8. Components of a 42-place GeMSAEC fast analyzer rotor. (*A*) Cover plate, (*B*) 42-place transfer disk, (*C*) upper cuvet rotor clamping plate, (*D*) upper glass window annulus, (*E*) black Teflon cuvet spacer annulus, (*F*) lower glass window disk, (*G*) lower rotor shell

small dams have been arranged to keep the liquid in each cuvet at rest, but not to interfere with liquid transfer into the rotor during rotation.

Emptying and Washing

The same syphons used to allow the air or other gas to stream through the reaction mixture are used to empty the cuvets, to allow a stream of wash water to flow through all cuvets, and to empty and dry them [4]. This may be done using either air pressure from the center, in the case of a closed rotor, or suction from the edge through lines which extend to a rotating seal below the rotor. The cuvets have Teflon walls and are therefore nonwetting. The size of droplets which will adhere to either the Teflon surfaces or the glass or quartz windows is a function of the centrifugal force used and the surface tension and density of the liquids. When a reaction mixture is expelled from the rotor, residual droplets are centrifuged into the syphon by brief acceleration to a higher speed, and then blown out during acceleration; approximately 0.2% of the reaction mixture remains [4]. One wash should therefore leave 0.0004% of the original volume. After the brief wash cycle, air is drawn through the rotor briefly during rotation to remove the last tiny droplets.

Syphons in centrifugal fields have certain interesting properties. If water is run continuously through the rotor at a few hundred rpm, the cuvets will fill and empty cyclically. At high speed, the liquid stream in the descending limb of the syphon

will break up into droplets. Liquid centripetal to the bend in the syphon will be drained out, and the syphon will merely behave as an overflow drain, but will not drain completely. Air pressure or suction will start the syphons only at low speed. When the centrifugal force exceeds the force generated by pressure, the syphon will not work, or will only drain until the two opposing forces are equal. The direct oscilloscope display (the so-called run monitor) allows the operator to see the syphon draining as a cyclical change in the peak height, to see at once whether a syphon is obstructed, and to detect dirt on the cuvet windows. The latter is esthetically unpleasant, but if it remains constant it will cause no difference in results, since the blank readings are stored and subtracted. The exception to this rule is the first cuvet, which should always have an absorbance less than all other cuvets. (Even this problem is easily taken care of with suitable software.)

The cleanup cycle usually takes only a few minutes. The system is then ready for another set of samples and reagents. The only changes required to shift to a totally different test are a shift in wavelength and a change in the computer program. In many instances even these are not required.

As with other aspects of this concept, we have striven to develop systems which would be useful today, but which can still be very much improved by further development.

We therefore do not yet know the shortest practical time for the wash cycle.

Temperature Control

The control of temperature is extremely important in reaction rate measurements. Several different methods for measuring and controlling the temperature of cuvet rotors and transfer disks have been developed in the Oak Ridge National Laboratory and by the manufacturers of commercial versions. These methods can restrict the variation to between 0.5 and 0.1°C [13,16,22]. There is, however, considerable variation in the time required to change from one temperature to another, suggesting the desirability of making as many measurements as possible at one temperature.

Data Reduction

One of the objectives of this work was to provide a suitable match between the human operator, the analytical machine, and the computer. The human requirements are that results be displayed in real time so that errors can be detected quickly, or the operator can assure himself that all is well. The "black box" syndrome occurs when the operator does not feel that he "sees" what is going on. The syndrome has little to do with how complex an electronic system really is. The run monitor, when fully understood, provides an instant, real-time indication of when the samples and reagents have passed into the cuvets, whether the reactions are proceeding properly, and whether any values fall outside the expected range. The human eye and brain are very competent at evaluating patterns provided they are presented rapidly enough. If data points are presented slowly, as is often done on a strip chart recorder, interest wanes rapidly. The run monitor display does not allow evaluation of the rate of change, however, and does not indicate whether a reaction is linear. A processed signal is therefore required to display rates.

The rate display shown in Fig. 9, employing a storage scope, shows each reaction in real time in a separate square, with absorbance (0—2) along the ordinate and time along the abscissa. For closer inspection of the results obtained in one cuvet, one may call for a single cuvet display through the

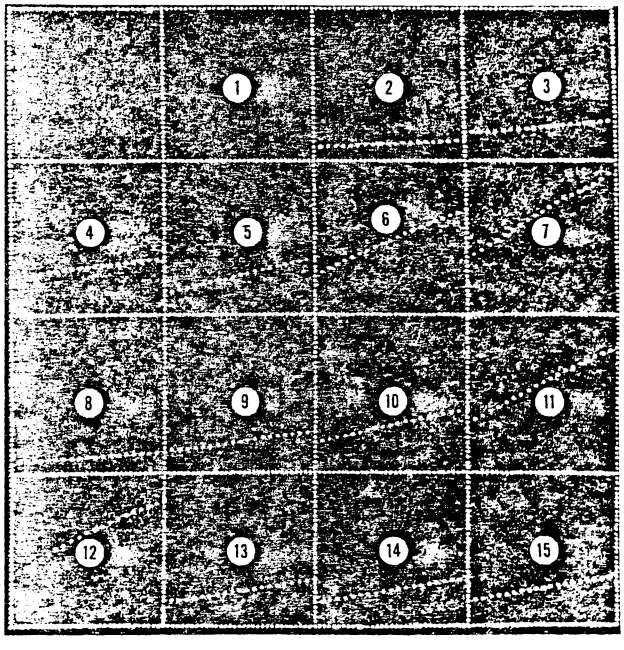

Fig. 9. Real-time display of reaction rates in a 15-place cuvet rotor. Each square (except the one in the upper left-hand corner) corresponds to one cuvet as numbered. The ordinate of each square corresponds to a range in absorbance from 0 to 2 on a linear scale, while the abscissa represents time. The first cuvet is the water blank and hence the plot is flat and along the base line. The remainder of the squares show actual reactions in progress

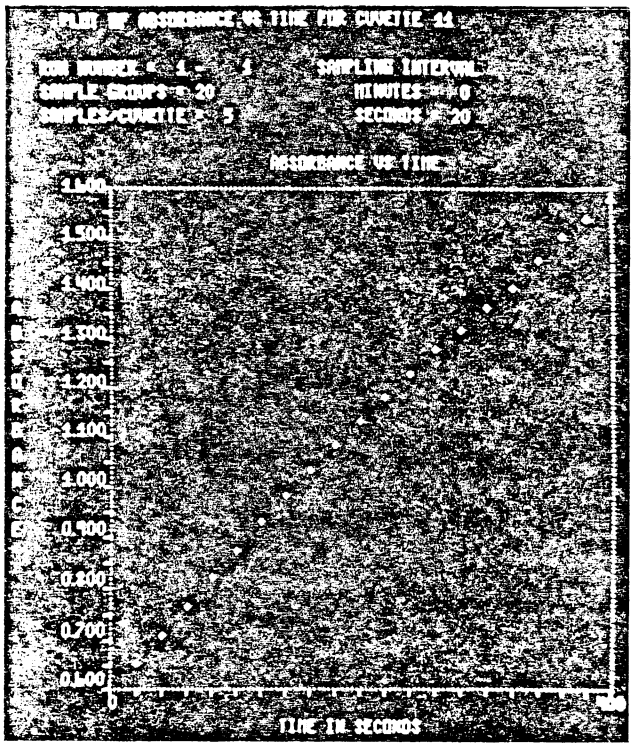

Fig. 10. Storage scope display of results from cuvet 11. Computer controls abscissa display to correspond to data taken. Absorbance range displayed may be controlled from the keyboard

Teletype, giving the results shown in Fig. 10. Here the operator may specify the absorbance range, but the computer correctly displays and marks off time to match the data taken. Other data on the analysis are also displayed in alphanumeric form; these include the interval between data points, the total number of data points taken per cuvet, and the number of individual absorbancy readings taken and averaged into a data point. If five absorbancy readings are averaged for each data point, and twenty data points are taken, then with a 42-place cuvet rotor (and counting the dark current measurements) a total of 4300 measurements are made during a single analysis. This points up a salient fact about GeMSAEC-type fast analyzers: *The data must be reduced to as close to final form as possible before leaving the machine.* If this is not done, the analyst will be inundated with paper. Therefore, where plots are desired these should be provided directly in publishable form. Hard copy should be in international or standard units.

A variety of programs have been written for fast analyzers [10, 18, 19, 29], many of which are available as ORNL reports [11]. PDP-8/E computers have been used almost exclusively. Much work remains to be done, however.

It is now quite evident that a kineticist will be able (provided he has all the solutions prepared) to do a planned series of several hundred analyses in a morning, place all in storage on a disk, and then rapidly generate plots of activity vs pH, ionic strength, inhibitor concentrations, etc.; calculate K_m's; and generate the family of curves familiarly seen in papers on enzyme kinetics in a few minutes. He will also see, before his solutions have deteriorated, what new data he requires. Rapid interaction with data through the computer will, I believe, become routine.

Automation

As mentioned, automation involves feedback control, as distinguished from mechanization, which does not. It is unfortunate that the well-defined word "automation" has been so widely used in clinical chemistry without understanding what it means. Since I have already suggested the word "cybernetization", we may as well begin to use it. The scientific question, however, is where do we *need* cybernetization in the clinical laboratory. There is no question that we need mechanization, and more of it. Cybernetization is a quite different problem.

In my view we need it for two reasons: The first is to provide automatic error correction or indication. (Where only error or failure indication is provided, the operator closes the servo loop.) Thus if two very different density solutions are included in the reaction mixture (for example a sample containing 40% sucrose) then the mixing air can be arranged to come in short bursts with absorbance measurements between. The air mixing is continued until the absorbance measurements are either constant or differ by a constant but very small amount, indicating that mixing is complete and that the reactions are proceeding. Additionally loss of signal may automatically shift into place a backup light source.

The operator may be included in the loop in several ways either through error signals that are printed out in plain English (or German) or through signal lights which flash indicating electronic failure, no reaction in any cuvet, mechanical failure, or the exceeding of temperature limits.

An immediate second use for cybernetization (feedback control) is to save time. A series of analyses may be started without having the total measuring time preset. The computer would arrange to take readings at short intervals initially to see which

reactions were proceeding rapidly. Data would be taken until certain statistical requirements had been satisfied. When this had been done, the machine would shut itself off, drain and wash all cuvets, and signal readiness for a new transfer disk which could, of course, be placed in position automatically. In this case the rate at which analyses were done would depend on the measuring interval required to get satisfactory data on the slowest reaction. The computer may also decide which reactions have proceeded too rapidly and should be rerun with a more dilute sample.

Transfer Disk Loading

While most analytical systems have been developed in a logical order beginning with sample and reagent measuring and addition, GeMSAEC fast analyzers were actually developed in the reverse order, with the computer and its role in the analysis as the first consideration. (Concepts which did not naturally interface with computers and make use of their capability for rapid data processing were discarded.) For this reason the last thing which we considered was the actual loading of the transfer disks. A loader adapted from a commercially available pipetter is shown in Fig. 11 which is used with the 15-place system [14]. The coefficient of variation of replicate samples ranged from 0.25 to 0.91%, depending on the volume delivered. Samples and reagents may be loaded on one disk in 3.25 min. Note that all three manufacturers of fast analyzers have loading systems, each with its own special features.

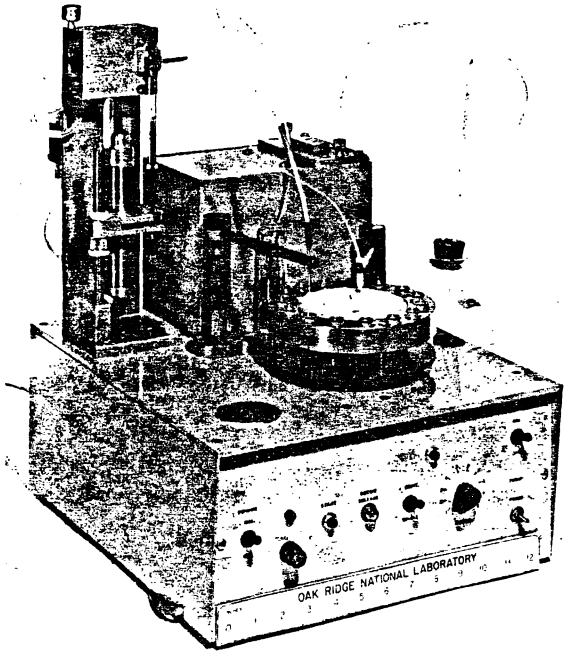

Fig. 11. Transfer disk loader for 15-place system (from a paper by C. A. Burtis *et al.* [14])

Since a number of loaders may be operated concurrently, the rate of transfer disk loading does not limit the total analysis rate of the system.

Dynamic Loading

Quite early in the course of this project it was realized that liquid introduced into a properly designed spinning rotor would be equally apportioned between all cuvets [5]. The stream, rotor speed, and surfaces in contact with the liquid must be carefully designed to achieve precise *dynamic stream segmentation*. This opens up several new possibilities which may be outlined as follows:

1. Samples only may be loaded into the transfer disk. The reagents may then be added during rotation.
2. Multiple sequential reagent addition is possible.
3. Different reagents may be loaded into different cuvets via the transfer disk, and one diluted sample added during rotation. In this way a series of different analyses may be made on one sample.
4. By using tiered rows of entrance ports to the cuvets, and with those at different levels connected to different cuvets, reagents or samples may be added at different times to different cuvets during rotation (J. Molloy, personal communication).

Use of Centrifugal Force in Volume Measurement

Thus far we have used centrifugal force chiefly to move liquids and to hold them in position. It may also be used to make precise volume measurements [2]. Almost any cavity may serve provided that it is overfilled slightly and that the meniscus is subsequently flattened with sufficient force. The position of the cavity may then be changed so that centrifugal force empties it.

Syphons may also be arranged to make volumetric measurements and to then be drained using vacuum or air pressure [7]. Much work remains to be done to fully exploit these principles. Recently some of them have been adapted for use in space.

Evaluation in the Clinical Laboratory

The 15- and 42-place GeMSAEC fast analyzers in routine use in the laboratory of the Health Division of the Oak Ridge National Laboratory are shown in Fig. 12. Data on the methods used are included in

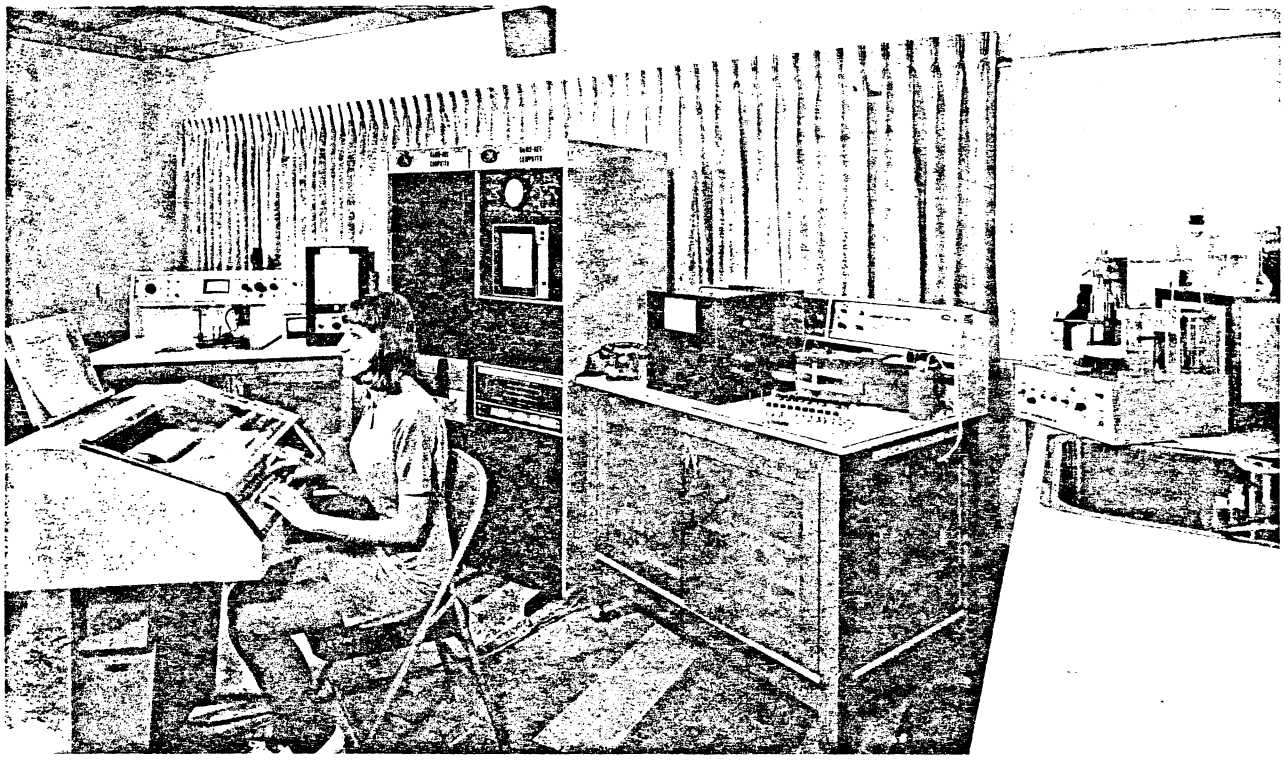

Fig. 12. Complete analytical system with both 15- and 42-place cuvets, sample and reagent loaders, computer, cathode-ray tube displays, and Teletype in the clinical laboratory of the ORNL Health Division

Table 1. while the rate of analysis in practice and reagent costs are shown in Table 2. Note that completely automatic loading of transfer disks was not available for the 42-place system when these data were taken.

Computer Analysis of Data

Software development will increase rapidly as more computerized systems become available. Several interesting possibilities however are now beginning to be exploited.

The first is simultaneous analysis of one sample using several different assays to produce a diagnostic enzyme profile [30]. The enzymes chosen were SGOT, SGPT, and GLDH. Since the conditions of measurement were identical in each, enzyme ratios calculated from the data are more meaningful.

The second is the measurement of reaction rates for reactions usually carried to completion, and the use of selected time intervals during the reaction either after an interfering reaction has been largely completed or before it has been started. This approach has been taken to the measurement of serum creatinine [16].

The most complete study of computer analysis of fast analyzer data thus far is that of Tiffany and coworkers at Oak Ridge, who compared end point and kinetic enzyme assays for the substrates glucose (hexokinase/G-6-PDH), urea (urease/GLDH), and uric acid (uricase) [30]. Reactions to completion usually require that blanks be run to allow compensation for absorbance of the reaction mixture at zero time. However, by taking a series of 20 readings at very short intervals at the start of the reaction and extrapolating back to zero time, the blank absorbance may be calculated and subtracted from the absorbance at the end of the reaction (Fig. 13). Thus no standards and no blanks are required. But the method has the distinct disadvantage of requiring a relatively long time.

Rate reactions using enzymes as reagents to determine substrate concentration also require no blanks, but require a series of standards. For many reactions conditions can be arranged so that pseudo first-order kinetics obtain, making the rate linear with concentration. Either the initial rate may be obtained by a least-squares fit to a series of data points, or two measurements may be made at

Table 1. Reaction volumes and methods used in the clinical analyses of various blood constituents utilizing a GeMSAEC fast analyzer as the analytical tool

Blood constituent	Reagent volume (μl)	Sample volume (μl)	Method and reference
Protein	400	10	Biuret[a,b]
Albumin	400	10	Haba[c,d]
Glucose	400	2.5	Barthelmai and Czok[e]
Enzymes			
AP	400	20	Bessey *et al.*[f]
CPK	400	50	Oliver[g]
LDH-L	400	50	Wacker *et al.*[h]
LDH-P	400	20	Wroblewski and LaDue[i]
SGOT	400	50	Karmen[j] Henry *et al.*[k]
SGPT	400	50	Wroblewski and LaDue[l] Henry *et al.*[k]

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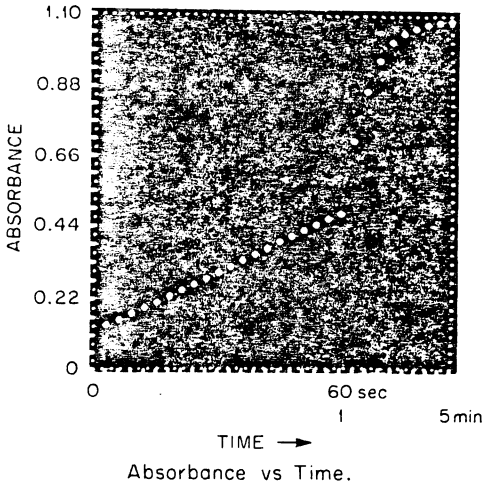

Fig. 13. Extrapolation to zero time for blank determination in a reaction run to completion (from a paper by T. O. Tiffany *et al.* [29])

fixed intervals. Nonlinear kinetics are not a problem when standards are used and when the computer is equipped with a curve-fitting subroutine.

Analyses in Space

During prolonged space flight in close quarters at zero gravity, and on recycled consumables, many physiological changes may be expected and some have been seen. It is important that as complete clinical chemistry analytical facilities as possible be available during flight. This raises a constellation of interesting problems. No clinical chemist will be available in the space vehicle. Only a very small amount of space in the space vehicle and a small launch weight load are allowed. Reagents must be stabilized to last the entire trip. The astronaut must

Table 2
Analytical results obtained from multiple analyses of a single serum sample by using the 42-place GeMSAEC fast analyzer

Analysis	No. analyses	Analytical results		Sample volume (μl)	Reagent cost (cents per test)	Time	
		Mean	C.V. (%)			min/disk	samples/h[a]
Protein	37	6.79 g/100 ml	0.7	10	0.1	16	139
Albumin	37	3.48 g/100 ml	0.7	10	0.1	16	139
Glucose	37	91.2 mg/100 ml	0.5	2.5	6	17	131
Enzymes							
AP	41	44.1 U/l (30°C)	1.1	20	4	18	137
CPK	41	25.6 U/l (30°C)	1.6	50	10	20	123
LDH-L	41	55.2 U/l (30°C)	2.1	50	4	19	129
LDH-P	41	147.1 U/l (30°C)	1.3	20	3	17	145
SGOT	41	13.4 U/l (30°C)	5.9	50	4	17	145
SGPT	41	11.0 U/l (30°C)	4.7	50	5	19	129

[a] Extrapolated values.

Note: Reproduced by permission from [13].

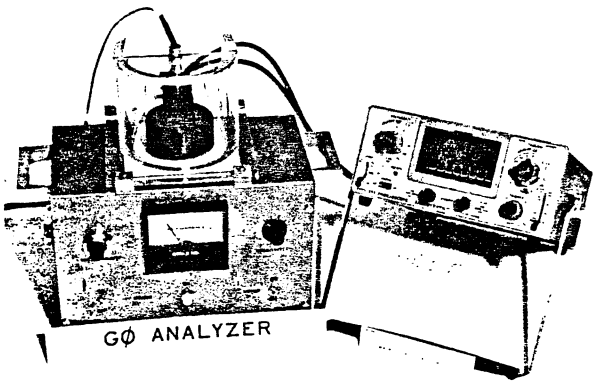

Fig. 14. First prototype of the Gravity-Zero (G ⌀) miniature analyzer for space use

draw his own blood and operate the entire machine. Pipetting at zero gravity is hazardous and ordinarily does not work. Spectrophotometry is beset with air bubble problems, and the transfer of liquids is extremely difficult. One answer is to perform all analyses in a centrifugal field—i.e., to provide "artificial" gravity in the analytical device itself. Since the GeMSAEC[2] does this, it was a natural choice for adaptation to the NASA Skylab program. However, a series of very difficult technical problems must be solved before the concept can be used in a space environment.

The first problem is miniaturization. A decision was made to use disposable rotors with preloaded reagents.

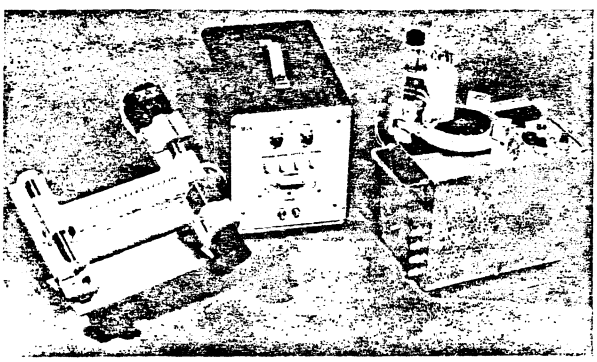

Fig. 15. Second prototype of G ⌀ system presently in use to develop analytical procedures for use in space [from a paper by W. F. Johnson *et al.*: Clin. Chem. 18, 762–766 (1972)]

2 GeMSAEC is an acronym for the National Institute of General Medical Sciences and the U.S. Atomic Energy Commission who sponsored most of this work. The same acronym has recently been trademarked by one of the manufacturers. Commercial versions of the system described are available from Union Carbide Corporation and Hoffmann-La Roche (CentrifiChem), Electro-Nucleonics Inc. (Gemsaec), and American Instrument Company (Rotochem).

Table 3. Desirable determinations for the G ⌀ analyzer

Glucose
LDH
SGOT
SGPT
BUN
Protein (Total)
Alkaline Phosphatase
Inorganic Phosphate
Ammonia
Acid Phosphatase
Bilirubin
Calcium
Chloride
Sodium
Potassium
Magnesium

It was hoped that this would allow all tests required to be done on one blood sample to be done simultaneously. The first prototype of a Gravity Zero (G ⌀) Analyzer is shown in Fig. 14 [8]. The total reaction volume for each reaction is 100 µl, and the optical path is 0.5 cm. Subsequent versions (see Fig. 15) [15] have incorporated refined optics, electronics, speed, and temperature control. Three data reduction alternatives are provided. The first is telemetry of a partially processed signal to Houston for computer processing. The second is onboard data processing using an onboard computer. The third is use of a mechanical printer which prints out plots of absorbance vs time.

The analyses presently planned for the G ⌀ system are listed in Table 3. One unique feature under development is a provision for preparation of serum from whole blood in the same disposable rotor used for chemical and enzymatic assays [27].

While many difficult problems remain to be solved, it is probable that miniature fast analyzers will one day find wide use in pediatric and emergency laboratories and in small hospitals and clinics.

Conclusions

What is the future of the GeMSAEC fast analyzer concept and what work remains to be *done*? Is there, for example, any reason to develop faster systems with a higher throughput? Is miniaturization important?

One of the major reasons for constantly striving to increase the speed and precision of measurements, to reduce sample volumes, and to lower the cost of individual tests is to be able to return to a classical principle of analytical chemistry—to demonstrate the precision of an analysis by repeating it two or more

times, i.e., to run them in duplicate, triplicate, quadruplicate, etc. If this is done on a random basis, that is, if the duplicates or triplicates are not consecutive, then one also has a check on sample identification. At present costs, with present work loads, and with present equipment, this approach to quality and identification assessment is almost entirely out of the question. With very fast inexpensive computerized analyses this would not necessarily be the case.

How fast is fast, and what limits speed of analysis? We may begin by asking how much time is taken to make one measurement as the rotor is spinning. The sample-and-hold circuit samples the photomultiplier signal for approximately 2 millionths of a second (2 μsec), so that only a very tiny fraction of the peak plateau is actually used. Actual measuring time is therefore not limiting, and rotors could be made to spin much faster than they do without running into trouble. Actually in the analytical ultracentrifuge, absorbance measurements are made and digitalized at 60000 rpm routinely [28].

With a 42-place rotor spinning at 600 rpm, the interval between signals from consecutive cuvets is 2.38 msec or approximately 1000 times longer than the actual sampling interval. The time between data points used to determine reaction rates is usually 5 sec or more, although for extrapolating to zero time absorbance it may be much less. It is obvious from these considerations that the reading intervals could easily be in the 100 msec range or less providing the reactions are proceeding with sufficient rapidity to warrant such fast data collection, and provided that the reactions could be started rapidly enough.

As mentioned, optical measurements may be made on solutions in analytical rotors spinning at 60000 rpm whereas we are usually working at only 1% of this speed. The possibility of higher rotor speeds is therefore wide open for exploration and should make possible reaction initiation by adding substrate quickly *during* high-speed rotation, possibly in the form of a fine spray.

The final question concerns limitations on reaction rate measurement. It is here that the inherent precision of the system becomes valuable, because with each increase in precision, the time interval required for rate measurement decreases. However, there are also biochemical methods for shortening reaction times which may be employed. Most reaction rate measurements have been set up for manual performance. This means that the rates are adjusted to allow manual adjustment of the spectrophotometer, operation of a stopwatch, and data recording—all during the reaction. By altering the relative concentration of reactants, the rates can often be very much increased, as is shown by the popularity of stop-flow measurements. From a purely physiological viewpoint, there is good reason to attempt to measure enzyme activities at concentrations closer to those which occur in the body. We conclude therefore that it is now technically feasible to initiate reactions, make rate measurements, and complete many of the calculations in less than 1 sec, entirely in the millisecond range. To make this routine will require very much additional work.

In conclusion, GeMSAEC fast analyzers in the form initially developed are now coming into general use for both kinetic and reaction-to-completion measurements. However, the several concepts embodied in the system have, as yet, not been fully explored. We will watch with keen interest the evolution of these ideas at subsequent sessions of the justly famed Biochemische Analytik.

Acknowledgement. It is a pleasure to list the help and advice of D. D. Willis, W. W. Harris. E. L. Candler, C. D. Scott, D. W. Hatcher, D. N. Mashburn, R. H. Stevens, L. H. Elrod, J. M. Jansen, M. T. Kelley, C. A. Burtis, T. O. Tiffany, W. F. Johnson, and J. C. Mailen.

This work represents part of a continuing effort on the part of the USAEC, the National Institute of General Medical Sciences, and the National Aeronautics and Space Administration to put new technology to use in the interests of health.

May I express my deep appreciation to the Deutsche Gesellschaft für Klinische Chemie and to Boehringer Mannheim GmbH for making this award possible.

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