

Computer Interfaced Fast Analyzers

New instruments afford quantitative analyses of biological samples in several minutes.

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Fast analyzers (1) are analytical instruments which provide output data in a form and at a rate suitable for direct input into a computer. The simple basic principles, developed during the past 2 years (2-4), give promise both of increasing the rate at which many biochemical analyses and clinical tests may be performed and of increasing the precision. The research and the clinical requirements which gave rise to this work and the present status of development are reviewed in this article.

At present the workload of the hospital clinical laboratory is increasing at an estimated rate of 15 percent per year, and any widespread screening studies are expected to overtax the existing facilities (5). Methods for mechanizing analyses have been developed as a result of the trailblazing work of Skeggs which led to the development of the AutoAnalyzer (6). In this device a continuously flowing, air-segmented liquid stream carries a sample past a series of stations or positions where reagents are added or where processes including heating, dialysis, incubation, filtration, or absorbance measurement are carried out. Use of the system has enormously increased the number of analyses which may be done in a given time and on a given sample. Recently a number of devices have been introduced in which discrete samples and individual glass or plastic reaction vessels are used; these vessels are moved past stations where additions, reactions, or measurements occur (7). Even so,

an enormous clerical workload usually remains, and estimates of the percentage of time that personnel devote to this purpose in clinical laboratories have ranged from 20 to 70 percent. At first glance it would appear that computers should be easily adapted to solving this problem. In practice this has been only partially true. One difficulty has been that the rate at which data are generated, the rate at which an analyst can evaluate it, and the rate that a computer can reduce it to final form are mismatched.

Automation

Automation involves feedback control to produce self-corrective or decision-making systems (8). True automation has rarely been applied to biochemical or clinical analyses; what has been done is more properly described as mechanization.

In practice, a chart record may be made for visual inspection as data are gathered, but numerical results may not be available until long after all analyses are complete. Automation requires some method of monitoring a reaction throughout its entire course to ascertain that the reagents are properly made, that the temperatures are those required, that proper temperature compensations are made, that the absorbance measurements are made at the proper time, or that proper dilutions of the samples have been made. With

enzyme assays, for example, a lag period often occurs before a linear reaction rate is obtained. Deciding when to read and when to stop reading is best done by a computer.

For automation, it is essential that the analytical results be obtained in sufficient time to be used to control some aspect of the analysis. If analyses are performed sequentially by an analytical train traversed in minutes or hours, this cannot be done. The advantages of true automation, while self-evident, remain to be demonstrated.

At present the large clerical component in the laboratory workload (namely, sample identification and data storage, intercomparison, and compilation) suggests that automated system development begin with the computer and work back toward the problems of wet chemical analysis. This is especially true if one proposes to attack the problems of sample misidentification and of quality control by doing multiple analyses of each sample in random sequence. If two (or whatever number) analyses of the same type on a sample agree, there is a high probability that the results are truly representative of the sample. Such multiple analyses can only be done if the cost per test is very small and if the number of analyses done can be very large.

Computer Interfacing

The objective is an analytical system in which a computer is interfaced to analytical devices in such a manner that it controls them. This objective has not been fully reached.

The basic problem with slow analyzers (about 20 seconds to 2 minutes between data points) has been that data is stored in the form of a strip chart record, of paper or magnetic tape, or of punched cards and complex interfacing is usually required. Baseline drift of mechanical, chemical, or electronic origin is indigenous to such

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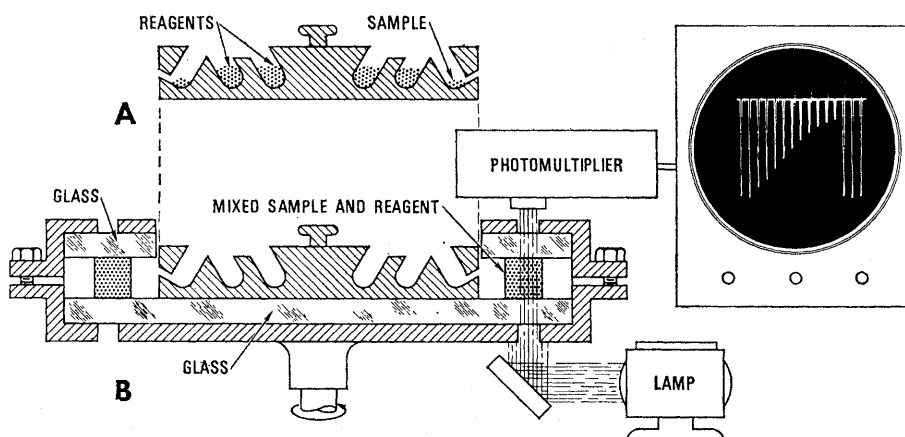


Fig. 1. Principles of operation of the GeMSAEC fast analyzer. Measured volumes of reagents and samples are placed in depressions in the fluorocarbon transfer disk shown in (A). The depressions are arranged so that reagents and samples are unmixed at rest, but can move radially without mixing with adjacent sets of reagents and samples. The transfer disk is placed in a cuvette rotor, as shown in (B), and rotated. Centrifugal force moves all liquids out into the rotor cuvettes which rotate past a stationary light beam as shown in (B). The signal obtained from the photomultiplier is displayed on an oscilloscope continuously. Fifteen separate reactions are therefore started at the same time and may be continuously followed. Gross errors in pipetting, reagent, or standard preparation may be observed, in the case of fast reactions, after a few seconds.

slow-output systems. If a computer is to be used efficiently, the data should be fed in directly at a high rate of speed and should be suitably initialized so that all signals are properly identified. If the data signals for 15 to 90 analyses can be produced in a small fraction of a second, electronic or other drift will be minimized within that set.

The possibility then exists of repeating all readings on each sample many times, averaging them, doing the necessary statistical analyses, and simultaneously deciding how many readings should be obtained for proper statistics, all in much less than a second. Programs making such decisions have been written for the photoelectric scanner for the analytical ultracentrifuge (9).

The data obtained during each short data-taking interval should include a measurement of the base-line current (called dark current); a water, air, or reagent blank; a series of standards, if required; and a series of unknowns.

It is at once apparent that these requirements preclude serial analysis of samples. Instead, analyses are best done in parallel with all reactions, additions, and other steps occurring for all analyses at the same time. The interval required for absorbance measurements must be a very small fraction of the reaction time (that is, less than 1 percent of it). In practice, this means that variations between mixing times and the time required for all photometric measurements should be

less than 0.1 second, and that the number of reactions run simultaneously should be large (15 to 90). These computer-oriented requirements have dictated the directions in which work on fast analyzers has proceeded.

Analytical methods in which absorbance measurements are used vary in complexity and involve sample and reagent measurement and transfer, plus one or more of the following: incubation at elevated temperatures, timed reagent addition, filtration, centrifugation, dialysis, solvent extraction, or distillation (3). A variety of problems must be solved if these are to occur simultaneously in each of a series of assays run in parallel.

In the initial studies described here we have concerned ourselves with only single-step reactions, that is, with analyses in which a sample and one or more reagents are mixed initially and read after a suitable interval. A number of such simple analyses are in common use and include the biuret test for protein, the anthrone test for sugars, and many assays for enzymes, especially those linked to nicotinamide-adenine dinucleotide. The principles on which fast analyzers with more complex reactions can be based are now at hand.

Cuvette Rotors

Centrifugation provides a driving force which has not been fully exploited to measure and transfer liquids. In the synthetic boundary cell of the analytical ultracentrifuge it was demonstrated that quantitative transfer of fluid can be achieved by centrifugal force and that the meniscus is flat (10). Air bubbles are quite obviously absent.

Traditional centrifuge tubes may be replaced by cuvettes, and these may be read during rotation with the use of a stationary light beam. Small cavities may be arranged centripetal to the cuvettes to hold samples and unmixed reagents at rest. During rotation these liquids flow into the cuvettes, and all cuvettes may thus be loaded at the same time (Fig. 1). The minimum

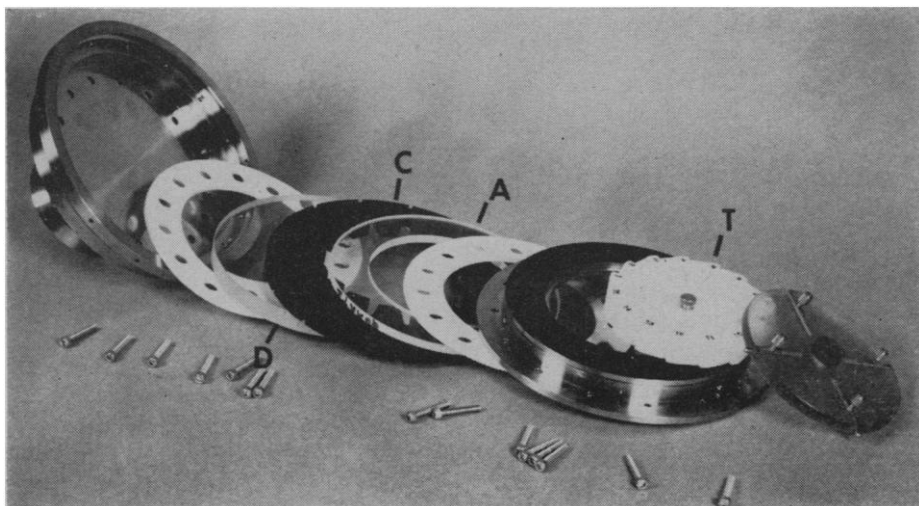


Fig. 2. Disassembled 15-place cuvette rotor. The slotted black Teflon cuvette ring (C) is compressed between a thick glass disk (D) and a glass annulus (A) to produce the cuvettes. Samples and reagents are placed in the transfer disk (T). Remaining components support the cuvette ring and glass components and attach them to the drive motor.

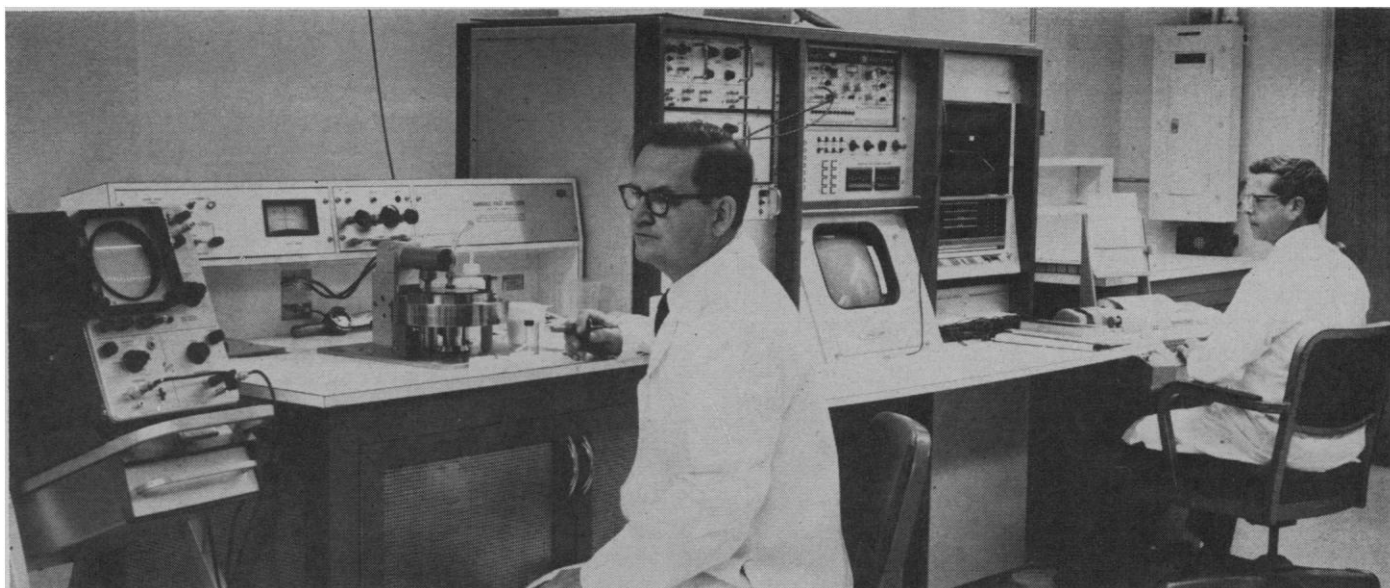


Fig. 3. Complete fast analyzer system. The oscilloscope is shown at extreme left, cuvette rotor and ancillary equipment mounted on table are at left center, and the computer, display, and teletypewriter are at right.

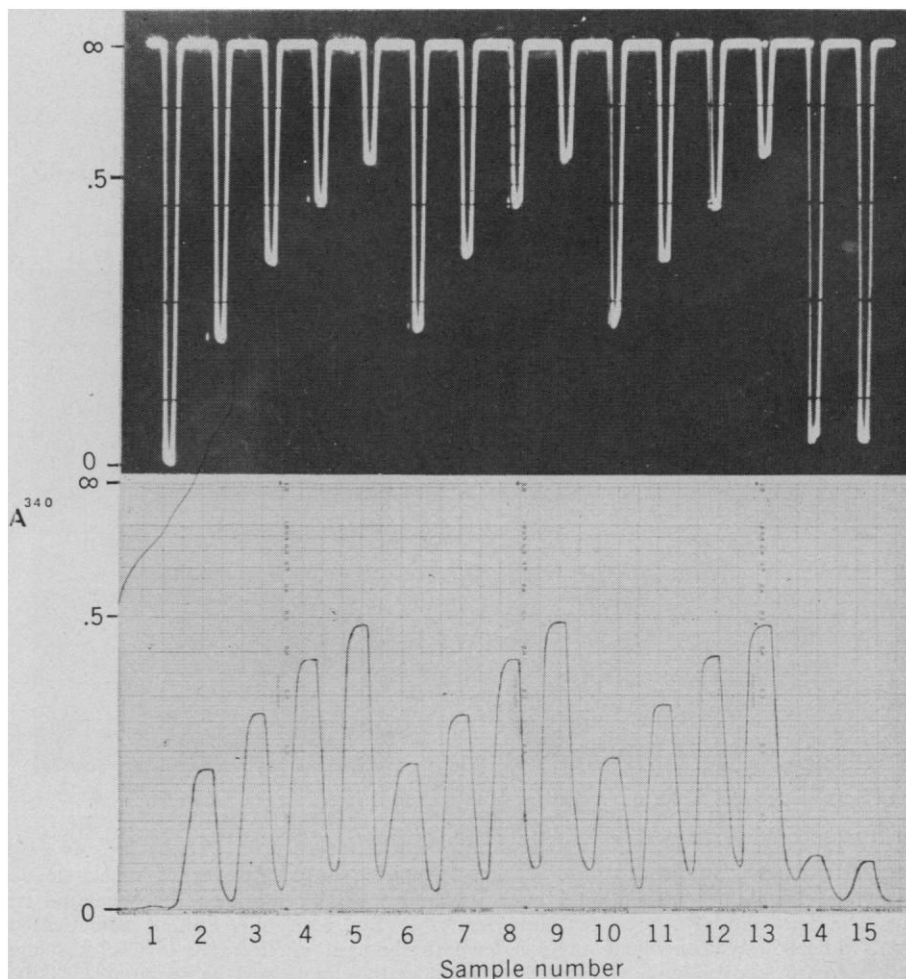
time required for reading all cuvettes is one rotation of the rotor. Only relatively low-speed rotors have been built thus far, and scanning times have varied from 50 to 100 milliseconds. The number of cuvettes has varied from 15 to 40.

The rotors are generally constructed of stainless steel, glass, and Teflon, the cuvettes being formed by slots in a Teflon ring compressed between a glass disk and a glass annulus. A disassembled 15-place rotor is shown in Fig. 2. The completely assembled system with computer is shown in Fig. 3. When a tungsten lamp is used as the light source, an interference filter is built into the photomultiplier housing.

For research purposes one system has been constructed with a Bausch and Lomb monochromator. Pyrex glass has been found satisfactory for the rotor end windows for wavelengths down to

340 nanometers, and fused quartz is used for work in the deeper ultraviolet. Photomultiplier leads and amplifiers must be constructed to prevent changes in signal shape due to capacitance.

Fig. 4. Comparison of types of charts obtained with GeMSAEC fast analyzer and with a continuous-flow analyzer. The upper chart was obtained with the Folin reagent for total protein; the lower chart was drawn to match and to indicate differences in inter-sample base lines. The upper chart was obtained with a water blank in cuvette (or sample) No. 1, and with 50, 100, 150, and 200 μ g of protein in a total volume of 500 μ l in cuvettes 2, 3, 4, and 5. This protein series is repeated in cuvettes 6 to 9 with the addition of 1.08 mg of sucrose per cuvette, and again in cuvettes 10 to 13 with 2.48 mg of sucrose per cuvette. Cuvette 14 contains a reagent blank and cuvette 15, a reagent blank plus 2.48 mg of sucrose. Photograph was taken 1 minute after the liquid was transferred. When an analytical reaction in a continuous-flow analyzer involves the destruction of a chromogen (rather than its appearance), the chart will resemble superficially that shown for the first analyzer.



Direct Display

A continuous oscilloscope display of the photomultiplier output provides the analyst with a visual indication of the progress of all reactions during their entire course.

The form of the display requires some comment since it is different from those obtained with continuous-flow analyzers, and, at first glance, may appear to be inverted. This occurs because the light beam is completely interrupted between cuvettes and therefore rises to the infinite absorbance (0 percent transmittance) value. In continuous-flow systems, on the other hand, the trace usually approaches a zero absorbance (100 percent transmittance) value at the bottom of the chart between samples, and the dark-current setting is determined only rarely. With the cuvette rotor, all parameters—dark current, water, or reagent blank, standards if required, and experimentals—are read during each rotation. The rotor system is thus an extension of the double-beam spectrophotometer concept to include a large number of solutions. A comparison of the types of peaks obtained with the cuvette rotor and with a flowing stream analyzer is illustrated in Fig. 4.

For many purposes a Polaroid photograph of the oscilloscope pattern is sufficient, and, in any case it acts as a reserve and convenient form of data

Table 1. Variations in fast analyzer transmittance readings. A standard deviation of 0.1 percent of the 100 percent transmittance scale value is equivalent to 0.00044 absorbance units. For each value in the table, 150 readings were taken.

Wave-length (nm)	Standard deviation of 100 percent transmittance	
	Filters (%)	Mono-chromator (%)
340	0.160	0.190
550	0.032	0.054
660	0.161	0.133

storage. The pattern indicates from the very outset the state of all reactions and indicates (i) errors in reagent preparation, (ii) off-scale results due to insufficient sample dilution, or (iii) instrumental failure.

Photoelectric signals from a disk which is attached to the drive motor reset the sweep circuit, before the first cuvette passes the beam, and control a ramp-voltage generator which keeps the pattern width constant when the rotor speed is varied (3).

Analog-to-Digital Conversion and Signal Identification

After the photomultiplier signal is passed through a buffer amplifier, it is fed into an analog-to-digital converter

and onto a PDP-8/I or PDP-8/S computer. The sweep-circuit reset signal indicates to the computer that a new data set is coming, and, since the signal occurs midway between the last and first cuvette, it is used also to signal that a dark-current reading should be taken.

Two methods have been used to read the cuvette signal peaks. In the first, an auxiliary train of pulses, each occurring two-thirds of the way through a peak plateau, is used to indicate when reading should occur (11). Absorbance is therefore read at the same place in a given peak. In the second system the synchronizing signal is used to trigger a sample-and-hold circuit of longer sampling time, which then allows the peak voltage to be read (12). The very short-term spurious spikes are removed by filtration.

Once a computer is "on line" with a fast analyzer, data may be obtained at a very rapid rate. Programs have been written which allow the operator to determine how many times he wishes the cuvettes read and which then print out both the average value obtained and the standard deviation. As well known, this error varies with absorbance in a nonlinear manner. This error has considerable bearing on how measurements involving rate changes are set up, and is the reason why blank solutions with a high absorbance are generally used in manual measurements

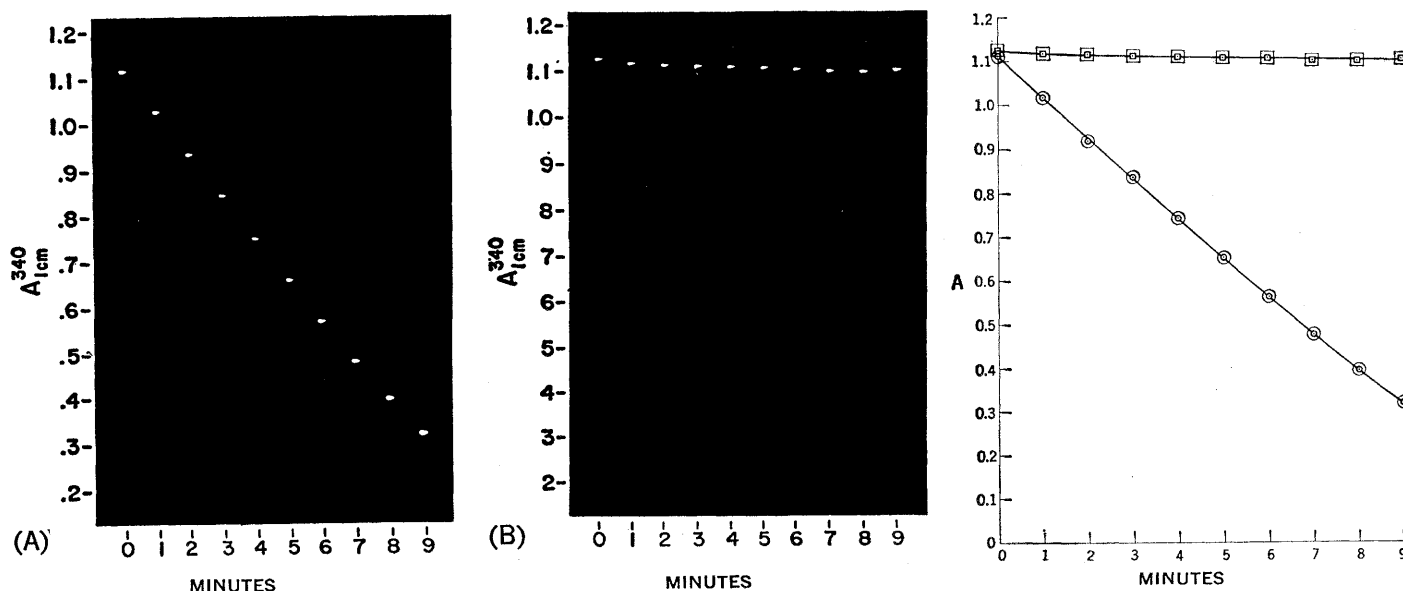


Fig. 5 (left). Photograph of a cathode-ray tube display of results obtained in a lactate dehydrogenase assay by a fast analyzer. All cuvettes were measured at 1-minute intervals, and the data from one experimental cuvette (A) and one control cuvette (B) are shown. The data were also typed out numerically. Fig. 6 (right). Plot of numerical data obtained from the same run shown in Fig. 5. Clinical and biochemical analysts have rarely used photographs of CRT displays. Before they are accepted, extensive inter-comparison with conventional plots such as given in this figure will be required.

involving reduced nicotinamide-adenine dinucleotide (NADH). Where great precision may be obtained by averaging many readings, this may be avoided. In our work, an analog-to-digital converter with a range of zero to 1000 has been used. The precision obtainable with wider range digitalization and refined voltage stabilization remains to be investigated. The standard errors observed at zero absorbance (100 percent transmittance) at three wavelengths are shown in Table 1. The errors arise from a variety of sources (13).

Programs and Analyses

Programs have been written to measure pathlength with the use of absorbance standards, to determine the standard error of multiple measurements, to fit a curve to a series of standards and print out the value of unknowns, and to follow rates of reactions by reading all cuvettes at intervals for a period of time set by the operator (14). Compensations for temperature, determination of maximum reaction rates, and conversion to standard enzyme units are easily added. In addition, all raw data may be displayed on a cathode-ray tube (CRT), and the results may be plotted on the same tube as calculated.

The initial work on the fast analyzer was done with the biuret (2, 15) reaction for total protein. During the very short history of these systems, they have also been adapted to determinations of total protein by the Folin reaction (16), to assay of cytochrome oxidase, to measurement of cholesterol (17), and to the measurement of lactate dehydrogenase in serum and plasma (18, 19). The last is chosen to illustrate some of the potentials of the system. The CRT display was photographed and used to prepare Fig. 5, while the printout data was used to prepare Fig. 6. The point to be made is that CRT displays of the absorbance plotted against time may be presented to the operator in rapid succession to allow him to evaluate the total process, with only the final single value of maximum rate expressed in standard enzyme units being printed out, as shown for three experimentals and three controls in Table 2. The same data are presented from two readouts in Figs. 4 and 5 to make this point: Initially, the oscilloscope pattern is photographed, all



Fig. 7. Small plastic volumetric measuring tubes and transfer disk used to hold tubes during centrifugal transfer of measured liquids into cuvettes (2). Tubes are filled, and the meniscus is flattened centrifugally. They are then placed in the rotor shown and the contents are moved centrifugally into the cuvettes of a cuvette rotor.

absorbance values and the rates of absorbance are printed out, and the curves of absorbance plotted against time are observed on the CRT display. As the analyst develops experience and confidence, the output will be gradually reduced to one number—the maximum slope. This experience sequence avoids uncertainties concerning actual events and allows the analyst to reinstate full data recall if desired.

In the manual (19) and continuous-flow (20) method for lactate dehydrogenase, the enzyme, buffer, and NADH solutions are incubated for 20 to 26

minutes before addition of the substrate, to insure removal of endogenous substrates. In the procedure now used, a blank is included, and its value is subtracted; the computer can determine when the change in absorbance with time is zero and add pyruvate during rotation, as described later.

Modifications

The simple concept of a transfer disk containing samples and reagents which are removed to a cuvette rotor by centrifugation may be modified in a variety of ways. The first of these is the inclusion of small syphons in the cuvettes which allow the cuvettes to be drained, either by air pressure applied to the rotor center or suction applied to the rotor edge (4). When air is forced back through the syphons during rotation, it produces rapid mixing of the cuvette contents—in 1 second or less. This modification reduces the turn-around time for analyses to less than 2 minutes, and in some instances to less than 45 seconds, depending upon

Table 2. Lactate dehydrogenase assay. Absorbances were read in a 1-centimeter cuvette rotor at 25.6°C (18).

Experiment	Change in absorbance per minute	
	With substrate	Without substrate
1	0.0906	0.0028
2	0.0903	0.0033
3	0.0908	0.0049
Average	0.0906	0.0037

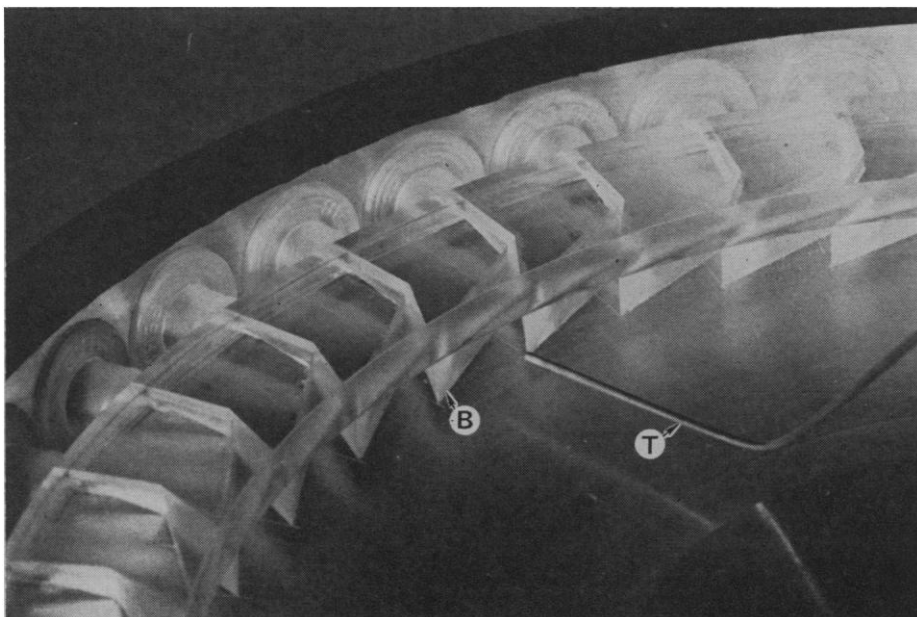


Fig. 8. Plastic blades (B) used in a cuvette rotor to segment a reagent stream flowing through tube (T) so that equal volumes are deposited in each cuvette. The reagents can be added to all cuvettes during the course of the analysis.

the amount of washing required and whether the cycle is run by the computer.

The centrifugal field prevents bubbles from adhering to the cuvette walls and tends to sediment precipitates which may be present. The centrifugal field may also be used to measure and transfer small fluid volumes efficiently. In devices for measuring volumes in the

microliter range either pipettes of sufficiently small bore that variations in meniscus shape are inconsequential or small captive pistons to suck the sample into a small tube with the force transmitted by air or liquid have been used. In the latter instance part of the liquid is expelled with the sample and serves as a diluting fluid.

In a centrifugal field, almost any

cavity can be used as a measuring device because the meniscus is flattened, and any fluid centripetal to the inner edge of the cavity is removed by centrifugation. Further, the centrifugal field can be used to fill the cavity initially. Air bubbles quickly disappear, and filling is not affected by differences in viscosity, density, or surface tension of the liquid measured. With simple plastic cylindrical vessels, ranging in contained volume from 30 to 125 microliters, average variations of less than $\pm \frac{1}{2}$ percent were observed in the volumes measured and centrifugally transferred from small fluorocarbon tubes (2). Freedom from errors due to density and viscosity differences between samples is especially important in the analysis of gradients, such as those recovered from zonal centrifuges where the sucrose concentration may be in excess of 55 percent by weight.

In the simplest case, a large number of tubes may be filled centrifugally and inserted in a suitable transfer disk (Fig. 7). The tubes may also be mechanically moved so that they are filled during rotation and rotated at rest to drain into the cuvettes when again accelerated (21). Cavities have also been designed which serve as measuring vessels at constant speed but drain into the cuvettes during rapid deceleration (so-called inertial transfer) (21).

When reagents must be added at

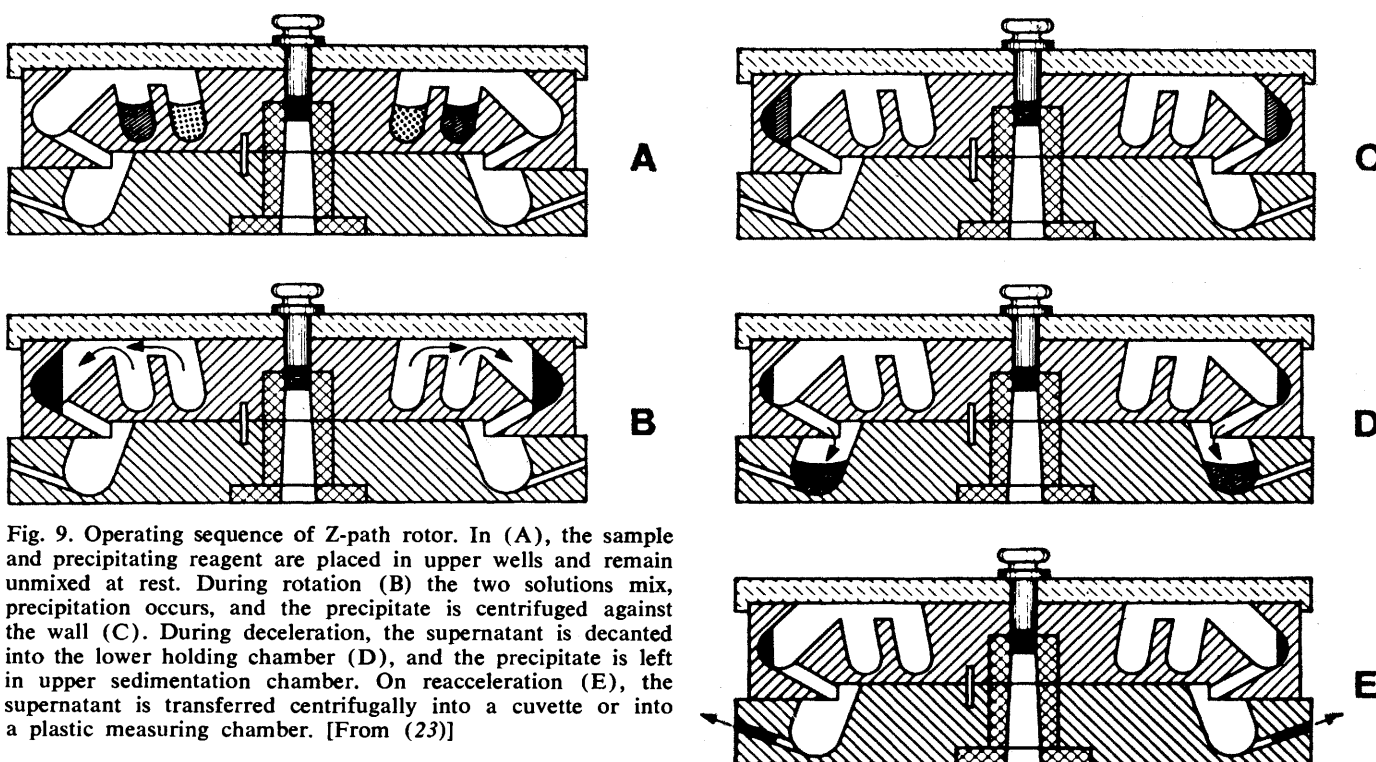


Fig. 9. Operating sequence of Z-path rotor. In (A), the sample and precipitating reagent are placed in upper wells and remain unmixed at rest. During rotation (B) the two solutions mix, precipitation occurs, and the precipitate is centrifuged against the wall (C). During deceleration, the supernatant is decanted into the lower holding chamber (D), and the precipitate is left in upper sedimentation chamber. On reacceleration (E), the supernatant is transferred centrifugally into a cuvette or into a plastic measuring chamber. [From (23)]

intervals, a technique termed dynamic stream segmentation has been used (22). Blades are positioned between the centripetal cuvette openings so that a very small reagent stream is equally apportioned, and each portion is channeled into one cuvette. With a simple plastic prototype (Fig. 8), the variation in the weight of the liquid delivered is ± 2 percent between cuvettes. When a non-absorbing reagent or substrate is added in a volume which is small compared with the total volume in the cuvette, and where its final concentration is not critical, then this technique is suitable in its present form. With further research, the precision of such additions "on the fly" should improve.

The transfer disks used to contain sample and reagents can be designed in a variety of ways. For example, they may be designed so that two or more solutions drain together through a small port leading to the cuvette. Alternatively, the radii and slopes of the cavities may be arranged so that the outer cavities drain into the cuvette at one speed, and each concentric cavity ring, as one proceeds from edge to center, drains at a different speed. Thus, programmed speed changes may be used to move samples and reagents sequentially and at predetermined intervals into the cuvettes.

One of the most interesting possibilities now being explored concerns packaging of measured volumes of reagent in the center transfer disk. Since the total volume for each test is 300 to 500 microliters, only very small amounts of reagents are required. Reagents in very small packages therefore would make possible a variety of individual tests to be run efficiently, and such packaging would eliminate the necessity of making up fresh reagents as

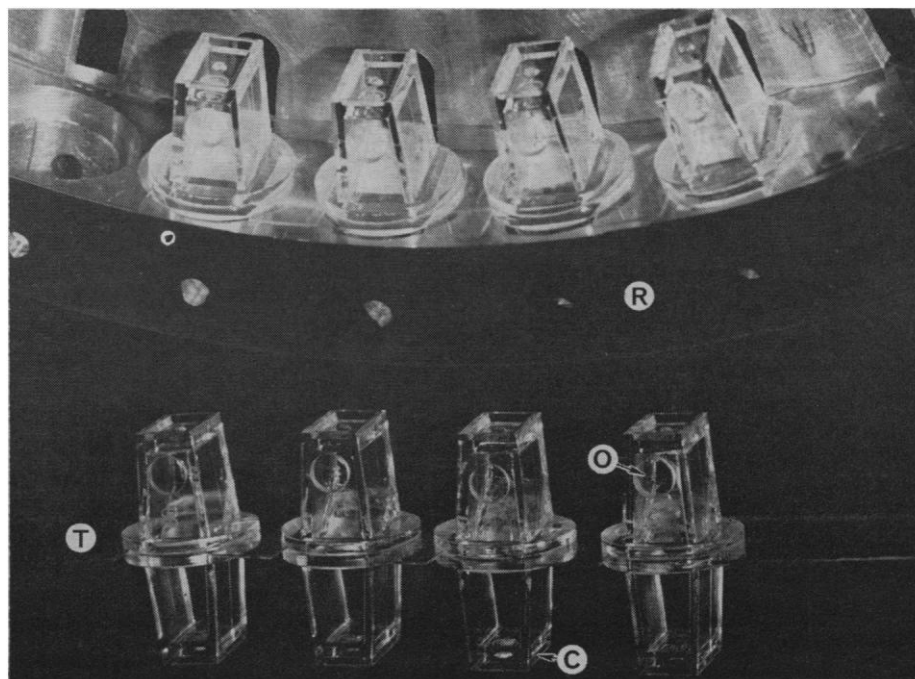


Fig. 10. Plastic cuvette belts used for multiple-addition reactions. The cuvette chamber (C) is attached to tape (T). The holding chamber aperture (O) allows samples or reagents to be added and then transferred centrifugally into the cuvette by means of rotor (R). The operating principles illustrated are shown in Fig. 11.

needed or of stocking large volumes.

The centrifugal field is useful for other purposes and has been used to drive samples or reagents through small ion-exchange or gel-filtration columns incorporated into the transfer disk (21). (For quite different purposes multiple, small ion-exchange columns have been incorporated into medium-speed rotors, and centrifugal force is used to drive the eluting solutions through the columns. The effluents are read as they pass through small flow cells in the rotor and are expelled at a radius greater than that of the input stream.) Filters may also be incorporated into the transfer disks to remove precipitates or to

retain proteins while smaller molecules are centrifuged through, thus providing protein-free filtrates.

The centrifugal field has been used in another way to remove precipitates in the self-decanting "Z-path" rotors (23) shown diagrammatically in Fig. 9. These are designed to provide cavities in which sample and precipitating solution are held unmixed at rest. During acceleration they are both quantitatively transferred to a chamber where the precipitate is centrifuged to the wall. This chamber is tilted so that the supernatant is decanted into a third chamber when the rotor is brought to rest. This simple device allows many clear super-

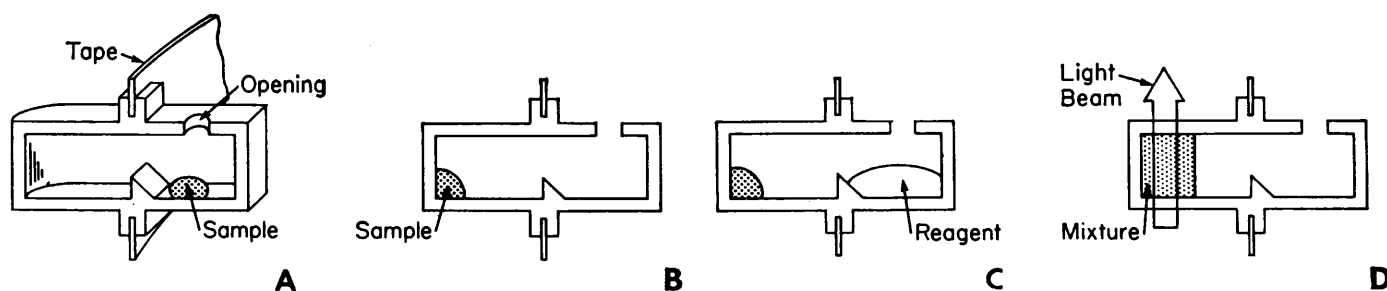


Fig. 11. The operating principles of cuvette belts. The plastic belt or tape (T) (Fig. 10) holds the cuvettes together. In (A), a small sample is added through aperture (O). During centrifugation, the sample is moved to the left cuvette chamber shown in (B). A reagent is then added in (C) and is moved centrifugally to the left cuvette chamber in (D). The absorbance of the analytical mixture is determined during rotation, a stationary light beam being used. The center barrier prevents liquids in either half of the chamber from mixing at rest. Reagents may be added to the right-hand chambers of a tape sequentially with an automatic measuring device. However, all transfers to the optical absorbance measuring chambers at left are made at the same time in all cuvettes in a belt during acceleration.

natants to be prepared simultaneously.

Syphons to measure and transfer samples and reagents have also been constructed and tested; these are drained by air pressure in a centrifugal field. These syphons present difficult fabrication problems which are only partially solved.

Cuvette Belts

A variety of designs for disposable plastic cuvettes attached to flexible belts or tapes has been proposed (24). These cuvettes are placed in rotors similar to cuvette rotors and read during rotation. An experimental tape and rotor is shown in Fig. 10 and is illustrated diagrammatically in Fig. 11. One advantage of such belts is that they may be loaded in one rotor, removed for incubation or heating, and read during a short interval in another rotor. Tapes having 40 to 100 cuvettes are feasible and, if read at rates of less than 2 minutes per tape, would yield many thousand analyses per hour.

Summary

We conclude that discovery of a variety of new principles makes possible the development of fast analyzers which are readily interfaced with and controlled by computers. However it is not yet possible to predict what configurations will prove most reliable and useful. For example, the reaction volumes may be varied greatly, and there appears to be no technical reason why devices having a total reaction volume of as little as 1 microliter and working at very high rotational speeds cannot be built. High-speed electronic computers have rendered secondary to present systems considerations of the cost or time required per addition, subtraction, or multiplication. The problem considered here is how to reduce the cost of individual analyses to the point where the cost of each is similarly inconsequential. The objective is not only to increase speed and lower cost, but also to solve

one of the most difficult problems in the clinical laboratory, namely, mismatching of sample and result. As previously noted, if each analysis can be done in duplicate or triplicate at random, then there is a very high probability that, if the results agree, they are correct. Statistical analysis of the results will also provide a constant check on precision.

In the lactate dehydrogenase assays presented, seven experimental assays with seven control were done in little more time than that required for assay manually. With larger rotors, the number may be easily increased. Even at this very early stage of development, when many concepts remain to be fully explored, the number of many kinds of tests done may be greatly increased, and the data in final form may be obtained quite rapidly. It is not too early to raise the question of the use to which these and similar devices will be put.

There can be no question of the advantage of increased speed and decreasing cost of clinical tests involved in diagnosis and patient care. Mass screening for occult disease has been repeatedly proposed as a desirable objective, and one which required only the solution of technical problems. In screening, the presumption is (i) that the natural course of a disease is sufficiently well understood so that benefit is derived from its discovery before symptoms appear and (ii) that resources are available which will make it possible to effectively treat the patient. In the case of infectious diseases, such as tuberculosis, these requirements have been met.

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