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These articles are intended to serve the readers of THIS JOURNAL by calling attention to new developments in the theory, design, or availability of chemical laboratory instrumentation, or by presenting useful insights and explanations of topics that are of practical importance to those who use, or teach the use of, modern instrumentation and instrumental techniques. The editor invites correspondence from prospective contributors.

LXXX. Centrifugal Analyzers—A New Concept in Automation for the Clinical Chemistry Laboratory

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The workload in hospital clinical chemistry laboratories has been steadily increasing at an average rate of 15% per year. In Babies Hospital Clinical Chemistry Laboratory the number of tests performed from 1972 to 1973 increased 19%. This upward trend is likely to continue as new and better diagnostic tests become available to help physicians make clinical decisions. To meet this increasing demand on the laboratory, new advances in clinical automation must be utilized.

A new concept in automation, centrifugal analysis, was recently developed at Oak Ridge National Laboratories (1-3) by Norman G. Anderson. At the present time, three different analyzers are commercially available in the United States. Although all are based on Anderson's original concept, there are some differences in their design and operation. This article will deal with the general theory and operation of centrifugal analyzers, descriptions of all three commercially available analyzer systems and applications of centrifugal analyzers in the clinical laboratory.*

THEORY

Centrifugal analyzers are multicuvet spectrophotometers that simultaneously and rapidly measure a single substance in a large number of discrete samples. The analyzer consists of a transfer disc, a rotor containing the cuvets, a light source, a photomultiplier, an oscilloscope, an analog-to-digital converter and a computer. A pipettor, which is a separate bench top instrument, is required with each analyzer.

The transfer disc is circular, constructed

of Teflon, and contains consecutively numbered positions into which samples and reagents are pipetted. The geometry of the disc is such that each position contains at least two wells (compartments) which separate the sample from reagent prior to analysis. The disc and cups which contain the sample to be analyzed are placed in the turntable section of the pipettor. The pipettor consists of two probes, one to dispense sample and diluent and the other to dispense reagent into the appropriate wells of the disc. The sample probe of the pipettor picks up a preselected volume of sample and moves to a position above the sample well of the disc. The sample is expelled from the probe into the sample well, followed by a preselected volume of diluent, usually water. By choosing an appropriate volume of diluent, carry-over from one sample to another can be eliminated. The reagent probe picks up a preselected volume of reagent, moves to a position above the reagent well of the disc and dispenses this volume. After each set of sample, diluent and reagent is pipetted into the appropriate wells in the transfer disc, the turntable moves to the next position. This process is repeated until all samples are pipetted. If there are not enough samples to fill all positions in the disc, a magnet is placed in the sample rack after the last sample. Water rather than reagent will be pipetted into every remaining reagent well of the disc. This saves unnecessary pipetting of expensive reagents. The last position in the disc is the reference position. Only water or reagent can be pipetted in this position. The volume of sample that can be pipetted ranges from 2 to 50 μ l. Pipetting of sample volumes of less than 5 μ l may result in a loss in precision. Depending on the design

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of the analyzer the number of positions in the disc will vary from 15 to 30 and the reagent volume from 300 to 750 μ l.

When the pipetting is completed, the transfer disc is manually removed from the pipettor, hand carried and locked into place in the thermostatically controlled rotor chamber of the analyzer. The rotor contains discrete cuvets corresponding to a numbered position on the disc, each with a path length of 1 cm. The analyzer is programmed for each analysis by selecting the wavelength, temperature, type of analysis and time intervals at which absorbance measurements are to be taken. A cover is placed over the disc and the entire rotor chamber including the transfer disc and cuvets is rotated. During the acceleration period, centrifugal force generated by the rotation of the rotor quantitatively transfers each set of samples and reagents into their respective cuvets. Mixing of solutions is accomplished by applying a vacuum which draws air through siphons which are connected to each cuvet. The entire process of transferring and mixing requires only a few seconds. Rotation of the cuvets is continued throughout the entire analysis. The rotor is revolved at high speeds, thus allowing all the samples and reagents to be transferred simultaneously into their respective cuvets and for the reaction in each cuvet to be started and measured spectrophotometrically essentially at the same time and under identical conditions.

The cuvets are rotated through a stationary light beam and the signal from the photomultiplier is amplified, digitized and stored in a computer. The absorbance is measured at preselected time intervals which range from a few seconds to several

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* A more detailed review has appeared in *Analytical Chemistry* (4).

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minutes. Each recorded absorbance measurement is the arithmetic mean of eight successive readings. This averaging process occurs during eight consecutive revolutions of the rotor and is completed in less than one second. At each time interval, the absorbances measured in each cuvet are subtracted from the absorbance of the solution (either water or reagent) in the reference cuvet and stored in the computer. The computer calculates the concentration of the constituent being measured from the change in absorbance. Thus, the effects of cuvet to cuvet variations due to scratches, dirt etc. are eliminated. Since, the readings in each cuvet are averaged readings taken at nearly identical times, and subtracted from the reading in the reference cuvet, centrifugal analyzers function as double beam spectrophotometers.

The products of chemical reactions that are taking place in each cuvet are detected by a photomultiplier. Throughout the entire reaction period the signal from the photomultiplier, for each cuvet, is continuously displayed on the oscilloscope. Thus, by looking at the oscilloscope, the operator can visually monitor the progress of the reactions in each cuvet and can observe any abnormal sample or instrument performance. The photomultiplier signal is passed through a buffer amplifier, directed to an analog-to-digital converter and subsequently to a computer. After the last measurements have been made, the computer processes the data, calculates and types the results. The solutions in the cuvets are emptied by applying pressure to the rotor chamber thus forcing the reac-

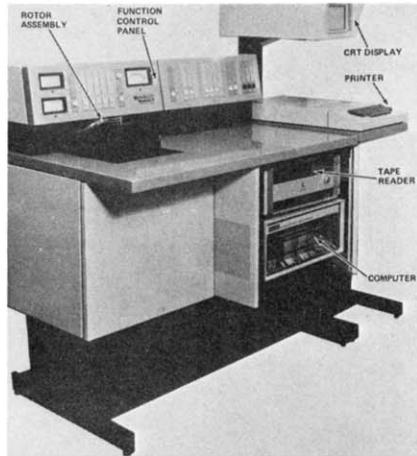


Figure 1. The Rotochem Centrifugal Analyzer System (American Instrument Company)

tion mixtures out through siphons and into a waste bottle. The cuvets are flushed with water and dried. Rotation is stopped and the analyzer is ready to use for the next analysis.

COMMERCIAL INSTRUMENTS

1. The RotoChem II Parallel Fast Analyzer system.

This instrument (Fig. 1) is manufactured by American instrument Company, Silver Spring, Maryland. The RotoChem system specifications are:

A. Transfer disc. The transfer disc con-

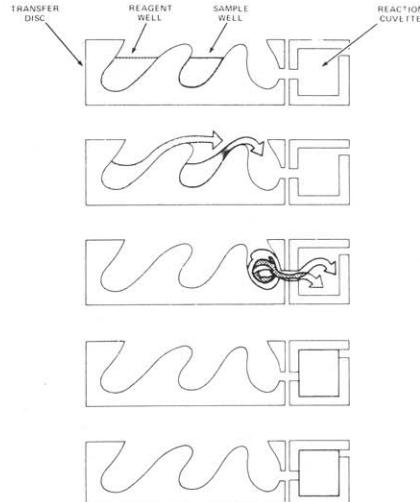


Figure 2. A cross section of the transfer disc showing the method of transferring and mixing of sample and reagent into the cuvet. (American Instrument Company)

tains 14 sample positions and one reference position. Each position has three wells, one for sample and diluent, one for reagent, and one for mixing the sample and reagents prior to entrance into the cuvets. A cross section of the disc is shown in Fig. 2.

B. Pipettor. The pipettor (Rotorfill 1), shown in Fig. 3, can measure from 2 to 50 μl of sample, up to 200 μl of diluent (water or reagent) and up to 500 μl of reagent. A 4:1 ratio of diluent to sample will, in most cases, eliminate carry-over. All positions on the disc can be filled with samples and reagents in less than three minutes. A

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third reagent can be manually pipetted into the mixing well of the transfer disc.

C. *Cuvets.* The minimum volume of solution that can be measured in each cuvet is 450 μl and the maximum volume 750 μl . All cuvets have a path length of 1.0 cm.

D. *Rotor speed.* The disc and cuvets are accelerated to a speed of 600 rpm at which all measurements are made.

E. *Cleaning of disc and cuvets.* After completion of each analysis the disc and cuvets are automatically emptied, washed and dried.

F. *Temperature control system.* A thermistor placed in the wall of one of the cuvets monitors the temperature and controls an electric heater. The rotor compartment is cooled by circulating cold water. The temperature in the cuvets is adjustable over a range from 20°C to 42°C.

G. *Optical system.* The light source is a tungsten halogen lamp. The instrument has an absorbance range of 0.0 to 2.0 and resolution of 0.0003. The wavelength range is 340–650 nm using six interference filters of 340, 405, 520, 550, 600 and 620 nm, each with a 10 mm bandwidth.

H. *Electronic system.* The computer, a PDP-8 (Digital Equipment Corporation) with 8k core memory, is interfaced to the centrifugal analyzer by a 12 bit analog-to-digital converter. The computer programs for all determinations are recorded on a 4-track magnetic tape cartridge and are loaded into the computer before each analysis. A high speed printer terminal keyboard provides the means for operator dialogue with the computer as well as a printout of the final data. The computer is

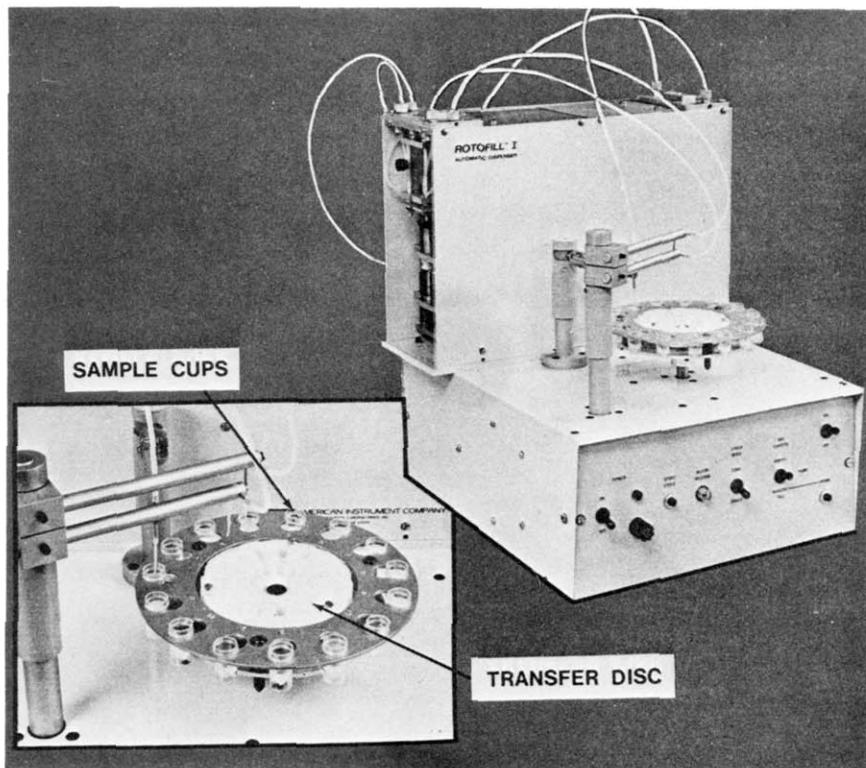


Figure 3. The Rotochem pipettor system. (American Instrument Company)

programmed so that all abnormal values, non-linear values and substrate exhaustion in enzyme assays are flagged on the data print out sheet. Figure 4 is a block

diagram of the RotoChem System.

I. The cost of the RotoChem Centrifugal Analyzer system is about \$55,000.

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2. The GEMSAEC Centrifugal Analyzer System

This assembly (Fig. 5) is manufactured by Electro-Nucleonics Inc., Fairfield, New Jersey. The GEMSAEC System specifications are:

A. *Transfer disc.* The transfer disc contains 15 sample positions and one reference position. The geometry of the disc is the same as that in the RotoChem System.

B. *Pipettor.* The pipettor (Fig. 6) consists of two Micromedic units (Micromedic Systems, Inc., Philadelphia) and can measure from 2 to 50 μl of sample, up to 200 μl of diluent (water or reagent) and up to 750 μl of reagent depending on the size of the pump. All positions in the disc are filled with sample and reagent in less than 3 minutes.

C. *Cuvets.* The minimum volume of solution that can be measured in each cuvet is 450 μl and the maximum volume 750 μl . All cuvets have a path length of 1.0 cm.

D. *Rotor speed.* The disc is initially accelerated to a speed of 2500 rpm and then gradually reduced to 400 rpm at which all measurements are taken.

E. *Cleaning of disc and cuvets.* After completion of each analysis, the disc and cuvets are automatically emptied, washed and dried.

F. *Temperature control system.* The temperature is controlled by pumping distilled water into a shroud which surrounds

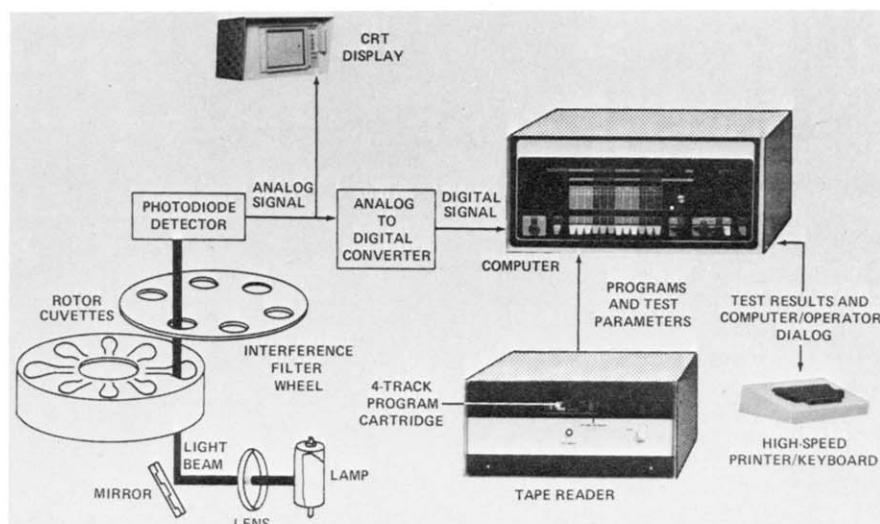


Figure 4. A block diagram of the Rotochem System. (American Instrument Company)

the cuvets. The temperature is monitored by a thermistor located in the wall of the shroud. A temperature range of 20° to 50°C can be obtained.

G. *Optical system.* The light source is a tungsten quartz iodide lamp. The absorbance range is 0.0 to 2.0 with a resolution of 0.0005. The wavelength ranges from 320–785 nm using a grating monochromator with a 5 mm bandwidth.

H. *Electronic system.* The computer is a PDP-8 (Digital Equipment Corporation) with 8K core memory interfaced to the centrifugal analyzer by a 12 bit analog-to-digital converter. The computer automati-

cally converts absorbance to serum test concentration, and flags abnormal values, non-linear values and substrate exhaustion in enzyme assays. The final results are printed by a Teletype or an optional high speed printer.

I. The cost of the GEMSAEC Centrifugal Analyzer System is about \$50,000.

3. The Centrifichem Centrifugal Analyzer System

This instrument (Fig. 7) is manufac-
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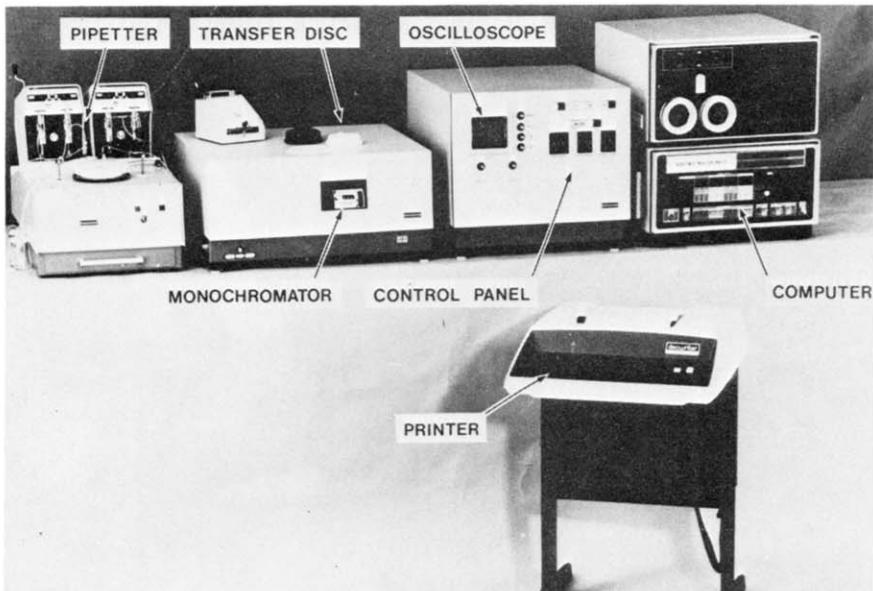


Figure 5. The GEMSAEC Centrifugal Analyzer System (*Electro-Nucleonics, Inc.*)

tured by Union Carbide Corporation, Tarrytown, New York. The Centrifichem specifications are:

A. *Transfer disc*. The transfer disc contains 29 sample positions and one reference position. Each position has two wells; one for sample and diluent and one for reagent. A cross section of the disc is shown in Fig. 8.

B. *Pipettor*. The pipettor (Fig. 9) can measure 2 to 50 μl of sample, up to 50 μl of diluent (either water or reagent) and ei-

ther 250 or 350 μl of reagent. A maximum of two reagents can be used with this system. All positions in the disc are filled with sample and reagents in three minutes.

C. *Cuvets*. The minimum volume of solution that can be measured in each cuvet is 300 μl and the maximum volume 500 μl . All cuvets have a path length of 1.0 cm.

D. *Rotor speed*. The disc and cuvets are accelerated to a speed of 900 rpm at which all measurements are made.

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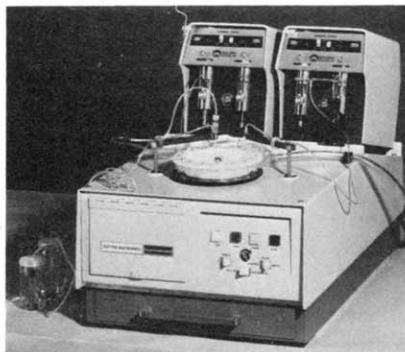


Figure 6. The GEMSAEC pipettor system. (*Electro-Nucleonics, Inc.*)

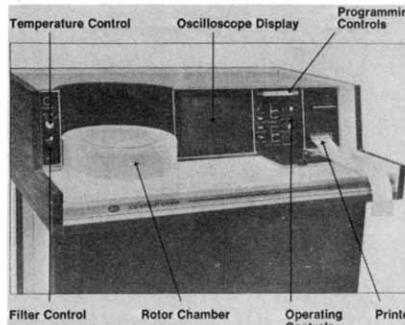


Figure 7. The Centrifichem Centrifugal Analyzer System. (*Union Carbide Corporation*)

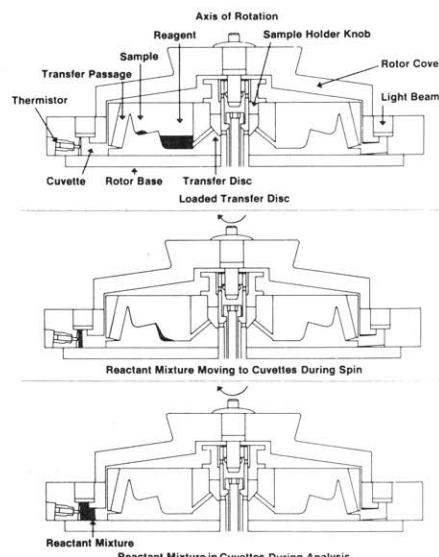


Figure 8. A cross section of the transfer disc. (*Union Carbide Corporation*)

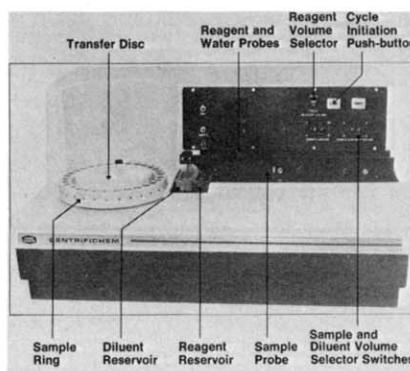


Figure 9. The Centrifichem pipettor system (*Union Carbide Corporation*)

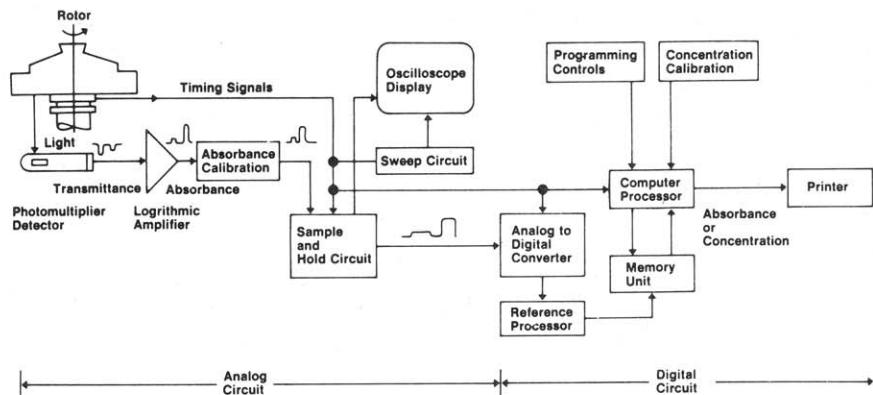


Figure 10. The Centrifichem analog-to-digital circuit system. (Union Carbide Corporation)

F. *Cleaning of disc and cuvets.* After completion of each analysis, the disc and cuvets must be manually emptied, washed and dried.

G. *Temperature control system.* Ambient air is drawn into the analyzer by a blower, passed over a heater and into the rotor chamber. The temperature of the cuvets is monitored by a thermistor located near the reference cuvet. Three temperatures are possible: 25, 30 and 37°C.

H. *Optical system.* The light source is a tungsten-iodide lamp. The absorbance range is 0.0 to 2.0 with a resolution of 0.001. The wavelength range is from 340–650 nm using six interference filters of 340, 405, 520, 550, 600 and 620 nm, each with an 8 mm bandwidth. A deuterium lamp and a 292 nm filter are also available.

I. *Electronic system.* The computer is an integral special purpose computer consisting of an analog-to-digital circuit and a printer. The results are calculated by the computer but with manual manipulation by the operator. Abnormal values, non-linear values, and substrate exhaustion in enzyme assays are not flagged on the print out sheet. Figure 10 is a block diagram of the Centrifichem System.

J. The cost of the Centrifichem Centrifugal Analyzer system is about \$32,000.

CENTRIFUGAL ANALYZERS IN THE CLINICAL CHEMISTRY LABORATORY

Three types of reactions can be performed on centrifugal analyzers, end-point, fixed-time and kinetic rate analysis.

A. End-Point Analysis.

End-point analysis involves measurement of the initial absorbance (A_0) at a time (T_0) before the reaction has started. This initial reading represents a serum blank and eliminates the need to analyze a separate sample to determine this value. The reaction is allowed to go to completion and the final absorbance (A_f) measured at

time (T_f). The change in absorbance ($A_f - A_0$) is used to compute the results. For example, glucose in serum can be determined by the hexokinase method. Glucose-6-phosphate dehydrogenase converts glucose-6-phosphate and NAD to 6-phosphogluconate and NADH. The increase in absorbance at 340 nm as NADH is formed is proportional to the glucose concentration. Initial absorbances are taken at 3.0 seconds and final absorbance at 6.0 minutes. The change in absorbance between 3.0 seconds and 6.0 minutes is used to determine the glucose concentration. Table 1 shows some serum constituents that can be determined by end-point analysis with a centrifugal analyzer.

B. Fixed-Time Analysis.

Fixed-time analysis measures the absorbance at time intervals of T_0 and T_1 . This change in absorbance ($A_1 - A_0$) is related to concentration, thus making this type of analysis faster than end-point analysis. For example, creatinine in serum can be determined by reaction with picric acid to form a red pigment (Jaffé reaction). By measuring the increase in absorbance at 520 nm between 40 sec and 2 minutes, interference from Jaffé-positive serum constituents (glucose, acetone, acetoacetic acid) can be minimized, since their reactions with picric acid are almost complete by the time the initial absorbance reading is taken. Table 2 shows some serum constituents that can be determined by a fixed-time method with a centrifugal analyzer.

C. Kinetic rate analysis.

Kinetic rate analysis is used to measure enzyme activity. Absorbance measurements are taken at times, T_0 , T_1 , T_2 , T_3 , etc. If the changes in absorbance (ΔA) at $(T_1 - T_0)$, $(T_2 - T_1)$, $(T_3 - T_2)$ are nearly identical, the ΔA 's can be used to determine the enzyme activity. For example, lactate dehydrogenase (LDH) activity in

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Table 1. End-Point Assays of Serum or Plasma Constituents That are Determined in Babies Hospital Clinical Chemistry Laboratory

Test	Sample size (μ l)	Method	T_0 (sec)	T_f (min)
Glucose	5	Hexokinase (5)	3	6
Phosphorus	10	Phosphorylase (6)	3	16
Total protein	5	Biuret (7)	3	6
Uric acid	8	Uricase (8)	3	5

Table 2. Fixed-Time Assays of Serum and Plasma Constituents That are Determined in Babies Hospital Clinical Chemistry Laboratory

Test	Sample size (μ l)	Method	T_0 (sec)	T_1 (min)
Creatinine	25	Jaffé (9)	40	2
BUN*	5	Urease (10)	35	0.75

* BUN = Blood urea nitrogen

Table 3. Rate Assays of Enzymes That are Determined in Babies Hospital Clinical Chemistry Laboratory

Test	Sample Size (μ l)	Method	T_0 (sec)	T_1 (Min)	T_2 (Min)	T_3 (Min)
Aspartate Aminotransferase (GOT)	40	Karmen (11)	90	2	2	2
Alanine Aminotransferase (GPT)	40	Wroblewski-La Due (12)	90	2	2	2
Lactate Dehydrogenase (LDH)	10	Wacker (13)	60	1	1	1
Creatine Phosphokinase (CPK)	15	Oliver (14)	150	1	1	1
Gamma Glutamyl Transpeptidase (GGTP)	30	Szasz (15)	30	1	1	1
Alkaline Phosphatase	10	Bowers-McComb (16)	30	1	1	1

serum can be determined by reaction of lactate with NAD to form pyruvate and NADH. The increase in absorbance at 340 nm as NADH is formed at time intervals of 1 minute is used to determine the activity of LDH. Table 3 shows some enzymes that can be determined by a rate method with a centrifugal analyzer.

CONCLUSION

Centrifugal analyzers offer the means to perform large scale parallel assays with microvolumes of sample and reagents. Samples and reagents can be processed under identical conditions of mixing, temperature, spectrophotometric measurement and timing, thus improving the precision and accuracy of the assay. The use of small volumes of sample makes centrifugal analyzers ideal for tests relating to pediatric and geriatric populations. By using small volumes of reagent, the cost per test is less than with other automated instruments. Babies Hospital Clinical Chemistry Laboratory, has been using centrifugal analyzers for routine and emergency tests, 24 hours a day, 7 days a week, for 3 years.

Future applications of centrifugal analyzers include measurements of specific proteins in serum by light scattering, simultaneous measurements at multiple wavelengths, and fluorescence measure-

ments of serum constituents.

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