AUTOMATION IN THE CLINICAL CHEMISTRY LABORATORY

ADRIAN HAINLINE, JR., PH.D.
Department of Clinical Pathology

TECHNOLOGIC developments in analytical chemistry have made it possible for the modern clinical chemistry laboratory to meet an ever-increasing workload despite a shortage of trained laboratory technologists. Furthermore, automatic machines have made possible an accuracy and a reliability seldom attained before in the laboratory. But the new and seemingly complicated equipment has brought with it a greater than ever need for mechanical, electrical, and electronic training for laboratory supervisors.

A number of technics are used to speed up various steps in analytic procedures that are performed in large numbers with repetitive steps; for example, it has been easy to automate pipeting. The Brewer automatic pipet (*Fig. 1*) uses a syringe driven

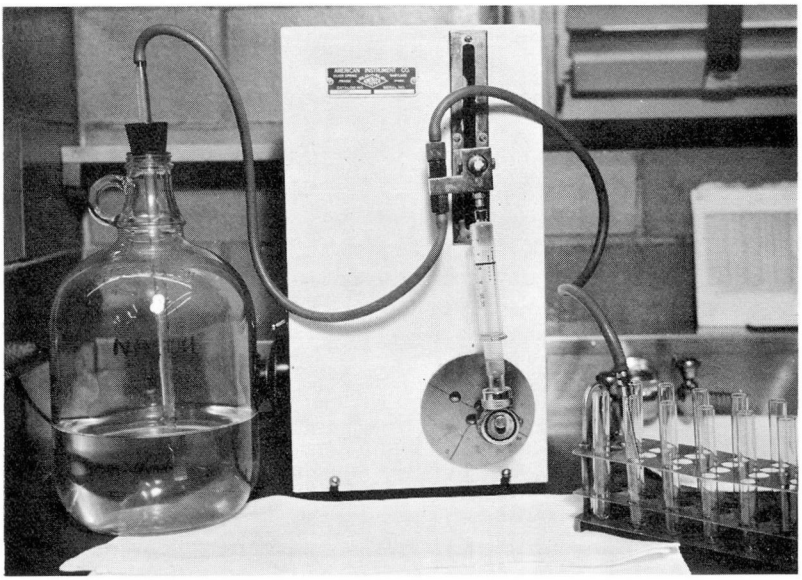


Fig. 1. Brewer automatic pipet: as drive shaft revolves, the plunger moves in and out of the syringe barrel, drawing in solution from a reservoir and pushing it out through a delivery tip.

by an offset cam and pumps, and delivers solutions through a system of ball-and-check valves to a delivery table. Another device used in some laboratories[1, 2] to eliminate the manual step of pipeting of sample and diluent consists of a buret that has a two-way stopcock. One side of the stopcock is connected to a vacuum line and can be used to pull the sample into the calibrated delivery tip. A turn of the stopcock connects to a diluting solution which flushes out the sample. An

automatic version of this is used for dilutions of microquantities, and is especially valuable in making hematologic cell-count dilutions (*Fig. 2*).

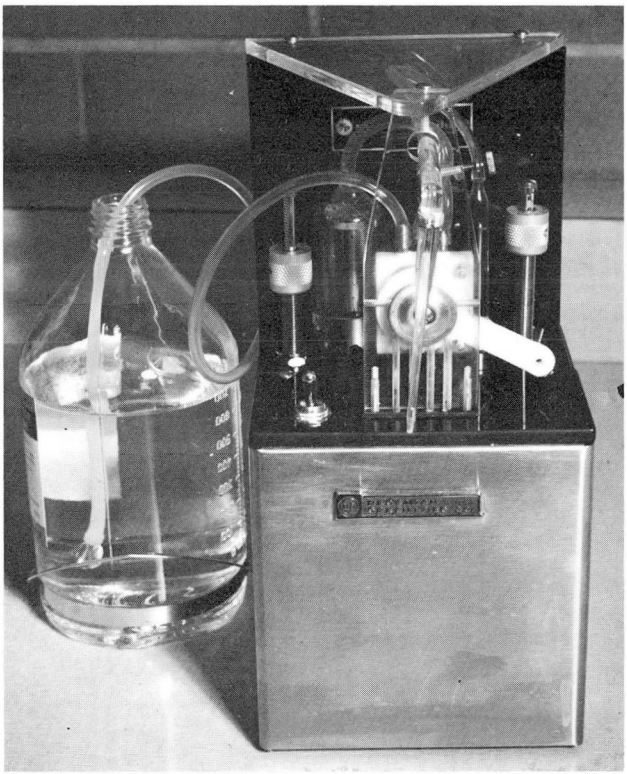


Fig. 2. Microdilutor.

*AutoAnalyzer.** Full automation in clinical chemistry came with the development of the AutoAnalyzer by Skeggs.[3] This machine is composed of a number of separate functional modules and uses a constant-flow system (*Fig. 3*). All manual processes have been replaced, including pipeting, transferring filtration, colorimetric reading, and recording (*Fig. 4*). Sampling rates are variable, and theoretically as many as 60 samples per hour can be run. The actual practical limit is about 50 different specimens per hour, because in practice, the run includes standards and blanks as well as samples.

The AutoAnalyzer system begins with a sample plate (*Fig. 5*) that holds thimble-sized polystyrene cups that can contain the various standards and specimens. A clockwork mechanism and cams move a sampling crook in and out of the specimen cups. The timing is regulated so that for 40 percent of the time interval the sample

**Manufactured by the Technicon Instruments Corporation, Chauncey, New York.*

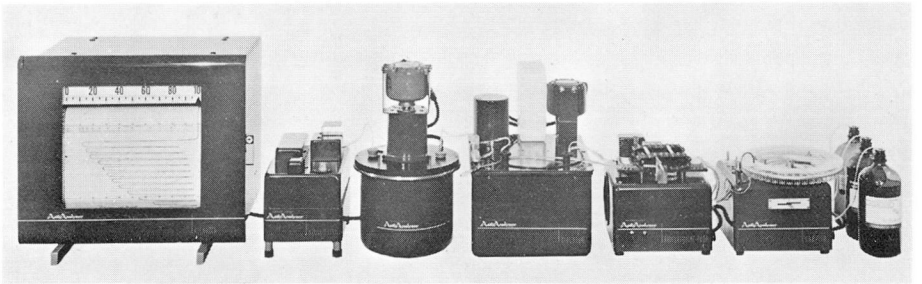


Fig. 3. View of AutoAnalyzer showing typical modules. (Courtesy of Technicon Instruments Corporation, Chauncey, New York.)

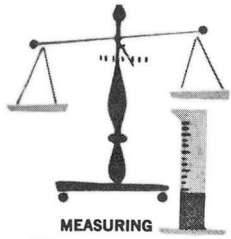
is withdrawn, and for 20 percent the crook is lifted between samples and is drawing air. Thus each sample is separated from the next by air.

The withdrawal of samples and movements of other liquids are accomplished by the motion of roller bars along a manifold (*Fig. 6*) of Tygon tubes stretched out against a platen in the pump. The diameter of the Tygon tubes controls the amount of fluid pumped. Changes in the quantities to be pumped can be made by using tubing of the appropriate diameter. Mixing of solutions is accomplished by transit through coils of glass tubing, the liquid being segmented with bubbles of air, which are pumped in continuously through one or more of the Tygon tubes. Thus, the pump and sample turntable modules accomplish automatically what is done manually with the pipet or buret—measuring out of samples and reagents. From this point on the instrument modules used vary with each type of procedure.

If protein can interfere in a test, it may be removed. The AutoAnalyzer system includes a dialyzer that permits the separation of crystalloids from proteins. The solutions pass through a spiral tube that has been formed by matching identical grooves cut into two matching plastic discs. The two discs (plates) are placed together with a thin cellophane membrane between them. The membrane thus divides the tube into two parts, through one part of which passes the sample. A recipient stream flows through the other part of the tube. Because the rate of dialysis is affected by temperature variations, the dialyzer plates are immersed in a constant-temperature bath.

In many procedures, it is necessary to heat the mixed solutions; the time interval is regulated by the length of glass tubing that is used, and the temperature is controlled by a heating bath in which the tubing is immersed (*Fig. 7*). To increase the time another glass coil may be added within the bath. Heating in such a bath is usually necessary to develop a color in the solution, and it is desirable that the intensity of this color be proportional to the amount of unknown to be determined just as is required in a manual analysis.

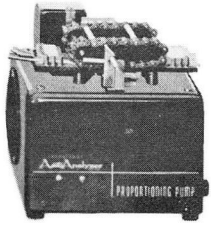
The exit side of the heating bath is connected to a colorimeter flow cell (*Fig. 8*). This cell is constructed so that a turbulence-free flow will pass between two optical windows that are interposed between a light beam of an excitor lamp and a photo-



MEASURING
PIPETTING • TITRATING
WEIGHING

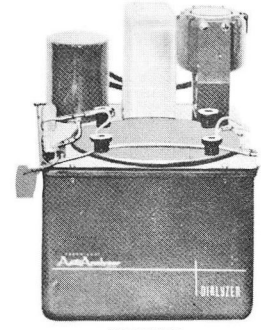
MIXING
AGITATING
STIRRING

PURIFYING
DISTILLATION • FILTRATION
CENTRIFUGING • PRECIPITATING

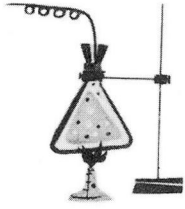


PROPORTIONING PUMP

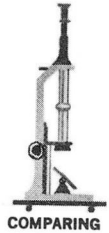
MIXING COIL



DIALYZER

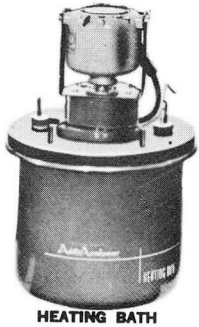


PROCESSING
HEATING • COOLING
INCUBATING



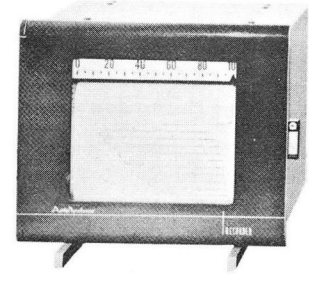
COMPARING
Ddensiometric • PHOTOMETRIC
COLORIMETRIC

RECORDING



HEATING BATH

COLORIMETER



CONTROL RECORDER

Fig. 4. Shows how functional modules of the AutoAnalyzer substitute for typical manual steps in analysis. (Courtesy of Technicon Instruments Corporation, Chauncey, New York.)

Fig. 5. Sample module. Tests-per-hour lever regulates the rate at which sample crook lifts and table turns. (Courtesy of Technicon Instruments Corporation, Chauncey, New York.)

cell (*Fig. 9*). As the intensity of the color increases, the light absorbed from this beam also increases, and the light falling on the sample photocell decreases. The reverse situation produces the opposite effect. The electrical output of the sample photocell is balanced against the output of the standard (reference) photocell, which receives an uninterrupted portion of the light from the lamp. These cells are so connected with a recorder that whenever the output of the sample photocell is out of electrical balance with that of the standard photocell (as would happen whenever the intensity of light passing through the solution in the flow cell changes), a servomechanism in the recorder restores the electrical balance. A recording pen traces these changes on a chart paper in the conventional system. The tracings are in the form of peaks the highest points of which are used for calculation of concentrations. Concentrations of unknowns are estimated from the values obtained with standards. By careful comparison with the sequence used for loading the sample plate, one can identify the various peaks with the samples.

Recent advances have replaced the recorders with automatic readout devices that either print results on a tape (*Fig. 10*), or activate a typewriter. The combina-

tion of the recorder and the readout device is valuable because the shapes of the tracings on the recorder may be useful in the diagnosis of trouble within the system.

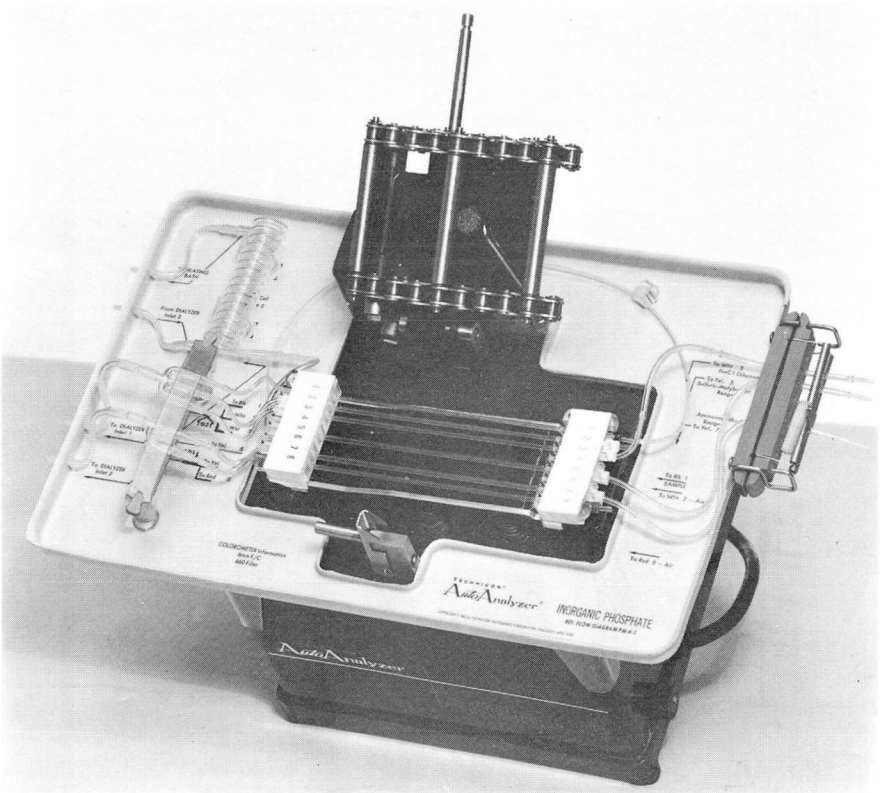


Fig. 6. Proportioning pump complete with manifold for inorganic phosphate determination. View from above shows roller head assembly open. (Courtesy of Technicon Instruments Corporation, Chauncey, New York.)

The main advantage of the readout is the avoidance of errors in the interpretation of the tracing peaks; also, the results are immediately available.

Illustrative example of automation. To illustrate how the AutoAnalyzer performs a complete analysis, the determination of glucose in blood is herewith described. The sample cups are filled with specimens of blood and are arranged in order on the sampler. The sampling crook withdraws blood from each cup for a definite period of time, thus controlling the volume of the sample. As the sample proceeds along the tubing it is combined with potassium cyanide solution. Bubbles of air also are pumped in to separate the solutions into many segments. The air helps to mix the sample and the potassium cyanide solution, and also helps to prevent retention of the aqueous solutions on the walls of the nonwettable tubing.

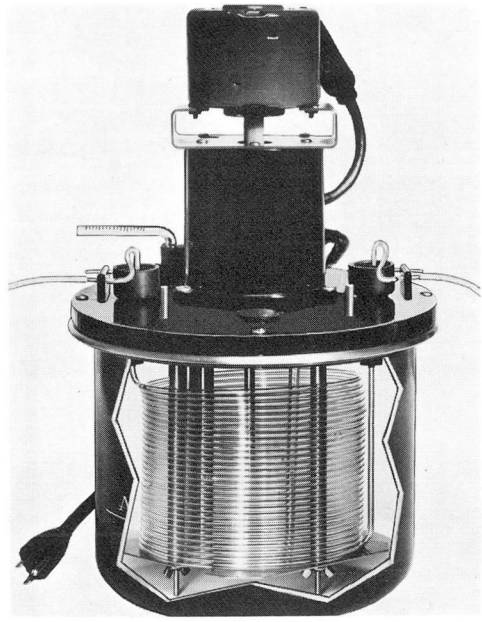


Fig. 7. Heating bath. Side view showing the glass coil. (Courtesy of Technicon Instruments Corporation, Chauncey, New York.)

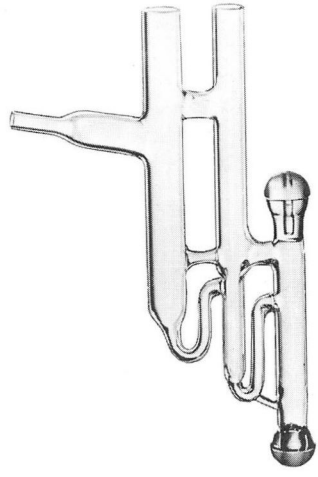


Fig. 8. Colorimeter flow cell, open type. Solutions enter from the side arm and exit from the bottom ball joint. Top ball joint assists in alignment. (Courtesy of Technicon Instruments Corporation, Chauncey, New York.)

After passing through a glass mixing coil the solution enters the dialyzer. The crystalloids, including glucose, diffuse through the cellophane membrane of the

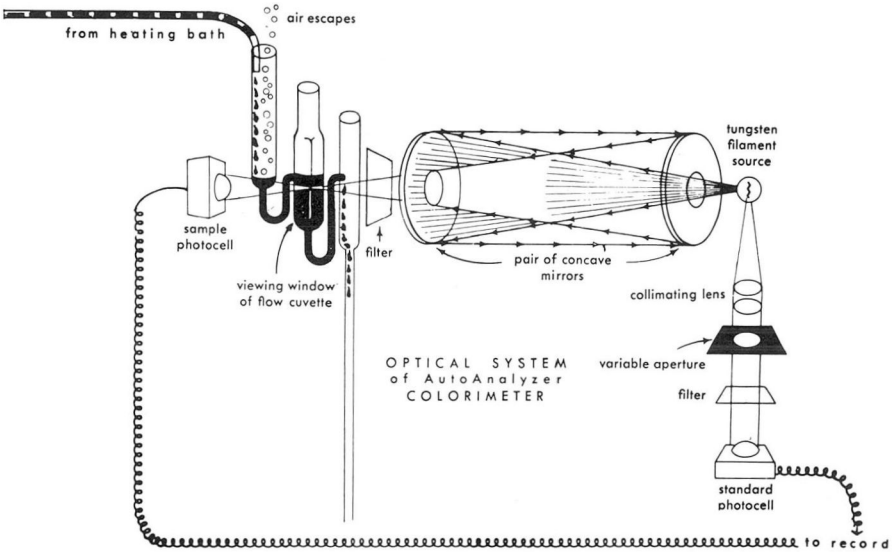


Fig. 9. Schematic diagram of colorimeter optical system. (Courtesy of Technicon Instruments Corporation, Chauncey, New York.)

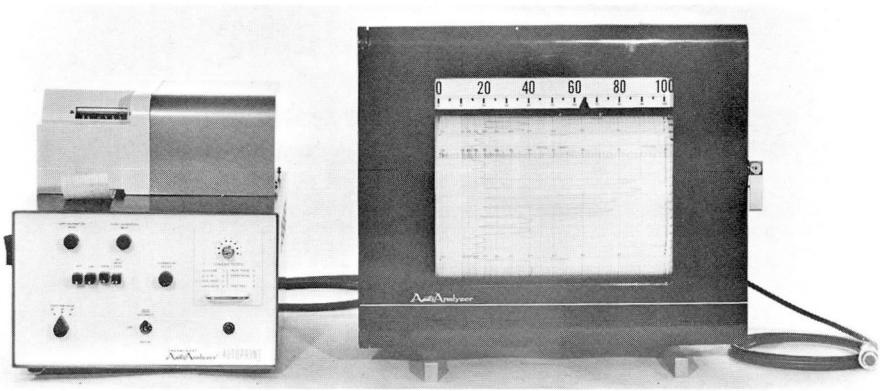


Fig. 10. Readout with recorder in use for the glucose determination. (Courtesy of Technicon Instruments Corporation, Chauncey, New York.)

dialyzer into a stream of potassium ferricyanide. The nondiffusible substances continue on through the dialyzer and out to a waste drain. The ferricyanide stream leaves the dialyzer and enters a 95 C. heating bath. After about seven minutes of transit through the coils of this bath the glucose has reduced yellow ferricyanide to colorless ferrocyanide[4] in proportion to the amount of glucose present.

The exit tube of the heating bath leads to the colorimeter flow cell where the air escapes; the solutions pass through the cell and finally into a waste drain. The

recorder records the changes in light absorbance of the solution. In this analysis, the greater the glucose concentration in the original sample, the less dense will be the color in the final solution. Concentrations of glucose are estimated from a curve of absorbance against concentration prepared from standard solutions.

It is now possible for the AutoAnalyzer to perform almost any colorimetric analysis. One of the limitations has been the capacity of tubing to transport various solutions; with the newest tubing compositions, liquids such as sulfuric acid and other strong acid mixtures, and certain solvents such as alcohol can be pumped. It is possible to transfer liquids that cannot be pumped directly, by displacement from a container with a liquid that can be pumped.

Certain analyses that have required considerable manual dexterity and manipulation have been automated. For example, the Kjeldahl determination of total nitrogen is carried out by means of a continuous digestor (*Fig. 11*). The digestor

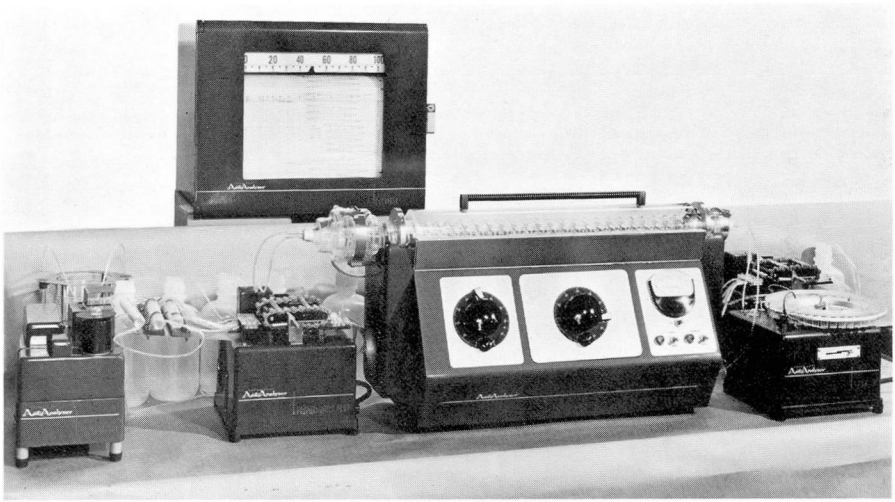


Fig. 11. Continuous digestor in use with other modules for the determination of total nitrogen. (Courtesy of Technicon Instruments Corporation, Chauncey, New York.)

tube continually turns, carrying the digesting sample through a heater by means of a spiral groove manufactured into the tube. By the time the sample reaches the end of the digestor, digestion is complete. A sampling tube removes a portion of the sample, which is mixed with other reagents to dilute it and to develop a color that can be read in the constant-flow colorimeter and is recorded on the recorder.

The constant-flow system works particularly well with the flame photometer for the analysis of sodium and potassium[5] (*Fig. 12*). The electrolyte analyzer system, which simultaneously analyzes each sample for sodium, potassium, carbon dioxide, and chloride content, eliminates certain of the components that would be used if the determinations were carried out individually. By judicious combination of

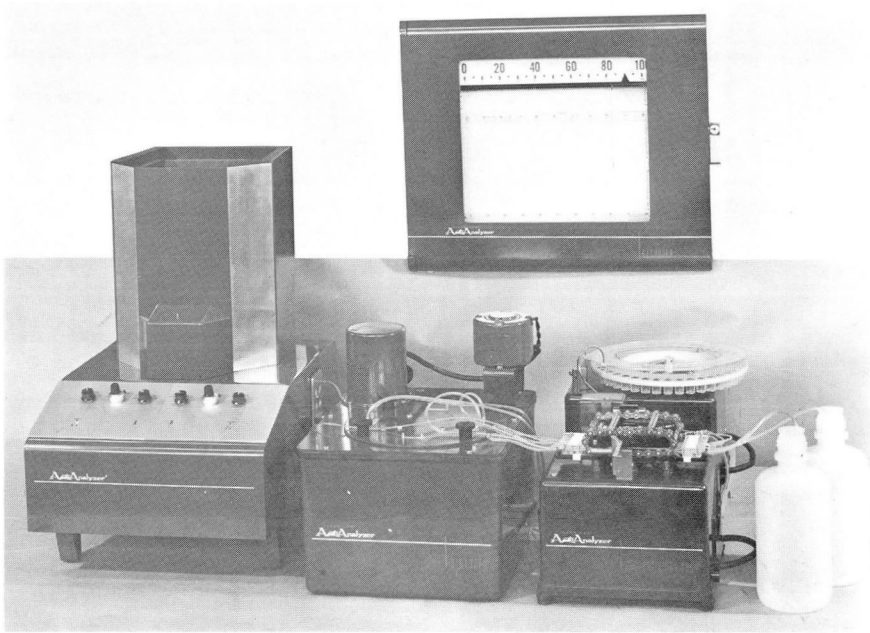


Fig. 12. Flame photometer. Recorder is equipped to record simultaneously sodium and potassium levels. (Courtesy of Technicon Instruments Corporation, Chauncey, New York.)

modules, one sample turntable, one pump, and one dialyzer may be used for the analysis of the four electrolyte components.

Many other innovations using this system have been devised or are in the process of development, in addition to improvements and changes within the system. However, all changes have been made so that the original equipment is still useful—an advantage of the modular system. The continuous filter is an intriguing development that will no doubt expand the usefulness of a constant-flow system (*Fig. 13*). The sample and the extracting solvent are pumped together into a mixing chamber where rapid mixing is assured with a motorized stirrer. A slurry of precipitated protein and solvent drop down onto a strip of filter paper moving continuously over a plastic plate. The precipitate remains on the filter paper and is carried forward while the filtrate is collected from below the plastic plate and is pumped elsewhere for analysis.

*Bench-Top Robot Chemist.** Another type of automatic device based on interrupted sequences is the Bench-Top Robot Chemist (*Fig. 14*). This apparatus performs various analyses in a fashion that more closely imitates the actions of the human analytical chemist than does the constant-flow systems.

Specific volumes of samples and other liquids are pipeted, are transferred, and are carried through analysis by a system of timers, relays, cams, and pumps. By

**Manufactured by Research Specialties Co., Richmond, California.*

means of the glass pipets that project out above the center of a turntable that contains the samples, the Bench-Top Robot Chemist can withdraw, can move, and can transfer samples to test tubes, can add reagents, and even can wash and dry the test tubes! At the proper time a pipet will transfer solution to a colorimeter (not shown) where the amount of color developed is estimated and then is recorded.

Such a device will fit in best where the continuous-flow system type of automatic analyzer is not so efficient. For example, the continuous-flow system is not at its best in procedures where long intervals of time are required; the Bench-Top Robot Chemist however, can more easily be programmed to handle long intervals. It appears to be superior to the constant-flow system when performing tests that require high heat, such as one would encounter in the typical nonprotein nitrogen or phospholipid analyses.

The main defect of the Bench-Top Robot Chemist may lie in its highly mechanical nature that limits its versatility and ease of repair. The AutoAnalyzer on the other hand is particularly easy to repair, to adjust, or to convert to other analyses. For specific tests, such as those using strong acids and hot digestions, or those in which one or more steps require extensive periods of time for incubating, such as

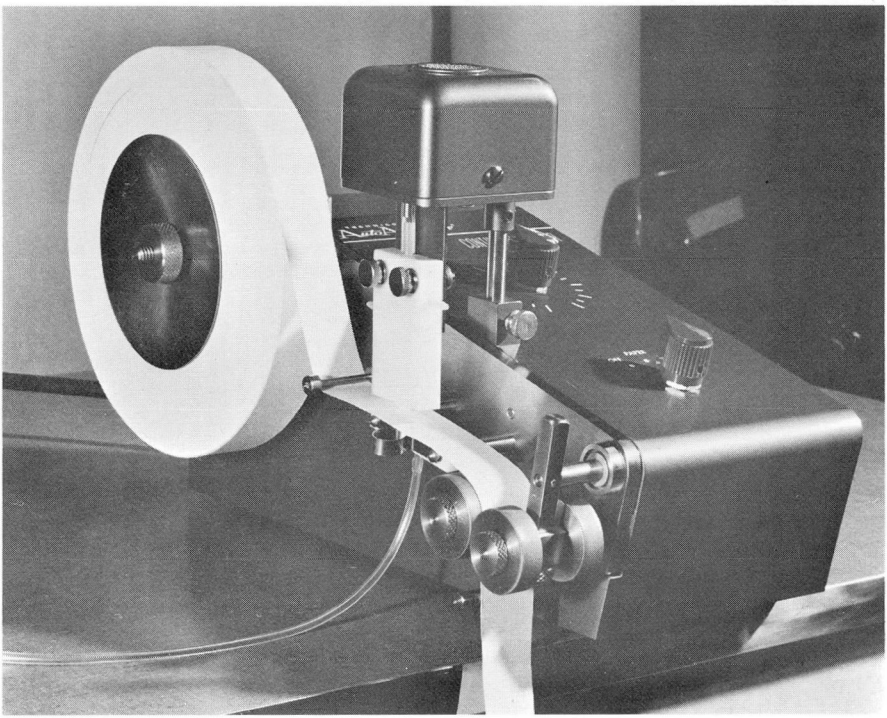


Fig. 13. Continuous filter. (Courtesy of Technicon Instruments Corporation, Chauncey, New York.)

Fig. 14. Bench-Top Robot Chemist. All working parts are contained within the cabinet; turntable and transfer pipets project above; complicated analysis may require two or more units placed side by side; colorimetric system is not shown. (Courtesy of Research Specialties Company, Richmond, California.)

in the measurement of enzyme activities,[6] the Bench-Top Robot Chemist has much to offer.

Comment

It is possible now to automate most of the tests used in the modern clinical laboratory. Those tests that require extraction with solvents such as the determination of fecal fats are not often automated. Among those for which automation procedures exist, a number of factors must be considered. An important factor is the work-load volume, because large numbers of samples are often best handled by automation. However, some laboratories do not limit the use of automation to those tests done in large numbers. In some instances automation may be the most practical way of performing an analysis. For example, column chromatography of amino acids or steroids is much more satisfactorily done with automatic equipment (*Fig. 15*);[7] performed manually these procedures are time consuming and require exacting meticulous care—the machine easily bears much of the burden.

One area of automation in clinical chemistry is of special interest: the use of the constant-flow systems for in vivo analysis. With automatic analysis available, physiologists and pharmacologists should be able to make measurements that were

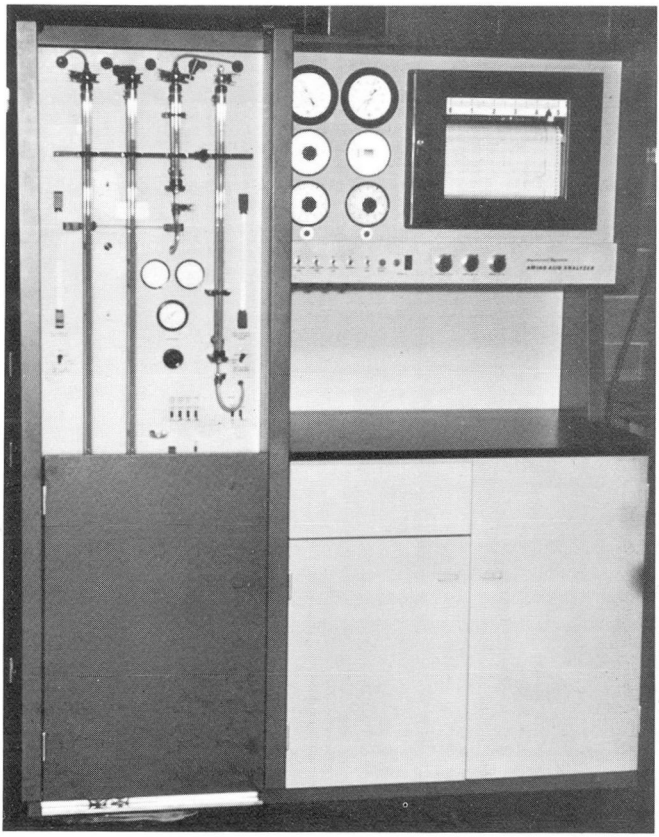


Fig. 15. Beckman Spinco Amino Acid Analyzer.

never before practical or even possible. Sampling rates at less than 0.1 ml. per minute will enable continuous sampling from many test subjects without fear of exsanguination. By proper placement of a catheter, sampling can be made from any part of the body.

Work of this nature on patients undergoing thoracic surgery has been reported by Clark.[8] Continuous monitoring of patients is done for electrolytes, pH, and blood gases during and after the surgical procedure. Special rooms for study with the equipment mounted in the walls, house the patients (*Fig. 16*). For analysis at the bedside of patients, carts have been made which contain the automatic analyzers.

Gradually the automatic analyzing instruments are bringing us closer to the realization of a dream held by many workers in the medical field. The well-equipped hospital of the near future may have an automatic laboratory in the walls of each patient's room. Attending physicians would know at a glance the level of a certain

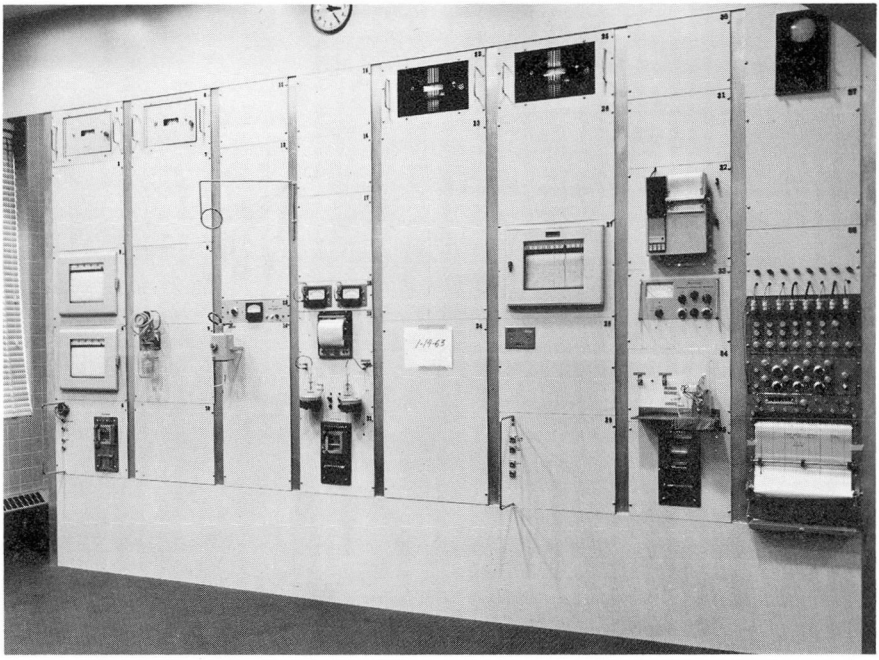


Fig. 16. *Analytical wall* designed for continuous direct monitoring of patients. (Courtesy of Leland C. Clark, Jr., Ph.D., Medical College of Alabama, Birmingham, Alabama.)

blood component at that moment, and what changes occurred since their last observations of the patient. It may even be possible that equipment that can analyze can also set up corrective measures. For example, when the blood sugar rises above certain limits, insulin could be automatically injected, and at low levels, glucose could be injected.[9]

One must not forget, though, that with its advantages, automation brings problems too.[10] First, it requires of laboratory personnel that they learn maintenance and repair of mechanical and electronic equipment, because sooner or later, trouble develops and someone must be able to repair the device quickly and properly. Secondly, the user of the automatic equipment tends to surrender surveillance to, and to place too much reliance on, the machine. There is also the tendency to rely upon the manufacturer of the machine, rather than on the clinical chemist's experience and ingenuity in the development and use of methodology for such devices. It is important to realize that machines are fallible, and that accuracy, even by automation, requires human aid (the difficulties experienced by several of the American astronauts are notable examples). Generally, when instruments are in error there are definite signs of difficulty which trained workers can recognize. In the hands of skilled operators, automatic equipment can often be as accurate and dependable as the best human technician. With the skill and tireless capacity of

automation for work, the future of medical care from the clinical laboratory viewpoint is indeed bright.

References

1. Seligson, D.: Automatic pipetting device and its application in clinical laboratory. Am. J. Clin. Path. **28:** 200-207, 1957.
2. Saifer, A.; Gerstenfeld, S., and Zymaris, M.C.: Rapid system of microchemical analysis for clinical laboratory. Clin. Chem. **4:** 127-141, 1958.
3. Skeggs, L. T., Jr.: Automatic method for colorimetric analysis. Am. J. Clin. Path. **28:** 311-322, 1957.
4. Hoffman, W. S.: Rapid photoelectric method for determination of glucose in blood and urine. J. Biol. Chem. **120:** 51-55, 1937.
5. Isreeli, J.; Pelavin, M., and Kessler, G.: Continuous automatic integrated flame photometry. Ann. New York Acad. Sc. **87:** 636-649, 1960.
6. Schwartz, M. K., and Bodansky, O.: Automated methods for determination of enzyme activity, p. 211-246, *in* Glick, D., editor: Methods of Biochemical Analysis, vol. **11.** New York: Interscience, 1963, 442 p.
7. Spackman, D. H.; Stein, W. H., and Moore, S.: Automatic recording apparatus for use in chromatography of amino acids. Anal. Chem. **30:** 1190-1206, 1958.
8. Clark, L. C., Jr., and Lyons, C.: Electrode systems for continuous monitoring in cardiovascular surgery. Ann. New York Acad. Sc. **102:** 29-45, 1962.
9. McCullagh, E. P.: Hormones—whence and whither. J. Clin. Endocrinol. & Metab. **19:** 1563-1574, 1959.
10. Hainline, A., Jr.; consulting editor: Automated and semi-automated systems in clinical chemistry. Introductory remarks, p. 3. Ann. New York Acad. Sc. **102:** 1-180, 1962.